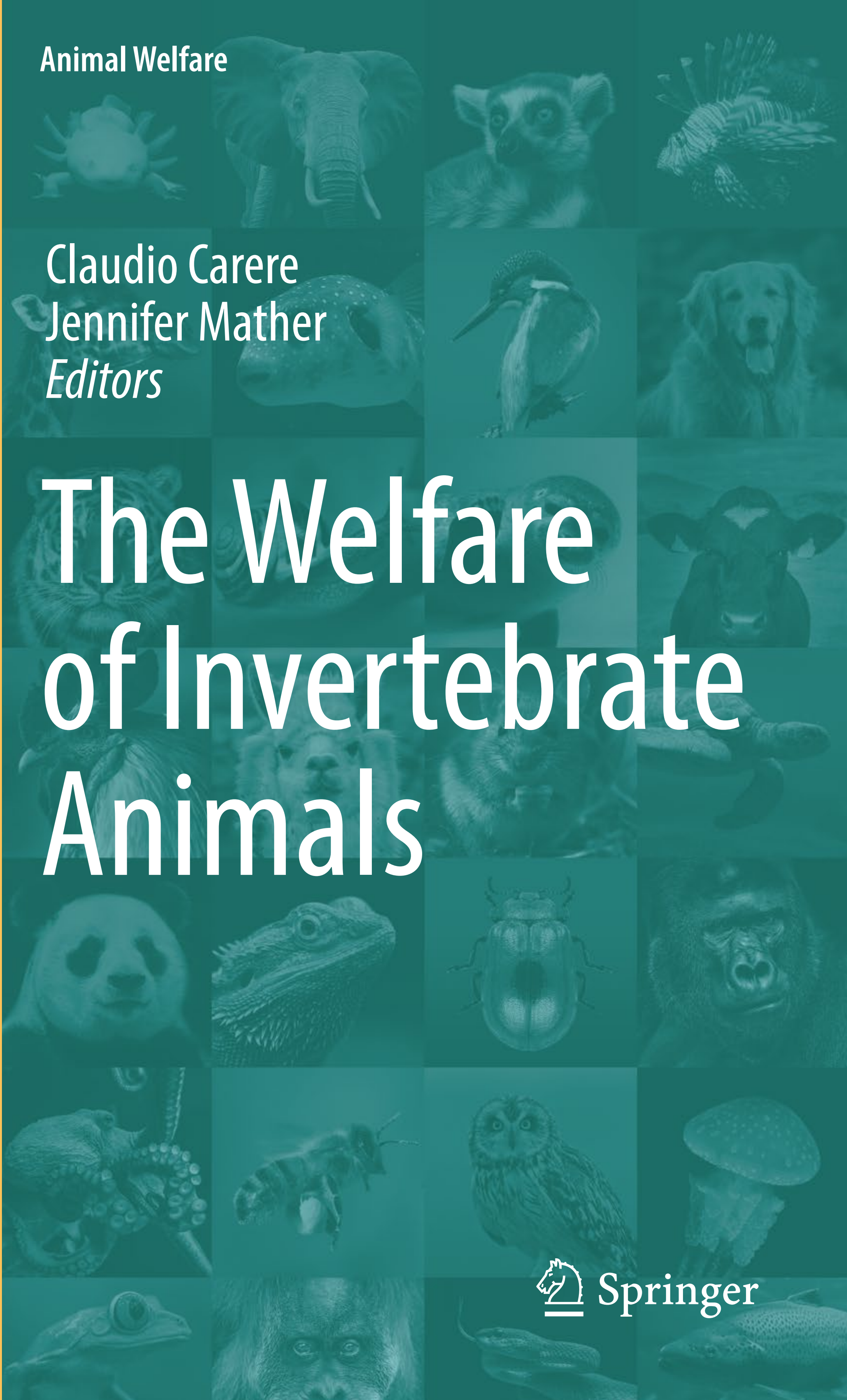
Animal Welfare



Claudio Carere
Jennifer Mather
Editors

The Welfare of Invertebrate Animals

Springer

Animal Welfare

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Claudio Carere • Jennifer Mather
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Editors
Claudio Carere
Department of Ecological and Biological
Sciences, Ichthyogenic Experimental
Marine Centre (CISMAR)
University of Tuscia
Tarquinia, Viterbo, Italy

Jennifer Mather
Department of Psychology
University of Lethbridge
Lethbridge, Alberta, Canada

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My Vote for the “Invertebrates”

At the time when I was a young student and quite some time beyond, most biologists were convinced: if you can’t even know what your best friend is thinking and feeling, how can you ever know what is going on in an animal? It was considered unscientific for such dogmatic reasons to seriously ask a question regarding the mental life, the thinking and feeling of an animal. You could not hope to find answers but would rather get lost, if you dared to delve into this black hole. Among widespread beliefs and attitudes were the following: (1) that we can never say anything about feelings or thoughts of animals; (2) that man is too distinct from animals to be compared with them; (3) that man is the centre of the world and human society has to take care of humanity only; and (4) that animals are dull and “bêtes machines”, as Descartes said, just things, which automatically follow their instincts in a preprogrammed way.

I am of course exaggerating. Darwin had firmly embedded man in the process of evolution and placed us on a continuum with animals a long time ago, and there have always been keepers of cats, dogs and horses and maybe even birds who would insist that their pets do think and feel and have emotions and personality. Obviously, it is easier to assign such capabilities to animals the closer these are to us humans phylogenetically. But invertebrates like crickets and fruit flies? For most listeners, it was at best an entertaining joke when I talked about the individuality of spider males, impressed by the differences of their courtship behaviour. Remember that Jane Goodall even met with difficulties when giving her chimpanzees in Gombe human names and personality in the 1960s.

Although much of the attitude prevailing at my time as a biology student still exists outside our field of expertise, big changes of our view of the world have occurred since. Many of us (though not enough yet) now better understand that humankind has responsibilities beyond itself or, put in another way, that we have to take care of the entire planet with all its diversity of life and environmental complexities. Nature is not just a machine endlessly producing for us only, or a big buffet table at which we can endlessly serve ourselves without paying. And wilderness is not just luxury. We have instead begun to better understand that all living beings are interconnected on this planet and depend on each other, that sustainability is a big issue and that nature cannot be treated as if it existed to serve our human needs and pleasures only. I hopefully assume that there is more awareness of biophilia now, following the psychoanalyst Erich Fromm and even more so the biologist Edward O Wilson: the appreciation of all living beings and a feeling of deep-seated connectedness. Some unpleasant changes critically endangering our own human welfare like overpopulation, food and energy shortage and climate change have helped to promote this point of view. A few societies only, mostly small and of little global influence, have kept the old traditions of their balanced view of nature. Unfortunately, they are unable to stop the dramatic impact of industrialization and the unlimited endeavour for our human well-being and materialistic success.

I am not an expert on questions of animal welfare but instead an experimental physiologist doing basic research. As is true for many biologists, my own biophilia implies a sense for the deep value of nature and an interest in its increasingly profound understanding, including an understanding of our human place in it. Apart from my professional interest in science, it is this background and wishful thinking which underlies my interest in animal welfare and the invertebrates.

I read through this book on the welfare of invertebrate animals with great interest and much appreciate that the topic is given so much attention. Apart from the presentation of practical cases like that of butterfly houses, bee keeping, spiders in captivity and captive cephalopods, experts also address questions of philosophy and ethics. I was pleased to find so many honest “might”, “could”, “probably” and subjunctives. No wonder we are still far from a clear understanding of nociception and pain, the integration of sensory information in the central nervous systems of most invertebrates and the selection pressures and fitness gains associated with much of invertebrate behaviour. Feelings and thoughts of a snail or an earthworm or a cockroach are still obscure issues. And of course, one cannot expect any overarching general recipe to provide welfare for such a large and diverse group of animals as are the invertebrates. From a zoological point of view, the “animals without backbones” are much too heterogeneous to be put into one phylum. Their diversity could not be greater, considering their Bauplan, their habitat, nervous and sensory systems and behaviour. A sessile sponge filter feeding in the sea, an earthworm digging through the soil, a spider building mechanical masterpieces of aery silken webs, a cricket singing love songs or a dragonfly manoeuvring masterly through the air: the differences could not be greater.

For the zoologist, the so-called invertebrates (not a serious terminology in taxonomy) comprise as many as 26 of a total of 27 phyla, the only additional one being the chordates which include us and all other vertebrates. “Invertebrates” make up for at least 95% of all animal species so far described. A few numbers illustrate their dominance impressively: insects c. 1,000,000 described species (and probably some 10 million existing) as opposed to vertebrates with only c. 66,000 species; even the arachnids are good for more than 100,000 species, with the spiders alone representing almost 50,000 described species. We are living in an age of insects and even more so of arthropods. Seen from this and an evolutionary point of view, it is frustrating that some people still talk about “insects (or even bugs) and animals”. Aren’t insects like all other “invertebrates” worth being called animals as well?

When we talk about the welfare of the “invertebrates”, we should also gratefully appreciate how important they have become in research, allowing us to reveal many fundamental secrets of life at all levels of organization, from molecular to organismal. The fruit fly *Drosophila,* the nematode *Caenorhabditis*, the honeybee *Apis*, the coelenterate *Nematostella* and others have become outstandingly important model organisms. Even spiders, disliked by many for no good reason, have made their way into experimental research asking serious questions of cognition and personality. Neurobiology owes the understanding of action potentials to the giant fibres of the squid *Loligo*, that of lateral inhibition to the horseshoe crab *Limulus* and that of learning and memory to a large extent to the sea hare *Aplysia.* And who had thought only some thirty years ago that the fruit fly and bees and jumping spiders might play a significant role in cognition research? Cognitive abilities of sometimes startling complexity now reach down far in the phylogenetic tree. Man is not so distinct from the rest of the animal kingdom anymore, including the “invertebrates”. Latest research has brought to light rather surprising deep homologies conserved throughout the metazoans. There are many correspondences of neural circuits in arthropods and chordates (vertebrates), and there is evidence for the conservation of neural ground patterns and genetic mechanisms underlying brain development. We have good reasons to assume an ongoing crucial role of “invertebrates” in biological research and the revelation of exciting new insights, not the least because the large majority of them have so far remained untouched by science.

Trying to be a bit more specific regarding “The Welfare of Invertebrate Animals” I would like to end with the following.

– The only and maybe trivial but best general recommendation that I can think of at present is to keep animals under conditions as natural as possible and to sacrifice as few of them as possible. To this end, it is crucial to learn and appreciate their way of life in the wild.
– “Invertebrates” will retain and even increase their particular value for research. Their well-being in the laboratories is also in the interest of the success of meaningful experiments.
– There is the possibility of pain and suffering of “invertebrates”, although there is still a lot of uncertainty. A particular difficulty still is to get away from too much anthropocentrism. However, despite all basic difficulties one should not stop to ask the relevant questions. “Invertebrate Animals” definitely should be included in our moral concerns.
– Considering the exciting recent discoveries regarding the nervous system and mental capabilities of “invertebrates”, we may expect many more surprises in the future revealing “higher functions” in animals, even including emotionality and intentionality.

To truly understand any “invertebrate’s” physical and mental condition will need more than an anthropomorphic comparison: it needs questions and insights derived from the particular lifestyle of the animal species concerned. Each species to a large extent will have to be considered in its own right, if we want welfare and avoidance of potential pain and suffering. And not the least, we must not forget about the welfare of billions of “invertebrates” in the wild and the necessity to protect and take care of their habitats.

This book is a much appreciated introduction to a broad spectrum of findings and questions associated with “invertebrate” animal welfare. It reflects the necessary and brave beginning of a change of our attitude towards the so-called lower animals, which after all are not as low as thought for a long time.

Friedrich G. Barth

Department of Neurobiology
University of Vienna
Vienna, Austria
August 2018

Animal Welfare Series Preface

Animal welfare is attracting increasing interest worldwide, especially in developed countries where the knowledge and resources are available to, at least potentially, provide better management systems for farm animals, as well as companion, zoo, laboratory and performance animals. The key requirements for adequate food, water, a suitable environment, companionship and health are important for animals kept for all of these purposes.

There has been increased attention to animal welfare in the West in recent years. This derives largely from the fact that the relentless pursuit of financial reward and efficiency, to satisfy market demands, has led to the development of intensive animal management systems that challenge the conscience of many consumers in this part of the world, particularly in the farm and laboratory animal sectors. Livestock are the world's biggest land users (FAO 2002), and the farmed animal population is increasing rapidly to meet the needs of an expanding human population. This results in a tendency to allocate fewer resources to each animal and to value individual animals less, for example in the case of farmed poultry where flocks of over twenty thousand birds are not uncommon. In these circumstances, the importance of each individual's welfare is diminished.

In developing countries, human survival is still a daily uncertainty, so that provision for animal welfare has to be balanced against human welfare. Animal welfare is usually a priority only if it supports the output of the animal, be it food, work, clothing, sport or companionship. However, in many situations the welfare of animals is synonymous with the welfare of the humans who look after them, because happy, healthy animals will be able to assist humans best in their struggle for survival. In principle, the welfare needs of both humans and animals can be provided for, in both developing and developed countries, if resources are properly husbanded. In reality, the inequitable division of the world's riches creates physical and psychological poverty for humans and animals alike in many parts of the world.

Increased attention to welfare issues is just as evident for zoo, companion, laboratory, sport and wild animals. Of growing importance is the ethical management of breeding programmes, since genetic manipulation is now technically advanced, but there is less public tolerance of the breeding of extreme animals if it comes at the expense of animal welfare. The quest for producing novel genotypes has fascinated breeders for centuries. Dog and cat breeders have produced a variety of deformities that have adverse effects on their welfare, but nowadays the breeders are just as active in the laboratory, where the mouse is genetically manipulated with equally profound effects.

The intimate connection between animals and humans that was once so essential for good animal welfare is rare nowadays, having been superseded by technologically efficient production systems where animals on farms and in laboratories are tended by increasingly few humans in the drive to enhance labour efficiency. With today's busy lifestyles, companion animals too may suffer from reduced contact with humans, although their value in providing companionship, particularly for certain groups such as the elderly, is beginning to be recognised. Animal consumers also rarely have any contact with the animals that are kept for their benefit.

In this estranged, efficient world, people struggle to find the moral imperatives to determine the level of welfare that they should afford to animals within their charge. A few people, and in particular many companion animal owners, strive for what they believe to be the highest levels of welfare provision, while others, deliberately or through ignorance, keep animals in impoverished conditions in which their health and well-being can be extremely poor. Today's multiple moral codes for animal care and use are derived from a broad range of cultural influences, including media reports of animal abuse, guidelines on ethical consumption and campaigning and lobbying groups.

This series has been designed to contribute towards a culture of respect for animals and their welfare by producing learned treatises about the provision for the welfare of the animal species that are managed and cared for by humans. The early species-focused books were not detailed management blueprints; rather, they described and considered the major welfare concerns, often with reference to the behaviour of the wild progenitors of the managed animals. Welfare was specifically focused on animals' needs, concentrating on nutrition, behaviour, reproduction and the physical and social environment. Economic effects of animal welfare provision were also considered where relevant, as were key areas where further research is required.

In this volume, the series departs from the single vertebrate species model to address the welfare of invertebrate animals, which form the majority of species on Earth. In the book, it becomes apparent that many species have levels of cognition that were until recently completely ignored and unrecognised. Although individual animals may have reduced cognitive powers compared with vertebrate animals, collectively they often function as a "superorganism". These often have extraordinary perceptive skills and advanced functional behaviour. Other species may display many of the same behaviours that are used as evidence that vertebrate animals are sentient, but the cognitive powers are not necessarily collected into a structure we call a brain. It becomes obvious as you read the book that there is no logic to using the possession of a vertebrate brain as a prerequisite for ethical status, which is currently usually the case in legal considerations of animal status. Indeed, convincing arguments are made for the attribution of a brain to some invertebrates. Furthermore, it is evident from the research described that functional significance should be recognised for some homologous structures to vertebrate sensory organs, which in many cases provide comparable performance in terms of pain avoidance, anxiety responses and other features of nociception. Thus, this book presents a compelling case for greater recognition of invertebrate animals in our moral compass. Not only is it clear that they are deserving of that, it is also apparent that their welfare is under threat from global warming, monocultural practices in agriculture and the various toxic substances that humans apply to the plants to maintain the monocultures free of disease. The number of animals involved in the human food chain, for example, vastly exceeds the number of vertebrate animals, so even if suffering is acknowledged to be less, the greater number compels us to introduce some control of their welfare.

Gatton, QLD, Australia Clive Phillips

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Contributors

Paul Andrews Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy

Association for Cephalopod Research 'CephRes' – a non-profit research organization, Napoli, Italy

Michael Boppré Forstzoologie und Entomologie, Albert-Ludwigs-Universität, Freiburg, Germany

Claudio Carere Department of Ecological and Biological Sciences, Ichthyogenic Experimental Marine Centre (CISMAR), University of Tuscia, Tarquinia, Viterbo, Italy

Laboratoire d'Ethologie Expérimentale et Comparée EA4443, Université Paris 13, Sorbonne Paris Cité, Paris, France

Gavan M. Cooke Department of Life Sciences, Anglia Ruskin University, Cambridge, UK

Robert W. Elwood School of Biological Sciences Queen's University, Belfast, UK

Graziano Fiorito Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy

Association for Cephalopod Research 'CephRes' – a non-profit research organization, Napoli, Italy

Viola Galligioni Association for Cephalopod Research 'CephRes' – a non-profit research organization, Napoli, Italy

Comparative Medicine Unit, Trinity College Dublin, Dublin, Ireland

Claudia Garrido BeeSafe, Leverkusen, Germany

Matjaž Gregorič Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia

Simona Kralj-Fišer Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia

Jennifer A. Mather Department of Psychology, University of Lethbridge, Lethbridge, Canada

Antonio Nanetti CREA-AA, Bologna, Italy

João Pereira Direcção-Geral dos Recursos Naturais, Segurança e Serviços Marítimos, DGRM/DRI, Lisbon, Portugal

Departamento do Mar e Recursos Marinhos, IPMA, Lisbon, Portugal

Simone Pollo Department of Philosophy, Università "Sapienza", Roma, Italy

Giovanna Ponte Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy

Association for Cephalopod Research 'CephRes' – a non-profit research organization, Napoli, Italy

Belinda M. Tonkins The College of Animal Welfare, Headland House, Godmanchester, UK

Richard I. Vane-Wright Durrell Institute of Conservation and Ecology (DICE), University of Kent, Canterbury, UK

Life Sciences, Natural History Museum, London, UK

Augusto Vitale Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Roma, Italy

Ernesto Weil Department of Marine Science, University of Puerto Rico, Mayaguez, PR, Puerto Rico

Alejandro Weil Center for Biospecimen Research and Development, NYU Langone Health, New York, NY, USA

Adriana Weil-Allen Department of Marine Science, University of Puerto Rico, Mayaguez, Puerto Rico

Chapter 1
Why Invertebrate Welfare?

Claudio Carere and Jennifer A. Mather

Abstract This introductory chapter sets up the roots and rationale of the volume while outlining its logical structure, contents and goals. It is about welfare, not of the captive laboratory or farm mammals and birds that we usually consider but about invertebrates, the other 99% of animal species. Invertebrates have been previously relegated to the category ‘things’ with no worry about what we do to them, but new research suggests that their behavioural and neurophysiological complexity was underestimated. Some invertebrates such as cephalopods, crustaceans and insects may feel pain and suffering and may have consciousness and awareness as well. Also, good welfare is going to mean different things to spiders, bees and coral animals. So we aim at taking animal welfare in a very different direction. We start by discussing why we need this book and what the value of non-vertebrate animals might be. The different chapters will focus on specific animal groups, tackling questions that are most appropriate to each one. What is pain in crustaceans, and how might we prevent it? How do we ensure that octopuses are not bored? What do bees need to thrive, pollinate our plants and give us honey? Since invertebrates have distinct personalities and some social animals have group personalities, how do we consider this? And, as in the European Union’s application of welfare consideration to cephalopods, how do the practical regulatory issues play out? This volume provides a first compilation of essays across invertebrate taxa illustrating how and why their welfare should be accomplished and instituted.

Information about what welfare of invertebrates might consist of is scattered and scant. Despite their widespread and worldwide utilization, invertebrates, with some

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C. Carere (✉)
Department of Ecological and Biological Sciences, Ichthyogenic Experimental Marine Centre (CISMAR), University of Tuscia, Tarquinia, Viterbo, Italy

Laboratoire d’Ethologie Expérimentale et Comparée EA4443, Université Paris 13, Sorbonne Paris Cité, Paris, France
e-mail: claudiocarere@unitus.it

J. A. Mather
Department of Psychology, University of Lethbridge, Lethbridge, Canada

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important exceptions, are often ignored in many biological surveys, and especially their welfare has received little and only recent attention (Carere et al. 2011; Horvath et al. 2013). One reason for this is that we tend to care about animals similar to us, and invertebrates look just very different; invertebrates were considered ‘things’, not animals, and the discussion as to whether ‘lower’ animals can even feel pain and suffering is burgeoning (see the online journal *Animal* Sentience, https://animalstudiesrepository.org/animsent/).The debate of whether crustaceans feel pain seems never-ending and with rigid positions, yet we need more experiments and data (Elwood 2016; Diggles 2019). Even with the 3R (Refine, Reduce, Replace) animal welfare approach (Zurlo et al. 1996), one of the tenets (‘replace’) was often considered to be replacement of ‘higher’ vertebrates with ‘lower’ invertebrates. Another factor leading to little consideration of the welfare of invertebrates was linked to the underestimation of their behavioural complexity and flexibility. Notably, research demonstrating cognitive abilities and even ‘emotionality’ is now spreading from the vertebrates to social insects, cephalopods, crustaceans and spiders.

Given these pressures, what kind of decisions can keepers and legislator make nowadays about invertebrates’ care and welfare assessment? Which directions should research on invertebrate welfare take? Think of the smart, sentient octopuses that have personalities, play and solve problems (Mather and Kuba 2013; Chaps. 8–10). Should we ensure that they are not bored? A committee for the American Zoological Association lays out procedures to do so (AITAG 2014). What of the lobsters that live in small social groups, is it reasonable to isolate them or crowd them into huge holding pens? Bees are major pollinators of our vegetable and fruit crops, how do we make sure they are free from diseases and not killed by pesticides? How do we ensure that spiders have good conditions of moisture, temperature and freedom from capture by other spiders? What do we do about the insects that are attracted to our crops? How, even, do we take sufficient care of coral animals so that the reefs that they form, each a major marine ecosystem, flourish and give homes to all the other animals that depend on them, and the tourists that visit them? Should such instances be accomplished, even formally? This volume includes reviews and essays across invertebrates, aiming primarily to fill this gap in an integrated manner and to provide exactly such knowledge, tools and recommendations, together with updated information and references about their diverse ecology, behaviour and physiology.

The book traces far back for its roots, to a conversation in the hallway at the University of Lethbridge. Gail Michener, a researcher who was also on the board of Canadian Council of Animal Care, came down the corridor and asked JM ‘Do you think of your octopuses as tissue?’ She said, having already understood their sensitivity, flexibility and intelligence, ‘Of course not. Why?’ It turned out that the US Department of Agriculture categorized all invertebrates for invasiveness of research procedures as the equivalent of dissected-out tissue (and they still do). Gail convened an ad hoc committee of JM, Ron O’Dor of Dalhousie and Dan Johnson of the Geography Department to look into the issue. After some debate and consultation with colleagues (Paul Lewis asked in the case of such associations, whether one would protect host or parasite), we concluded that cephalopods deserved the same protection as vertebrates, and they have retained that status for research in Canada since 1991. Sometime later, JM was attending the annual conference of the Animal Behavior Society and a group were discussing animal pain and suffering over dinner in the cafeteria. Since JM was teaching human sensation and perception as well as working with octopuses, she had been thinking of what those terms might mean for cephalopods. After the discussion, Stephen Zawistowski asked her if she would write a paper on invertebrate suffering for the *Journal of Applied Animal Welfare Science*, and it was published in 2001. During this time, her colleague Roland Anderson was writing about keeping octopuses in captivity, including their escape tendency, enrichment and release at the end of the lifespan (see Chap. 8). Not surprisingly, these views came together, and when the journal *Diseases of Aquatic Organisms* produced a special issue on invertebrates, they asked him and JM to provide a chapter (Mather and Anderson 2007). When the Institute of Laboratory Animal Research published a special issue on welfare and use of invertebrates in the laboratory and classroom, JM had begun to teach a course on human–animal interactions, so was able to write a piece for them on the philosophical background of and attitudes to invertebrates (see Chap. 2).

Welfare of invertebrates was an obscure topic until in 2010 the European Union (EU) decided to update their animal welfare legislation. What animals should be protected was included in the revision, and considerable debate ensued over the candidacy of crustaceans (see Chap. 7) and cephalopods (Chap. 9). Indeed, during the long period of ‘gestation’ of the new Directive, the issue of including crustacean decapods gave rise to various discussions and point of views, but the decision was eventually negative. While the writers concluded that only cephalopods should be protected, they extended this consideration past research to treatment of cephalopods in captivity, aquaculture and fishing. The EU set up an agency to look at issues such as methods of capture, training of workers in cephalopod welfare and anaesthetics, far beyond the narrow protection of the Canadian guidance for researchers (Chap. 9). Since the exit of Great Britain from the European Union, an organisation called Crustacean Compassion has begun to work for protection of that group in the UK as well (Chap. 7).

It was when CC started his PhD on personality of a common songbird in the late 1990s at the University of Groningen (the Netherlands) that, as a young PhD fellow being sure and proud of the originality of his project, he bumped with a mix of curiosity and disappointment into the seminal paper ‘Personality of Octopuses’ (*Journal of Comparative Psychology* 1993) by JM and Roland Anderson—probably the first paper ever to speak about animal personality, as seen from the title. In an invertebrate! Several years later—meanwhile the field of animal personality had been growing tremendously yet mainly on vertebrates—CC invited JM to write a chapter with David Logue for the volume on animal personality he was editing with primatologist Dario Maestripieri (published in 2013 by University of Chicago Press). The title of the chapter was ‘The Bold and the Spineless: Invertebrate Personalities’. The step from studying animal personality and becoming deeply interested in and worried about the scientific study of animal welfare was quite logical for CC, also boosted by the aforementioned new normative requirements in the European Union, as were most behavioural biologists at the time. Meanwhile, he became a member of the ASAB (Association for the Study of Animal Behaviour) ethics committee—a very stimulating task—and had been invited to work and supervise activities at the CISMAR (Ichthyogenic Experimental Marine Center, University of Tuscia) of Tarquinia in Italy, a new location on the Central Tyrrhenian coast with excellent facilities for breeding, housing and restocking of marine organisms, including crustaceans and cephalopods, and many enthusiast students. Surely, animal behaviour is a wide-ranging and integrative discipline, but CC had never worked with those marine animals previously. A couple of e-mail exchanges, some formalities and an invitation to JM to come over to CISMAR for a couple of months were quickly set up. Our long-term collaboration was firmly established.

The biggest difficulty for invertebrate animal welfare is what has been called 'the hard problem'. How do we know what animals unlike us think and feel—see Nagel about what it is to be a bat (1974). Welfare consideration is often extended to animals that have intelligence and cognitive ability, whose behaviour is not automatic but the result of thought and evaluation. In addition we want to know whether they can feel pain, that bodily disruption is not met automatically with response (nociception), but is avoided, or the stimulus situation is calculated for trade-offs (pain). Scientists talk of whether animals are sentient or conscious. Traditionally, researchers and philosophers have given some cut-off sentience value above which we will generate consideration (Chap. 2), though Birch feels that we should use a precautionary principle and be cautious about eliminating species or groups, based on our incomplete knowledge (Birch 2017).

Conversely, recent research on invertebrates has uncovered far more cognitive ability than we would have thought of even a couple of decades ago. Thus it is also necessary to consider welfare of spiders (Chap. 5), social insects such as bees (Chap. 4) and other insects (Chap. 3). And yet Reber (2016) has argued that a cut-off value is not appropriate, that consciousness is an inherent feature of some kinds of organic forms and that a small foundation of sentience is present even in very simple animals. In that spirit, and because they are such important bases for whole ecosystems, the book includes a discussion of Cnidarian corals' welfare (Chap. 6). Similarly, because all animals have distinct personalities, we argue that evaluation and treatment of them must be at the level of the individual (Chap. 10), a stance with which Regan would likely agree.

This book is ambitiously ground-breaking and may be seen as provocative by some colleagues. It's about the other 99% of the animals we share the planet with. It takes welfare into a whole new and challenging area. For some of them like the octopuses or lobsters, we should be considering their pain and suffering, maybe even their boredom. For others such as the bees, we need to know how to keep them well and contented, so that they continue to pollinate our plants and give us honey. For corals, we need to figure out how to encourage these ecosystem builders so that the animal diversity they support and we visit and enjoy continues to exist. Yet this is a challenge too. We consider welfare from the viewpoint of the different groups and also through universals such as individual personality and encroaching regulations. But it had to be done, as we need to consider the animals that live with us on the planet, and a dialogue needs opening in this area. The diversity of physiological and behavioural capacity addressed in the chapters is stunning, especially in contrast to the single or few species covered in other books within the animal welfare series. Finally, given the large economic impact of invertebrates, and their extensive use in biomedical research, developing attitude changes towards invertebrates will be beneficial for producers and researchers, while providing better welfare conditions for the animals. They are primarily aimed at those who keep invertebrate animals, for research or in service to the goals of humans, who have a practical reason to understand those in their care. Invertebrates are increasingly used in scientific research, so we must understand their needs so that they thrive. Philosophers interested in the views of humans to the animals with which they share the planet will find new evidence and material for their understanding. And students will want to investigate this new area, both to care for the animals they investigate and to learn new views and attitudes about them.

Yet it is a start, tackling the criteria and considerations that are helping to make a true wide-scale view of welfare for animals.

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Chapter 2
Invertebrates and Humans: Science, Ethics, and Policy

Simone Pollo and Augusto Vitale

Abstract In this contribution we will first briefly describe different ways in which invertebrates are part of our lives and how we interact with them. A special focus is the use of invertebrates in scientific research. After a review of the major fields of investigation utilizing invertebrates, we will argue that their use in research constitutes an interesting and inspiring case study. As an example, the relatively recent European legislation on the protection of animals in scientific procedures now contemplates cephalopods. For this reason, it appears that animal experimentation is a kind of relationship in which the awareness of welfare problems of invertebrates is relatively more advanced. This opens a series of considerations on public attitude toward invertebrates, ethical issues arising on the use of these animals in research, compared both with other kinds of invertebrate/human relationship (e.g., pest control) and with the regulation of research on vertebrates, and the related legislative aspects. One question that is addressed is whether such attention for the ethical implications in the use of invertebrates in scientific research is and/or can be extended to other aspects of our relationships with these animals.

2.1 Introduction

The invertebrates are animals which do not possess a vertebral column, a distinction mainly of exclusion. This absence of a vertebral column groups together animals as different as insects, worms, and sponges. The term invertebrate covers 36 phyla, with 8 common ones: *Porifera* (sponges), *Cnidaria* (coelenterates), *Platyhelminthes* (flatworms), *Nematoda* (roundworms), *Annelida* (segmented worms), *Arthropoda* (the largest phylum in the animal kingdom, including insects, crustaceans, and spiders), *Mollusca* (the second largest phylum, including clams, snails, and octopuses), and *Echinodermata* (sea stars, sea urchins, and sea cucumbers). Invertebrates

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S. Pollo
Department of Philosophy, Università "Sapienza", Rome, Italy

A. Vitale (✉)
Center for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy
e-mail: vitale@iss.it

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do not share common structural or behavioral characteristics, which makes it difficult to think of them as a homogeneous group in terms of welfare. The largest group, the arthropods, possesses a hard and chitinous exoskeleton, but many have no skeleton and only vague neural organization. Like all animals, however, invertebrates are defined as heterotrophs, as they need other organisms to feed on in order to survive.

Invertebrates have colonized all kinds of climates and can be found in every ecosystem on the planet and are the only animal that can be found in the extreme regions of the Antarctica. The numbers are staggering: about 97% of living animals are invertebrates (IUCN 2014). The insect species are estimated to be around one million, whereas the total number of invertebrate species should be more than 1,300,000 (over one and a half million of living species have been described in total; see IUCN 2014). Despite these numbers, it has taken some time for invertebrate zoology to emerge as a significant field of research in biology. One of the first significant contributions was by Jean Baptiste Lamarck who authored, between 1815 and 1822, the "*Histoire naturelle des animaux sans vertebres*" (de Lamarck 1815) (Lamarck is thought to be the first to use the term "invertebrate"). His classification of molluscs was by far the most advanced for those times, and he was the first one to separate the arachnids from the insects. Charles Darwin also gave his contribution, including invertebrates in his theory of evolution, with the publication of his famous work on earthworms and the formation of vegetable mold (Darwin 1881). Having spent a great proportion of his younger years collecting insects, he made descriptions and notes on some beetle species appeared in Stephens' *Illustrations of British entomology* (1829–1932), giving the young scientist a great sense of satisfaction and fulfillment.

At the turn of and during the twentieth century, the study of invertebrates gave very significant contributions to medicine, genetics, ecology, and so on. Specialized scientific journals and dedicated scientific societies were born, with an increasing degree of specialization toward particular groups of invertebrates and to even more specific subdivisions within a particular group. Just to cite a few: the Entomological Society of America was born in 1889 (*American Entomologist* became its official journal) and the Malacological Society in London in 1893 (publishing the *Journal of Molluscs Studies*). Many scientific periodicals are published today, such as *Journal of Invertebrate Pathology*, *Invertebrate Biology*, *Invertebrate Neuroscience*, and many others.

2.2 Invertebrates Are Part of Our Lives

The presence of invertebrates extensively exists in the lives of humans. Here our intention is not normative, but purely descriptive: as in many other cases of our relationships with other animals, invertebrates are "used" by humans for many purposes; we are aware that this is a rather anthropocentric point of view. However, evolutionary and cultural history of humans is linked with different ways of exploiting other animals (see Pollo 2016), and invertebrates are no exception. To present these cases is, in our opinion, relevant also in the light of considering the use of animals, which are potentially able to experience pain. Later on, the ethical aspects of the relationships we have with invertebrates will be discussed from a philosophical point of view.

So, invertebrates, like all the other animals, are an essential part of our lives. We eat them, we study them, and some of us keep them as "pets," for example, tarantulas and other spiders (in Italy exists an association, called Aracnofilia, dedicated to the study, protection, and education on spiders (www.aracnofilia.org)). However, some species of invertebrates inspire fear and/or disgust in people. For example, the term arachnophobia indicates a specific terror of spiders and other arachnids, like scorpions (see Hardy 1988; Kellert 1993; Prokop and Tunnicliffe 2008 for some examples of how some invertebrates are perceived). The crawly movements of these animals make them particularly "alien" to us. Some of these animals indeed can harm people, and others are considered as pests: for example, in 2017 the EPA, the US Environmental Protection Agency, has listed cockroaches, crabs, mosquitoes, ticks, and bed bugs, among household pests, dangerous for public health (EPA 2017). The majority of invertebrates are in fact harmless and play a major beneficial role in different ecosystems. Many insects and other invertebrates are the primary food source for a large number of vertebrates, supporting and keeping alive entire ecosystems (Chap. 6).

Invertebrates also form part of our economy. The honey bees, for example, are farmed for agricultural reasons (Chap. 4). Honeybee farming origins are believed to be from 15,000 years ago. Egyptian art representing collection of honey has been estimated to be at least 4500 years old. Jars containing honey were found in Tutankhamun's tomb. Up to the eighteenth century, the hive was destroyed in order to collect honey, but from the nineteenth century onward, moveable hives have been used, improving the farming efficiency while protecting the bees' environment. The inventor of modern bee farming is Lorenzo Lorraine Langstroth, who published the book *The Hive and the Honey-bee*, pioneering the idea of moveable hives (Langstroth 1853). To illustrate the importance of honey production, just in the United States alone, more than four million bee colonies produce annually 80,000 tons of honey, and countries like Russia and India follow with more than 54,000 tons produced annually. Silk is another fundamental product we derive from invertebrates. The use of this natural fiber is known and documented from ancient times, especially in Asia. Silk fabric was first developed in ancient China (the earliest example of silk fabric is from about 3600 BC), and later the Roman Empire heavily traded in silk. The International Sericultural Commission publishes data on annual global silk production, and in 2016 about 192,692 of silk in metric tons were produced (www.inserco.org).

Pearls represent another commercial use of invertebrates with a long historic tradition. Pearls can be natural or cultivated. The natural ones are mainly due to pure chance, when the fragile edge of the shell of a bivalve mollusc or a gastropod is attacked by a fish or invaded by a parasite. Cultivated pearls are created by humans by inserting a tissue graft from another oyster. From Canada to the Gulf of Mexico, freshwater river mussels are also harvested for the purpose of creating artificial pearls. Currently China is the major producer of artificial pearls, and it is estimated at about 95% of the world production, with about 1600 tons of pearls put on the market every year (www.sustainablepearls.org/pearl-farming/pearl-farming-world-map/).

Invertebrates are part of our diet too, and in very general terms, we eat a great number of molluscs: marine snails, clams, cephalopods, land snails, and shrimps, lobster, or crab can all be eaten. The most important consumers of molluscs are the Japanese: it is thought that more than 100 different species are eaten daily in Japan. Squid is considered a basic and fundamental element of the diet of the Japanese people (Kurokura 2004, http://www.noaanews.noaa.gov/stories2008/20080717_seafood.html). Eating insects is part of the normal diet of many populations around the world. Insects are commonly eaten in the Americas, Africa, and Asia; about 1000 species of insects are known to be eaten in 80% of the countries in the world (that makes about 3000 ethnic groups practicing entomophagy) (Ramos-Elorduy and Menzel 1998). Arab populations commonly consume locusts as part of their diet, whereas ants, termites, grasshoppers, and beetle grubs are eaten by African people. Entomophagy has been suggested as a possible solution to environmental pollution: insects emit less greenhouse gases than conventional farm animals and can be fed grown on organic waste (Premalatha et al. 2011).

Invertebrates are ubiquitous in mythological tales and artistic expressions; their strange appearance, far away from the classical four-legged vertebrate, contributed to making these species perfect monsters in myths and legends. The folklore of nearly every country refers to invertebrates, who are protagonists in myths and legends since ancient times. For example, John Batchelor, describing the folklore of the Japanese people Ainu, mentions the sea monster Akkorokamui, a gigantic octopus with arms 120 m long (Batchelor 1901). To cite a few more examples, bees were linked to the cult of Artemis in ancient Greece (Elderkin 1939), and molluscs are represented beautifully in pottery art from the Minoan era (see, e.g., Gill 1985). In art and aesthetics, designs based on invertebrates are widespread in jewelry and fashion. Thousands of stamps illustrate insects and other invertebrates. Furthermore, we cannot forget the famous “Flight of the Bumblebee” by Rimsky-Korsakov or a musical quartet from Liverpool that named itself after a group of insects (although slightly changing the correct term to relate it to musical beat).

An important question is whether the general public acknowledges and appreciates the different ways these animals are important. Most people have a limited appreciation of the many benefits we derive from invertebrates and suffering from anxiety, and antipathy avoidance toward insects and arthropods in particular is still widespread (Prokop and Tunnicliffe 2008). Scientists and conservation group members were exceptions to this trend, but they really represent minor groups within society. Education on the importance of invertebrates will have positive effects on young generations, but cultural biases are sometimes difficult to change (see, e.g., Prokop et al. 2010). Scientists are very aware of the important progress made in science studying invertebrates.

2.3 Invertebrates in Scientific Research

Scientists have been and are studying invertebrates for different purposes: systematic models and molecular biology, cooperation and mutualism, mimicry, and genetics, just to name a few topics. Around 300,000 papers on invertebrates can be currently found in the PubMed website. It is impossible to enumerate all of the contributions the study of invertebrates have provided to general biology, but we can remember some remarkable examples, both in basic and more applied research (for an exhaustive review, see Wilson-Sanders 2011).

The first example comes from the fruit fly (*Drosophila melanogaster*). Thomas Hunt Morgan established the famous "fly room" at Columbia University at the beginning of the last century, to study heredity and mutation. The fruit fly was thought to be an excellent model, due to the speed of reproduction and inexpensive housing facilities. Morgan performed a series of very simple, elegant experiments, which are still considered classics in genetics and are part of any course in genetics at most universities. One of the important achievements, through hours and hours spent at the microscope and magnifying glass, was the confirmation of the chromosomal theory of inheritance, and that some genes are linked and always inherited together. Thanks to his work on the fruit fly, Morgan was awarded the Nobel Prize in Physiology or Medicine in 1933. However, the contribution of the fruit fly to the development of biological studies does not limit itself to classic genetics. As Kohler points out in his book *Lords of the Fly* (Kohler 1994), the fruit fly became one of the first "model organisms." In other words, this little insect showed a significant adaptability to laboratory conditions, and to standardization techniques, allowing it to be the subject of many studies in genetics and developmental biology. Entire infrastructure of laboratories and research groups were built around the possibilities offered by this animal. As Ankemy and Leonelli pointed out, a model organism is an organism that can be standardized to fit multiple purposes of research, and the fruit fly presented these characteristics (Ankeny and Leonelli 2011). The study of this little invertebrate has changed the history of the study of genetics. Seminal studies on *D. melanogaster* have led to the Nobel Prize for medicine in 2017 on the molecular basis of circadian rhythms (Hardin et al. 1990; Price et al. 1998).

Another biological milestone, in the field of behavioral biology, comes from the work of William Hamilton on social insects in the 1960s. Hamilton studied the society of social insects, to find a solution to the paradox of altruistic behavior. How is it possible, in Darwinian terms, for an individual to behave in a way that benefits another individual, at its own cost? Hamilton, in a famous paper published in 1964 in the *Journal of Theoretical Biology*, proposed that eusociality arose in social insects (Hymenoptera) by kin selection, through a particular sex determination. This intuition was crucial for the birth of sociobiology and opened an entirely new theoretical framework to explain the different forms of altruistic behaviors. Not only in social insects but termites, bees, and ants were absolutely instrumental in opening this new theoretical territory (Hamilton 1964).

The use of invertebrates in applied science relies on the crucial concept of the animal model. An animal model in biomedicine can be defined as a condition which permits us to study the fundamental biological and behavioral processes, or a pathological process can be induced that resembles, at least a certain aspect, the same pathological phenomenon observed in humans, or in other animal species (see, e.g., www.merriam-webster.com/dictionary/animal%20model). This is a concept which implies that certain characteristics (anatomical, physiological, behavioral) are shared by different animals, which have been conserved through evolution. It is feasible to think that the most basic and functional biological characters (e.g., basic cell structure and functions) have remained intact, from one species to another. The presence of analogous systems between invertebrates and vertebrates, though convergent evolution, makes the use of invertebrate models particularly promising. However, it must be pointed out that many factors can influence the choice of a particular species as a model, including the difficulty in changing consolidated experimental traditions (Vitale et al., unpublished data). Never forget that in many instances we are comparing very different animals indeed, and the transition of information acquired from a species to another must be always done with prudence and conceptual clarity.

In the context of applied science, invertebrates have been and are crucial in the field of neuroscience. Perhaps, the most famous example is the sea slug, or *Aplysia*, which is very distant from us in phylogenetical terms. The study of the nervous system of this little animal is straightforward as the animal has 20,000 very big brain cells (almost visible to human eyes), which makes it easier to study (humans have about 100 billion nervous cells). Neuroscientist Eric Kandel and colleagues have studied memory and learning using this mollusc as a model, and their discoveries resulted in the Nobel Prize in 2000. A focus of their research was the primary reflexes of *Aplysia*, which consists of the retraction of the gill and the siphon, in adverse conditions. Due to the characteristics of its nervous system, it was possible to study the behavior of the synapses during the withdraw reflex, which led to finding functional correlations between this mollusc and mammals. *Aplysia* has become a powerful model to study learning and conditioning (at a cellular level) in other organisms (including our own species). For example, the properties of the synapses displayed in the tests on conditioning involving dopamine neurons in the *Aplysia* are directly related to behavioral responses such as addiction in mammals (see, e.g., Baxter and Byrne 2006).

In recent years, basic and applied research have greatly benefited from the use of very simple invertebrates such as the flatworms or *Platyhelminthes*. Within the *Platyhelminthes*, the planarians are known for being able to regenerate any part of the body which has been damaged. This ability is due to the presence of pluripotent stem cells. Researchers have discovered a protein that is required to maintain stem cells active in planarians and could also be involved in pluripotent stem cells of mammals. Many questions are still to be answered. Are stem cells responsible for the regeneration of each organ, or what activates stem cells when regeneration is needed (Adler et al. 2014; Rossant 2014)? Another example from these invertebrates comes from the study of aging: the round worm (*Caenorhabditis elegans*) has been extensively used to study longevity. In particular the discovery of the gene age-1, able to increase lifespan of *C. elegans* when mutated, has provided important insights into the factors contributing to aging in vertebrates (Friedman and Johnson 1988; Lopez-Otin et al. 2013). However, also in this case, much remains to be learned about these mechanisms. Future use of other invertebrates will provide new models that will be very useful (see, e.g., Murthy and Ram 2015).

The use of invertebrates in experimental research opens a series of important ethical dilemmas.

2.4 The Moral Status of Invertebrates

As previously stated many invertebrate species are part of human life because they are used as food and research subjects (and to a very far lesser extent, kept as companion animals). And like many other nonhuman living beings, they are affected by environmental effects of human civilization. Many are deeply intertwined with human life and have rarely been regarded as deserving any kind of moral consideration. In popular culture metaphoric references to invertebrates mainly address something or someone without particular value (if not disgusting and depreciable), an example being when someone threatens to “crush him/her like a bug.” Very rarely positive values have been attached to invertebrates in novels and fables (probably bees are the invertebrates with the best reputation). Bad fame is not limited to insects, “octopus” (*piovra* in Italian) is a nickname for mafia, and calling someone a mollusc means blaming him/her for a weak character. Separation of invertebrates from the human beings seems to be wider and deeper than the separation of vertebrates, and here we cannot address and analyze all of them. But we can just mention the role that phylogenetic distance could play in making human empathy with invertebrates more difficult than with many vertebrates (especially mammals).

Besides a commonsense attitude toward nonhuman animals, since the 1970s philosophical ethics has dedicated systematic analyses on the topic of nonhuman animals’ moral status. Pioneering works of philosophers such as Peter Singer (1975, 1979) and Tom Regan (1983) have set the agenda of theoretical discussions about animals’ moral value and human responsibilities and obligations toward them. Their work founded the research field of “Animal Ethics” that is articulated in many different theoretical views. Many are in favor of reforms of the many instances of human/animal relationships (farming and lab experimentation are the most important). Animal ethics combined with knowledge and reflection about nonhuman life (ethology, just to mention one of the most important) fostered societal changes in favor of the recognition of animals’ moral status and legal protection. The process of systematic inclusion of animals into the domain of law began during the nineteenth century when the first laws aimed at protecting animals from cruel treatment and regulating animal experiments were issued by UK Parliament (Ryder 2000). It has only been during the twentieth century that animals have been systematically included into political agendas and processes of law making of Western countries. The Directive of the European Union regulating the use of animals in scientific procedures is an excellent example of the steady process of widening the circle of legal protection to nonhuman animals. In the previous version of the Directive, regulations were limited to vertebrates, but the current Directive now in force includes cephalopods among the animals whose welfare has to be taken into account if they are used for scientific purposes.

The EU Directive states invertebrates are currently on the threshold between disregard and consideration. Common sense attitudes are among the causes of the fact that moral consideration of invertebrates seems more difficult to be socially recognized, but they are not the only reason. Mainstream animal ethics seems to have devoted little attention to invertebrates. Normative theories like Singer's and Regan's link to moral status the possession of cognitive capacities that have been denied invertebrates for the most part. Singer's utilitarianism recognizes moral value to animals capable of feeling pain and pleasure. Regan's deontological right theory grants the status of a "subject of a life" to any organism endowed of the capacities, to be self-conscious and have beliefs, feelings, desires, and memories. The most influential animal ethics normative theories set the baseline for the admittance into the circle of moral consideration at a level that seems met by a good number of vertebrates species but not invertebrates for the most part. When those theories were developed, poor scientific evidence of invertebrates' sentience and cognition was available. The ethical approaches developed as alternatives to standard utilitarian and deontological ones, like those referring to the ethics of care (Donovan and Adams 2007), appear to fail in providing a convincing framework able to grant some moral consideration to invertebrates. Sympathetic attunement with invertebrates is more difficult than with vertebrates, and this makes it more unlikely to ground recognition of invertebrates' moral status in empathy.

The most recent scientific developments about sentience in invertebrates require philosophers interested in animal ethics to review their views. If subjective experience can be recognized in some invertebrates species (Klein and Barron 2016), then some kind of moral consideration ought to be afforded to them. The scientific understanding of invertebrates' mental capacities plays a key role in philosophical discussion. If the scientific evidence about invertebrates' sentience increased the eventual recognition of their moral status, this could be just part of a more articulated (and complex) theoretical discussion. What would invertebrates' moral status mean from a practical point of view? As Peter Singer recently stated, recognition of invertebrates' sentience would mean that the Earth is populated by a quantity of subjective experiences incredibly higher than that commonly believed (Singer 2016). Taking seriously such an enlargement of the domain of organisms deserving some kind of moral respect seems quite problematic. "From the practical point of view, what kind of consequences for human behavior and practices could entail the recognition of the invertebrates' moral status?"

It seems difficult to accept such a change of the moral scenario by human beings. Singer himself raises the question of what kind of success invertebrates' rights could have in a world where the recognition of the moral value of animals much closer to humans is so far from being a matter of universal consensus.

Skepticism about possible outcomes of recognition of invertebrates' moral status should not prevent scientific and philosophical discussion from proceeding and to call for the reform of human behaviors, practices, and institutions. As in the case of more consolidated fields of ethical, political, and legal reflection about human/animal relationships, it is reasonable to expect that reforms will be produced starting in selected fields of interaction. Given what it is happening in the practice of laboratory research, it is likely that invertebrates will start to gain consideration and respect.

2.5 Sentience

In the process of moral recognition of invertebrates, the discussion about sentience becomes crucial, especially when it comes to the legal protection of this particular group of animals. Sentient animals are protected by the Directive 2010/63/EU. Legislators have set a threshold for protection of certain animals above others, but in biological terms thresholds are difficult to identify for characteristics such as sentience or awareness: characteristics gradually vary between different organisms, following a evolutionary gradient.

Sentience can be simply defined as the capacity of feeling. However, sentience is one of the most slippery and difficult-to-grasp concepts in animal behavior. A certain consensus exists that sentience usually refers to the ability to feel and have subjective experiences. These experiences can be both aversive and attractive, so sentience can be associated with the possibility to feel "pain" and/or "pleasure." In particular, the feeling of pain can be intended as an unpleasant experience related to the damage of tissues or organs (Fiorito 1986; Duncan 2002). In a recent short article, Vallortigara affirms that sentience, intended as feeling an experience, has not necessarily got to do with advanced cognition (Vallortigara 2017). However, the search for sentience is frequently seen as the search for higher and higher cognitive abilities in animals. It is also argued that sentience can also be intended in a broader sense, that is, the whole experience of an animal in its own environment, as well as its own body. The obvious reference to this kind of sentience is the famous Nagel's article on "feeling as a bat" (Nagel 1974). There is a link between the two kinds of sentience, animals can have a sense of their own body and movements (it is hard to think differently for any kind of moving creature) but not necessarily associate this kind of sentience with pleasant or unpleasant feelings. Broom (2013) describes sentient beings as animals able to distinguish between their actions and others who can act on the basis of memory and experience. To emphasize excessively on the cognitive and intellectual side of consciousness may lead us to overlook other aspects that are equally important. It does not take much intellectual effort to experience pain, fear, or hunger. This is not of secondary importance, especially if we recall the words of Jeremy Bentham "The question is not Can they reason? Nor, Can they talk? But Can they suffer?" (Bentham 1789). Sentience is a very important controversial issue and so is the definition of "pain." Pain can be intended as an unpleasant experience, but because this characterizes pain as a personal experience, we can only infer indirectly which animals experience pain (apart from our species which can directly declare that it feels pain).

An interesting question in the present context is: "Do invertebrates have those characteristics which indicate that they are sentient and feel pain?". The Animal Health and Welfare Scientific Panel of the European Food Safety Authority (EFSA) was asked in 2005 to examine evidence of sentience in invertebrates. The literature survey indicated the presence of higher brain centers, a possibility of the presence of nociceptors, and a likely presence of nervous pathways connecting nociceptors to the brain centers, where "higher brain centers" have not to be intended sensu mammalian brain. Different invertebrates have nervous systems, which differ immensely, but, as the the nervous system becomes more complex, they can develop structured cephalic ganglia, which integrate inputs coming from sensory systems (Zullo and Hochner 2011). One very interesting example is the case of the octopus, in which much of the sensory processing take place in the peripheral parts of the arms and not in a centralized brain (Carls-Diamante 2017). This observation questions the idea of considering a "higher brain centre" as a requisite for sentience, even more so because the cephalopods are included in the list of sentient animals protected by law. It appears that biology sometimes is required to fit within a consensus of terminology and concepts among legislators and scientists.

Cephalopods are particularly interesting because they are the invertebrates normatively recognized as sentient. These animals respond to noxious stimuli (Andrews et al. 2013), but do they actually feel pain? Learned avoidance of electric shock, as well as sensitization and hyper-responsiveness after injury, were observed in cephalopods a long time ago (Boycott 1954). The discovery of nociceptors in cephalopods has occurred relatively recently, and in 2013 nociceptors responsive to mechanical and electrical stimuli were described in a squid species (*Doryteuthis pealeii*) (Crook et al. 2013). Cephalopods show behavioral and neuronal plasticity (Yasumuro and Ikeda 2011). All of these could be indications of the ability of these animals to experience pain, but it is still to be confirmed by more evidence. However, on the basis of the existing evidence, EFSA requested the inclusion of the cephalopods under the protective umbrella of the forthcoming new European Directive on the protection of animals utilized in scientific procedures (then published in 2010, Chap. 9).

What about the other invertebrates? Neuronal plasticity is not limited to cephalopods, and structural changes in synapses and neurons due to external stimuli have been also recorded *Orthoptera* (Pfister et al. 2013; Pfluger and Wolf 2013), as well as other invertebrate species (see, for a review, Pyza 2013). Neurons and neuronal circuits generating responses to noxious stimuli have been observed in nematodes (*C. elegans*) and fruit fly (*D. melanogaster*) (Tobin and Bargmann 2004).

Who has sentience then? If we are satisfied by a simple definition of this concept, in the terms of "feeling" a pleasant or aversive experience, then the reaction to pain could be a parameter we could use. We have evidence showing many invertebrate species could experience pain, and we have already mentioned the cephalopods, but then reactions to aversive stimuli have been recorded in other marine invertebrates, such as the sea slug and hermit anemone (see St. John Smith and Lewin 2009 for review). But again, do these animals feel pain?

It is clear that associating sentience to a particular species is not an easy task. Opinions are contrasting both in terms of the terminology used and scientific arguments in favor of sentience, awareness, and consciousness in animals (see, e.g., Duncan 2006). It appears to be a problem very difficult to understand. It is a matter of consensus among researchers, with the scenario still confused.

It is worth mentioning here a controversial point of view, expressed by Marian Dawkins, who does not think we really know whether animals are conscious. Dawkins argues against anthropomorphism and claims of animal consciousness, which lack firm empirical evidence. Instead, animal welfare arguments must focus on science, appreciating the critical role animals play in human welfare. In the end, she argues, it is human self-interest that will drive changes in our treatment of animals (Dawkins 2012). Her view can be considered rather cynical but interesting, because it opens the question of how we should proceed in discussing the topic of moral recognition of nonhuman animals and the reasons underlying such recognition.

However, in the face of persistent uncertainty in granting (in a rather anthropocentric and patronizing perspective) sentience to a particular species of invertebrate, the precautionary principle on animal sentience appears to be justified.

2.6 The Protection of Invertebrates

Among the invertebrates, cephalopods are now legally protected in the European Union across the Member States. The Directive 2010/63/EU, different from the previous Directive 86/609/EEC, brings under its protective umbrella "live cephalopods" (both adults and juveniles) (European Parliament 2010). In 2003 the EU Commission asked for a technical expert working group (TEWG) to give their opinion on the protection of invertebrates, in consideration of the revision of the EU Directive 1986/609. The TEWG proposed: "Inclusion of any invertebrate species should only occur on the basis of sound scientific evidence as to their sentience and ability to feel pain…" (TEWG 2003). It must be noted that the EU Commission has taken a very clear position to extend protection to cephalopods. One could argue, as already mentioned in a previous section of this chapter, that "the scientific evidence…ability to feel pain" is far from being scientifically proven, and still a matter of methodological and terminological consensus (see, e.g., Ponte and Fiorito 2013). A further opinion (EFSA-AHAW 2005) has recommended the inclusion of decapod crustaceans, referring to work on defensive behavior of crabs to aversive stimuli. However, this recommendation was not incorporated in the final version of the new Directive, due to strong objection expressed by the biomedical research community (Member of EC, pers. comm.). This decision to protect cephalopods adopted by the European Commission came with consideration of the literature on cognitive abilities of these animals, improved understanding and assessment of animal welfare. The British were pioneers in this respect. The welfare of *Octopus vulgaris* was included in 1993 under the scope of the Animals (Scientific Procedures) Act (Animal Act Order 1993). The Directive 2010/63/EU adopted for all of the 28 Member States a norm on the welfare of cephalopods, which was already present in the British national law. Other countries are now considering the welfare of cephalopods. Switzerland now regulates experiments on cephalopods and decapod crustaceans, and Norway does the same with squids, octopus, and crustaceans (and honey bees). Some states of Australia regulate the use of cephalopods through the National Health and Medical Research Council's Code, and New Zealand includes in its legislation consideration for octopus, squid, crab, lobster, and crayfish. It is interesting to note what has been declared by the Canadian Council on Animal Care: "cephalopods and some other higher invertebrates [that] have nervous systems as well developed as some vertebrates, insofar as they may experience from little to severe pain, stress, discomfort or other suffering" (see Tonkins 2016 for review).

Still, cephalopods are protected, and decapods not. Cephalopods are therefore considered sentient, and decapods not. It is our opinion that this distinction is not based exclusively on scientific ground. Perhaps it is based on the fact that the terms "pain" and "suffering" are still not well defined and understood. So, who are we going to protect? Based on what? Maybe the precautionary principle can help us. The original version of this principle argues that "where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (United Nations 1992). The principle has been formulated in relation to environmental policy, and we can translate the phrasing "threats of serious or irreversible damage" to, for example, pain and/or sufferance inflicted to animals (invertebrates in our case) in the practice of animal experimentation.

Birch (2017) poses the question about the resistance by the scientific community to put in practice this principle, when it comes to protection of animals used in research possibly creating significant bureaucratic problems to studies performed on nematodes or fruit flies (see, e.g., Bioscience Sector 2009). One possible danger would be to see scientists perform invertebrate experimental work outside the EU, to escape the bureaucratic burden associated with project applications. In this case welfare conditions would not always be guaranteed at a European level. However, in our opinion, invertebrates not protected by European law are in the hands of the good will of the scientists using them for research. This would not necessarily mean that their level of welfare is always at a very high standard. Realistically speaking, it is not feasible to apply the precautionary principle to any living organisms (do bacteria have sentience? And nematodes?): a bar has to be set somewhere. Birch asks every pertinent question about what indicators should we use to apply the precautionary principle to a particular set of animals (Birch 2017). The suggestion, which we find sharable, is that the possession of nociceptors are not enough, but the information acquired by nociceptors must be centrally integrated with information coming from other sources. And then what about the octopus with its “intelligent arms”? An animal, which shows a physiological or behavioral response due to this kind of process could be a good candidate for the precautionary principle. By the way, decapods fall into this category (Elwood and Appel 2009; Magee and Elwood 2013; see Chap. 7). This does not solve the question on “who has sentience and who has not,” but it merely gives us an indication on the parameters on which humans draw a line. In terms of treatment of invertebrates, as in any other animals, the law can help us to set up minimal standards, but then it comes down to the sensibility of each researcher how to treat their experimental subjects in their daily laboratory practice, and the need to promptly publish scientific results must not be an excuse to overlook the welfare needs of the experimental subjects.

2.7 Conclusions

For many decades the whole scenario of human/animal relationships has been subjected to moral scrutiny by parts of public opinion, which is continuously increasing. Such scrutiny is the main reason for the process of transformation of such relationships with animals in different areas. Until now most attention has been devoted to vertebrates (and not all of them). The immensely diversified group of invertebrates is the new territory for this process of transformation. We have endeavored to show how diversified, deep, and large the presence of invertebrates in human life is. Given the nature and the extent of such a presence, reasoning about their moral status and human responsibilities toward them is strongly recommended. Use of invertebrates in scientific research is a good case study for this, because it seems to be the most advanced field of human/animal interaction with respect to the reasoning about animal status and human duties. This is also the case for invertebrates, with legislations regulating lab research starting to include some invertebrates among species worthy of some kind of protection. The key issue in reasoning about invertebrates’ protection is the scientific assessment of their capacity for sentience. The capacity to empathize with nonhuman emotions has a key role in triggering human moral reflection about the status of animals and our responsibilities toward them (Aaltola 2013). The attunement with the emotions of vertebrates is easier because of their phylogenetic proximity with us (although sometimes phylogenetic proximity is not what counts the most). The lives of the most of invertebrates are somehow “alien” for human beings. Empathy and imagination are more likely to foster sympathetic concern for mammals than for crustaceans, because for humans to imagine what it is like to be a calf is much easier than trying to put themselves in the shoes of a lobster. For this reason, empathic concern alone seems not enough to systematically and consistently put invertebrates under the focus of moral consideration.

Reasoning about invertebrates’ moral status and their legal protection springs out from a possibility to extend protection already in force for other animals. The case of the extensions to cephalopods of the EU Directive is exemplary. As we have demonstrated, such a reasoning can be usefully driven by a well-balanced use of the precautionary principle. Prudence should be balanced by a reasonable knowledge of the capacities for sentience and suffering of the nonhuman species used for scientific research and other human purposes. Moral reasoning alone can never be enough for determining moral responsibilities toward invertebrates (and in general all the nonhuman world). It must be always accompanied and inspired by scientific understanding and knowledge.

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Chapter 3
Welfare Dilemmas Created by Keeping Insects in Captivity

Michael Boppré and Richard I. Vane-Wright

Abstract The challenging issue of animal welfare has focused mainly on furred and feathered vertebrates. However, unnoticed by most people, literally billions of insects are kept in captivity, in increasing numbers, and traded for a great variety of purposes. Arguably the most successful animals on Earth, insects are ignored or actively disliked by most people. Not just the different appreciation of insects by humans but the diversity of insects, and the diversity of their ecosystem services, shows that a discussion of insect welfare requires different criteria than vertebrate welfare. Their biology is very different, and insects are far less tolerant of suboptimal conditions. As a result, successful insect breeding programmes must necessarily fulfil basic welfare requirements. Insect natural history illustrates the complexity of practical welfare, even without fundamental consideration of insects as animals that have intrinsic value and their own agency, and the extent to which they are conscious or not and may or may not suffer pain. The great variety of insect lifestyles and lack of accessible information about industrial breeding mean that it is impossible to set general standards for insect welfare or provide meaningful evaluations of current practices. The best guidance that can be offered is to 'keep insects under as natural conditions as possible'. However, even this cannot be adhered to. Conditions in live butterfly exhibits involve compromises. Insects released in billions as biocontrol agents often involve x-ray sterilisation or transgenic procedures and pose environmental risks. For insects bred for human food and animal feed, euthanasia is a pressing issue. Numerous questions and ethical and welfare dilemmas are raised. Despite this, formulation of an Insect Welfare Charter based on respect, and the need to pay more attention to insects, is encouraged, preferably also addressing insects living in the wild.

M. Boppré
Forstzoologie und Entomologie, Albert-Ludwigs-Universität, Freiburg, Germany
e-mail: boppre@fzi.uni-freiburg.de

R. I. Vane-Wright (✉)
Durrell Institute of Conservation and Ecology (DICE), University of Kent, Canterbury, UK

Life Sciences, Natural History Museum, London, UK

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3.1 Introduction

At first mention, insect welfare in captivity sounds a highly specialised subject, relevant to few people. Moreover, while trillions of organisms, not just insects, are killed by habitat destruction, pesticide use and other human activities, concern for captive insects might seem naïve or even a dishonest distraction. Who cares about insects anyway? However, encouraging more respect for insects in general could create better awareness of the many insects in captivity and vice versa, as well as the damage being done to the biosphere by relentless growth in resource consumption. When the facts are set within an ecological framework, the subject becomes relevant not only to scientists and philosophers but also the public. Yet, as we explore below, the issue reveals numerous paradoxes and ethical dilemmas.

While discussing in some detail welfare of bred butterflies—'good' insects for most of us—we mainly consider the multiple uses for which insects are nowadays kept in captivity and bred in billions and the diverse welfare factors involved. Although the issues go well beyond natural science, insights from insect physiology, behaviour and ecology are essential for proper understanding.

Cultural differences affect attitudes towards insects. In advanced economies, these differences are multifold, non-homogeneous and conflicting. Here we take a largely European perspective. We have also chosen a focus on commercially used insects in captivity. Working at the interface between philosophy, natural sciences and the humanities, our arguments necessarily range from 'soft' to 'hard'.

We do not have space to address all of the rich and complex aspects of insect welfare in depth or comprehensively. We needed to restrict citations to a minimum and—whenever possible—we preferably quote recent reviews. Unfortunately, numerous current issues are not dealt with in formal publications, and relevant discussion is often only available from web or grey literature sources. A major problem is the lifestyle diversity of species in captivity in combination with a lack of sufficiently detailed information necessary to permit assessment of their living conditions. Our contribution thus illuminates the breadth or dimensions of the subject but cannot provide proper welfare analyses. Needless to say, insects are animals, and welfare issues discussed for vertebrates are applicable to insects, too—however, insects are in many respects very different. Although final answers to the numerous questions raised cannot be given, we hope to stimulate discussion by providing an overview together with relevant basic ecology and entomology. We also highlight various 'insect welfare dilemmas' that arise in this context.

Terminology

To appreciate the text, it is necessary to comment on two terminological matters: (1) We differentiate various ways of keeping insect in human custody and call it *rearing* when early instars of insects are collected in the wild and kept in captivity until adulthood; *breeding* when several generations are continuously kept in captivity; *farming* when insects are kept outdoors but on plants especially provided for them—in the literature these terms are not uniformly, often synonymously, used; and also 'culturing' (which implies breeding) and 'raising' (which implies rearing).

(2) We here subdivide antagonists (natural enemies of animals) into *predators* (which kill and typically feed on more than one, mostly non-specific prey), *parasites* (which live in or on one or more hosts and do some harm but without necessarily killing them), *parasitoids* (in which each larva feeds on or in a single (typically specific) host, which usually dies as a result) and *pathogens* (protozoans, fungi, bacteria and viruses that cause diseases—in many cases these are transmitted by parasites).

3.2 Insects Around Us and Our Attitudes Towards Them

Everybody during his or her life kills many insects, intentionally and unintentionally. Who will not smite a biting mosquito? As individuals we kill insects when driving a car or playing on a meadow; as societies we destroy habitats of insects and kill many in the course of pest management—not only target species but also uncounted numbers of nontarget, often beneficial ones ('collateral damage' on a vast scale). Even vegetarians (and vegans) among us cannot avoid consuming insects accidentally (Gorham 1979). However, none of these facts invalidates the need to address the issues of insect welfare.

Human attitudes to insects generally seem to fall into three categories:

Dislike: The great majority see insects as bad, dangerous and/or repellent (Kellert 1993)—they are feared or disliked because some sting and transmit diseases, many are annoying and some destroy stored food or clothes, while others are pests in gardens, agriculture and forestry. Many people are phobic of insects and even find them 'revolting' (Lockwood 2013).

Like: A few insects, including colourful butterflies, scarabs, ladybirds and dragonflies, are appreciated and in various cultures receive not only aesthetic appreciation but also have spiritual associations (Hogue 1987; Kritsky and Cherry 2000; Manos-Jones 2000) or are liked for their products (honey) or as 'gardeners' friends'.

Ignorance: The vast majority of insects play no acknowledged role in human life—not even their existence is recognised. Taxonomists have only catalogued a fraction of the several million insect species believed to exist (Stork et al. 2015), and in general very little or even nothing is known of their biology. Admittedly, the majority are small or tiny creatures with a cryptic appearance, often only apparent for short periods of time, or generally not found or seen without special searching and technical aids. This limited knowledge seems largely responsible for the fact that insects do not get the attention they deserve. We share the planet with as few as 5500 species of mammals but over a million insects have already been named and millions more will follow. Insects are different and extremely diverse (see Sect. 3.8), and so unsurprisingly there is much more consistency of opinion regarding vertebrates than insects. Scientific names often reflect human attitudes—beautiful butterflies named after Greek goddesses (e.g. *Troides helena*, *Speyeria aphrodite*), flies named after disagreeable habits (e.g. *Haematobia irritans*, *Calliphora vomitoria*).

We conclude that most humans differentiate between *some* insects but usually consider them only from a personal, anthropocentric view, unaware of their importance in the ecosystems on which all of us depend (Schowalter 2013). Insects play vital roles in ecology and thus—although mostly indirectly—serve human interests ('ecosystem services', e.g. Losey and Vaughan 2006; Stout and Finn 2015). Few insects are in fact dangerous to humans. The biocentric view that *every organism has the right to live* conflicts with the common opinion that *the only good insect is a dead insect*. A unified view has never existed and likely will never be realised, making consideration of insect welfare something of 'a suicide mission' almost bound to end in failure. But a far better informed picture needs to be drawn, because insects are nowadays more relevant to human life than ever before (see Sect. 3.4). We need to pay insects far more respect and far more attention. It is thus good to note that their importance was recognised by the Council of Europe (1986) in their Charter of Invertebrates.

3.3 Insects in Captivity: Historical Changes

Many people worldwide have long made use of insects (Table 3.1), notably opportunistic gathering for food (see Sect. 3.10) and medicine (entomoceuticals; Dossey 2010; Cherniack 2010; Mishra and Omkar 2017a). Only two species have been kept permanently in captivity: about 2500 BCE humans started to use silk and domesticated the silk worm moth (*Bombyx mori*). Even earlier, wild honey was harvested and the honeybee (*Apis mellifera*) subsequently domesticated (see Roffet-Salque et al. 2015), originally for honey and beeswax and recently for assuring pollination in orchards (see Sect. 3.10).

The use of insects as weapons of war, tools of terrorism and instruments of torture has been a practice, even mentioned in the Bible, continued over many centuries (Lockwood 2009, 2012). This represents a transition between wild insects and those held in captivity, and a very obvious connection between welfare and ethics.

Humans live unintentionally with many insects under one roof: silverfish, house flies, cockroaches, meal moths, rice weevils, clothes moths, etc. (Bertone et al. 2016). We now store an increasing diversity of food and other materials in our homes, with the likelihood that additional insect species will become 'uninvited guests'. Such inquilines, like 'pests' in agriculture and forestry, are combated in many different ways.

For many centuries the relations between humans and insects changed little. People did not need to know much about insects because life was largely sustainable (although the word had yet to be coined) and, despite the steady increase in land area transformed for agriculture and forestry, environments remained essentially fully functional for a long time. However, after the mid-eighteenth-century European industrial and agricultural revolutions, followed by the mid-nineteenth-century 'marriage' of science and technology, the situation changed dramatically (White 1967; Berger 1980).

Table 3.1 Necessarily incomplete overview of the diversity of insects kept in captivity, both now and in the past, and the numerous purposes served

Use	Insects	Order	Sample genus	Trade 'product(s)'	Comments	Sample reference(s)
Historic uses						
Special products	Silkworms	LEP	*Bombyx*	Silk from cocoon (pupae)	Pupae also sometimes used as food for farm animals or humans. *Bombyx mori* is domesticated, does not occur in the wild and cannot fly. More recently also 'wild silk' from cocoons of Saturniidae as home industries	
	Honeybees	HYM	*Apis*	Honey, wax, royal jelly	Honeybees are not strictly kept in captivity	
Warfare	Wasps	HYM	*Vespula*		Not only were stinging insects fired into enemy strongholds; bioterrorism also includes insects carrying diseases or causing damage in agriculture	Lockwood (2009, 2012)
Fun	Crickets	ORT	*Gryllus*	Individual adults	Enjoyment of insect song in homes or competitive fights	Judge and Bonanno (2008)
More recent uses						
Research					Uncountable numbers of species and individuals have been and are being reared or bred for studying their physiology, behaviour, genetics, development, etc.	
Hobby	Gentles	DIP	*Calliphora*	Maggots by weight	Maggots bred en masse and traded as bait for fishing	
	Butterflies, moths	LEP	Any	Mainly eggs, pupae, adults in small numbers	Amateur entomologists have long reared insects in captivity for collections; insect lovers also keep various insects as pets, nowadays an increasing trend due to wider availability of interesting exotic species	
	Ants	HYM	*Atta*	individual living queens		
	Beetles	COL	*Pachnoda*			

(continued)

Table 3.1 (continued)

Use	Insects	Order	Sample genus	Trade 'product(s)'	Comments	Sample reference(s)
Feed for small animals	Crickets	ORT	*Gryllus*		Commercial large-scale breeding as food for pets at home, zoos, laboratory animals, etc.	Finke and Oonincx (2014)
	Locusts		*Schistocerca*			
	Mealworms	COL	*Tenebrio*	Larvae by weight		
Medicinal therapy	Blow flies	DIP	*Lucilia*	Maggots in medicinal kits	Maggots clean necrotic flesh from open wounds, releasing antibiotics and promoting healing; applied since middle ages, now on industrial scale	Fleischmann and Grassberger (2003)
Most recent uses						
Entertainment	Butterflies	LEP	*Danaus*	Adult butterflies by dozen(s)	For ceremonial release (in- and outdoors) at weddings, funerals, birthday parties	Pyle et al. (2010)
Education/ edutainment	Grasshoppers	ORT	*Romalea*		Live exhibits as continuous cultures in zoos, museums and butterfly houses	
	Beetles	COL	*Dynastes*			
	Butterflies	LEP	*Morpho*	Pupae by dozen(s)	Live exhibits in zoos, museums and butterfly houses, usually not bred	Boppré and Vane-Wright (2012)
	Butterflies		*Vanessa*	Eggs by dozen(s)	Rearing kits for schools to demonstrate different life stages and metamorphosis	
Conservation	Butterflies and others	LEP	*Lycaeides*		Endangered or locally extinct species are bred in captivity to eventually be released and establish a new population in the field; environmental risks exist re *Wolbachia*	Hughes and Bennett (1991); Pearce-Kelly et al. (2007); Crone et al. (2007)

Pest management	Phytophagous wasps	HYM	*Tetramesa*		Mass-produced parasites for field release to combat exotic weeds, often unsuccessful and associated with environmental risks by influencing nontarget populations	Moran et al. (2014)
	Moths	LEP	*Cactoblastis*			Capinera (2008: 696)
	Phytophagous flies	DIP	*Urophora*			Pearson and Callaway (2003)
	Lacewings	NEU	*Chrysopa*		Biological control of insect pest populations in agriculture and greenhouses but also in households by predators	
	Ladybirds	COL	*Delphastus*			Riddick and Chen (2014)
	True bugs	HET	*Dicyphus*			De Clercq et al. (2014)
	Parasitoid wasps	HYM	*Encarsia*	Infested substitute host or artificial eggs	Industrially mass-bred parasitoids for field release to kill pests or for use in private households against insects feeding on stored products	Sithanantham et al. (2013)
	Fruit flies	DIP	*Ceratitis*	Sterile males	Industrially mass-bred conspecific males for release to reduce fertility of wild females; often in the course of ‘eradication programmes’	Krafsur (1998); Dindo and Grenier (2014)
	Blow flies		*Cochliomyia*			Wyss (2000)
Pollination	Solitary bees	HYM	*Megachile*	Pupae	Industrially mass bred for release to pollinate alfalfa	Peterson and Artz (2014)
	Bumble bees		*Bombus*	Queens, international	Pollination of greenhouse or field crops, with various environmental risks such as spread of pathogens	Owen et al. (2016)
Food/protein for humans	Crickets	ORT	*Achaeta*	Adults or powdered specimens	Currently for restaurants or as ingredient of power bars or for insect burgers; in the future to provide basic food and/or to substitute vertebrate meat	van Huis et al. (2013, 2014); van Huis (2015); Shockley and Dossey (2014); Dossey et al. (2016); Mishra and Omkar (2017b)
	Grasshoppers		*Locusta*			
	Mealworms	COL	*Tenebrio*	Larvae or powdered specimens		
Feed for poultry and fish	Saprophagous flies	DIP	*Hermetia*	Puparia or as meal/flower	Saprophagous flies are reared on animal dung and/or organic waste in increasing amounts to recycle it and obtain, at the same time, eco-friendly substitute for fish meal to feed poultry and fish	Khusro et al. (2012); Riddick (2014)
			Musca			Hussein et al. (2017)

COL Coleoptera, *DIP* Diptera, *LEP* Lepidoptera, *HET* Heteroptera, *HYM* Hymenoptera, *NEU* Neuroptera, *ORT* Orthoptera

Modern agriculture and silviculture have created superabundant crops, often in the form of monocultures. Certain formerly unnoticed insects can now multiply rapidly on these resources, and, as a result, they can become pest populations. This has brought us into a new type of conflict with nature—albeit an altered nature largely of our own making. The monocultures, supposedly necessary to feed our burgeoning population, have encouraged the invention and widespread release of poisons (pesticides): insecticides to kill insects directly, as well as herbicides to kill 'weeds'—which for many insects are larval or adult hostplants. Further, keeping stock in large numbers (e.g. sheep, cattle) brings disease-causing and disease-transmitting insects into focus (e.g. flystrike, blue-tongue; see Sect. 3.10).

Over the same time period, more and more people live in cities—currently more than 50%. City dwellers are increasingly alienated from nature, according to some resulting in undesirable psychological conditions such as biophobia (Orr 2004) and 'nature-deficit disorder' (Louv 2005; Dickinson 2013).

Counter-intuitively, these changes have resulted in taking more and more insects into human custody (Table 3.1). Widespread use of non-specific pesticides has enormous impact on nontarget organisms (many of them beneficial) and even entire biocoenoses. The search for more 'environmentally friendly' means of biocontrol *s.l.* includes industrial production of various antagonists (see Sect. 3.10), seen as desirable not only to control outbreaks of native species but also invasive exotics—themselves often moved around as a result of our ever-increasing global trade. Honeybees, in many areas, having become slaves of crop industries, as well as many other pollinators and insects in general are in decline, and a pollinator crisis is being debated (Levy 2011; Martin 2015). We seek to support pollination by breeding huge numbers of solitary bees and bumblebees in captivity for targeted release (see Sect. 3.10).

With the human carrying capacity of the Earth arguably already exceeded, as a result of this overexploitation, we face a food crisis, in particular a lack of protein (Drew & Lorimer 2011). In response, it is suggested we produce insect protein on an industrial scale for food and feed (see Sect. 3.10), and terms such as 'minilivestock' for mass breeding of insects are coined (Defoliart 1995, Paoletti 2004; see Sect. 3.10). More and more species of insects are now used in research, education and art (Table 3.1).

Perhaps reflecting a reaction against our disconnect with nature, an increasing number of people now keep insects as pets, or as a hobby (including 'urban beekeeping'). In pre-industrial societies, this was probably very rare—although, for example, it has been a custom in China since the eighth century to keep crickets as 'pets', to enjoy their singing and especially fighting (Judge and Bonanno 2008). With the arrival of Internet communication and fast courier services, it has become easy to obtain exotic insects for hobby purposes, including walking sticks, beetles, cockroaches, praying mantids, ants and grasshoppers. Interestingly, not only 'nice' insects but also 'interesting' ones are thus taken into human custody. The Internet also provides background information on pet insects, including 'care sheets' aimed at proper husbandry. Many zoological gardens now exhibit some insects too, and over the past 30 years, dedicated live exhibits of butterflies for edutainment (butterfly houses; see Sect. 3.9) have added significantly to the growing number of insects in human care.

3.4 Insects in Captivity: A Necessity?

A list of insects held in captivity and their major uses (Table 3.1) may come as a surprise for many of us because captive insects largely escape our attention. Table 3.1 gives an overview not only of the numerous uses but also the range of insects involved and indicates the diversity of their lifestyles.

The rather small number of species regularly held in captivity belongs to about one third of the 27–30 recognised orders (major groups) of living insects: grasshoppers and crickets (Orthoptera), stick insects (Phasmatodea), cockroaches (Blattodea), plant lice and assasin bugs (Hemiptera), lacewings (Neuroptera), beetles (Coleoptera), true flies (Diptera), butterflies and moths (Lepidoptera) and ants, bees and wasps (Hymenoptera). While the number of species bred does not exceed several hundreds, the number of individuals is in the order of trillions (see Sect. 3.10). The different groups (and usually individual species) have various and often very different lifestyles and require their particular requirements to be met fully if they are to be maintained successfully in confinement (see Sect. 3.8).

For basic research, innumerable species of all kinds have been taken into the laboratory, mostly for limited periods (although *Drosophila* species have been bred for over a century). Because of space limitations, we say nothing further here about the welfare of insects used in basic research (but see Crook 2013).

Great differences in the numbers of insects held captive are due to who keeps them, and their purposes: hobbyists just keep a few individuals for a while, or typically rear a single generation; in contrast, commercial businesses continuously breed insects en masse as feed for zoo and pet animals, silk, education, pest management and pollination. The greatest numbers are (or potentially will be) bred for pest control and obtaining insect protein as food and feed (see Sect. 3.10). To be able to produce chosen insects on an industrial scale, we presume that during the initial search for optimally efficient breeding regimes, huge numbers may be sacrificed (see Sects. 3.9 and 3.11). The number of individual bred insects already exceeds the number of vertebrates held in captivity.

Not many insects currently kept in captivity could readily be substituted without impact on our daily lives, or our environment—even if we would not be directly aware of it. Commercial production of silk from domesticated as well as wild silkworms to make a minority luxury product can be seen as unnecessary because alternative materials are available. However, for the sake of our managed ecosystems and human well-being, breeding certain insects in captivity (and often later releasing them; see Sect. 3.10) has become a necessity and will become even more important in the future.

While the use of commercially produced parasitoids, for example, serves the profit of some farmers (as well as those producing the insects), breeding and releasing antagonists seems, at first sight a least, far healthier for our societies, more sustainable for the environment and more ethical (cf. Pimentel et al. 1980) than application of non-specific insecticides. The only alternative would be largely to abandon monocultures and return to small-scale farming—likely a fantasy in our globalised world, despite possible greater per-hectare productivity, sustainability and conservation potential of the latter (e.g. Perfecto and Vandermeer 2010).

3.5 Human Attitudes to Insects and Insect Welfare

In parallel with increased captive insect breeding, human views about nature in general and animals in particular have been changing. Despite concerns about harm, cruelty and disrespect to animals going back to the philosophers of ancient Greece and long expressed by some eastern religions, in the modern west the term ‘animal welfare’ only came into common use over the past 50 years. There is public debate about ‘humane’ keeping of chicken, cattle and other livestock, and zoos are improving their ways of keeping exotic animals to provide their specific requirements (Kohn 1994).

Animal welfare is thus a current issue (see Eadie 2012) receiving more and more public attention. While ‘insect’ does not appear once in Eadie’s overview, to our surprise, in Animal Welfare, a journal published by the Universities Federation for Animal Welfare (UFAW 2017), insects so far seem not to be an issue either.

The conservative philosopher Sir Roger Scruton (1998) has written: ‘. . . it is only with a certain strain that we can care for the wellbeing of individual insects, even though we recognise that they suffer pain and fear, and are often hungry and in need like the other animals’. Although insects belong to the Kingdom Animalia and are thus undeniably ‘animals’ in a scientific sense, Scruton accurately describes the *dis*affection many people feel towards them. Speciesism towards insects is thus unlikely to be challenged by anyone other than animal rights activists. And this is enshrined in the laws of many countries which (deliberately) *ex*clude insects (and indeed most animals other than vertebrates) from legislation concerning cruelty and welfare. Before the law of most countries insects are literally of no account.

Sekimizu et al. (2012: 226) suggested using the silkworm moth *Bombyx* for drug testing in Japan, instead of ‘sacrificing model animals’ such as mice and rats, to circumvent animal welfare requirements. As they put it, ‘“Animal” addressed in the Act can be defined as a “vertebrate animal”. If we can make use of invertebrate animals in testing instead of vertebrate ones, that would be a remarkable solution for the issue of animal welfare’. Clearly Sekimizu and co-authors considered insects—from legal, welfare and perhaps moral, ethical and emotional perspectives—to be qualitatively different from vertebrates.

In contrast and at the same time, slowly and not yet universally, insects are also coming into consideration (Horvath et al. 2013; Broom 2013). Discussion is so far largely limited to the most obvious cases of insects that are either liked (e.g. butterflies) or perceived as ‘good’ in some way. In particular, extensive discussion about bees, sometimes treated ‘unnaturally’ and ‘disrespectfully’ (Imhoof & Lieckfeld 2015), has raised awareness about the treatment of insects in human custody and posed moral and ethical questions. It is a serious issue although parodied by some (e.g. www.insectrights.org). More significantly, the now widespread focus on bees as pollinators to the exclusion of all else runs a real risk of oversimplifying general understanding of ecosystems, with attendant risks for biodiversity (Smith and Saunders 2016).

The exhibition of live butterflies in windowless rooms as objects of art by Damien Hirst at Tate Modern has resulted in some public protest (Nikkhah 2012; Cashell 2009: 159). Increased awareness has also made people reflective about other practices. The traditional way of obtaining silk from silkworm cocoons, involving ‘cruel’ killing of the pupae by boiling, is now also subject to public criticism, and silk is increasingly questioned by some as an unethical product (Plannthin 2016). The application of pesticides is mainly discussed in relation to harming environments, but the ethics of en masse killing of insects in our environments is also a matter of discussion (e.g. Pimentel et al. 1980; Lockwood 1996; Bentley and O’Neil 1997).

Does the fact that some people condemn the use of silk on grounds of cruelty indicate a fundamental change in (western) perceptions of insects? While a majority of people still do not like ‘creepy crawlies’ and care little for their welfare, increasing concern is being expressed about human responsibilities towards an ever-wider range of organisms. This has been termed ‘ethical extensionism’ (Engel 2008; see also Favre 1979; Singer 2011).

Traditionally, Western philosophy generally limited moral concern to living human beings. Extensionists claim this speciesism was based on false understanding of ethics and moral theory and that our concerns should be expanded to include humans yet unborn, arguably all sentient animals and even, according to some, all living organisms. There are two major variants of extensionism—utilitarian and deontological.

As Blackburn (2001) has suggested, ‘We are often vaguely uncomfortable when we think of such things as exploitation of the world’s resources’. Utilitarian extensionism regards organisms as natural resources which we need to deal with or treat in an ethical manner. This can be reduced to the claim that any organism capable of experiencing pain has an interest in avoiding suffering. According to Singer (1975, 2011), this then requires humans to take account of the interests of all sentient organisms. In our context the question then becomes: are insects sentient, and can they experience pain as a result of human activity (see Sect. 3.7)?

In fundamental contrast, the deontological or rights approach, as advocated by Regan (1983), is based on the notion that all conscious creatures are ‘experiencing subjects-of-a-life’ (ESLs). Their own welfare and needs are important to them without regard to other organisms, including humans. In effect this is an extension of Kant’s respect-for-persons principle (Dillon 2016)—which, if accepted, leads to the conclusion that we have a duty of respect towards all ESLs because of their independent agency and own intrinsic value. Consequently, they should never be treated solely as a means to some (human determined) end (Engel 2008). Or as Samways (2005: 11) has put it, specifically in our context here, ‘Do we have the right to assume that insects do not have rights?’

There are further, more nuanced, variants of extensionism, some of which Engel (2008) explores. For example, Paul Taylor’s (1986) synthesis leads to an egalitarian biocentric ethic which argues that ‘every living organism deserves equal moral consideration’ (Engel 2008). By extending such equality to every living being, Engel asks if Taylor has thereby taken extensionism ‘to an absurd extreme’. Thus ‘Respect for all living things would require settling conflicts between persons and non-persons in ways that are fair to both’ (Dillon 2016). This might sound good in theory, but in practice to decide on conflicts that inevitably arise between the supposedly equal interests of different organisms in general, one is reduced to making up more or less *ad hoc* rules (as Taylor himself tried to propose). Do we really need a complex rule book to decide upon the morality of smiting that mosquito? To many people such arcane arguments only seem important to those of us fortunate to live a life of plenty—are we in danger of inventing a ‘luxury problem’ in more than one sense of the word? So we are left with irresolvable ethical dilemmas.

Another variant (not without its own considerable problems: Sander-Staudt 2017) is ‘care ethics’—which seeks to ground moral treatment of animals ‘not in rights or utilitarian considerations but in our sympathy for animals and relationships with them. By grounding moral duties in sympathy and relationships, care ethics avoids some of the more problematic elements of other animal welfare positions’ (Engster 2006). Donald Broom holds the view that our actions towards others, including other animals, should be based on obligations (cf. Lockwood 1987), stating recently that ‘all animal life should be respected and studies of the welfare of even the simplest invertebrate animals should be taken into consideration when we interact with these animals. Even if we do not protect the animals by law, we should try to avoid cutting an earthworm in half, mutilating a snail or damaging the wing of an insect’ (Broom 2014: 200). But even if we are willing to accept notions of interests, rights, care ethics or obligations, in the end the question is not, for example, if we should keep insects in captivity at all, or how we might justify which ones we keep and for what purposes, but something far more operational—how and on what basis can we establish appropriate welfare practices when we ‘interact’ with insects, justifiably or not?

Human Attitudes to ‘Nature’

Humankind has not just different attitudes to insects but to nature in general. Not only the beauty or ugliness of different organisms is a matter of individual taste and influenced by culture but also their behaviour. In nature what many westerners would call ‘brutal’ is common (hunting and killing), and there is much cheating which we do not want as standard for us. We do not need to accept rules of nature as rules for human behaviour, but we do need to acknowledge species-appropriateness. We are a different species!

If we accept that, does respecting organisms in captivity mean taking care of them appropriately, on their own terms so-to-speak, even if this, potentially, is not appropriate for us? Among ourselves we mostly aspire to accept different cultures. So in parallel, don't we have to accept nature even with its often, in our terms, ugly and cruel ways? We have to accept the existence of insects, even ‘dangerous’ ones, and respect their individual lives. Do we therefore accept that our feelings and values with respect to living conditions and lifestyles are fundamentally different to the reality of insects in the field and that we cannot apply our (culturally determined) views to nature? Or do we want to establish ethical rules superior to ‘rules in nature’ because of our disgust?

We can change nature significantly, we can even modify organisms genetically—but not the intrinsic rules of life. Nature inevitably entails struggle and competition, and, except within a few social mammals such as monkeys, elephants and cetaceans, the life of the individual largely appears ‘unrespected’ (see Sect. 3.7). In thinking about insects in captivity—which moreover unlike many vertebrates never become companions, or ‘bond’ with their captors—to develop any principled ethical basis, we will have to decide between human culture-appropriate vs species-appropriate measures. Thus ‘nature’, depending on this decision, may or may not offer us a basis for establishing principled, ethical guidelines (see Sect. 3.7).

3.6 What Makes Insects Different, What Differences Occur Among Them, and How Do These Differences Relate to Welfare in Captivity?

Insects have basically the very same functional organisation as ourselves and other vertebrates, including nervous, digestive, respiratory, motor and reproductive systems. But with millions of species and countless trillions of individuals, abounding in every terrestrial ecosystem from the poles to the tropics, including high mountains, and fresh and brackish waters, they are often considered one of the most successful groups of organisms on Earth. What can explain this?

Insects are invertebrates, and, although belonging within the major phylum Arthropoda, they are in many ways incomparable to most other groups of organisms. They share with all arthropods jointed limbs, and their special cuticle confers numerous advantages (Watson et al. 2017). Other factors that arguably contribute to their success include small size (imposed by respiratory constraints and their exoskeletal structure); short generation times (often only days or weeks); high reproductive rate; sophisticated sensory and locomotor abilities (including powered flight in the adults of most species); countless, often specific biotic relationships with plants and other organisms; and metamorphosis (Gullan and Cranston 2010). Mayhew (2007), however, challenged a number of these ideas as lacking decisive evidence—but still accepted ‘complete metamorphosis’ as one of the most likely key factors. Metamorphosis has many important consequences for insect biology—and thus for thinking about insect welfare.

Metamorphosis In those taxa exhibiting incomplete metamorphosis (hemimetaboly: grasshoppers, cockroaches, lice, etc.), the first instar that hatches from the egg is somewhat like a small adult—but lacks functional wings and genitalia. It then grows through a series of discrete stages (‘instars’), each separated by moulting. The last larval moult gives rise to the adult, the final and only stage that can fly and reproduce.

In contrast, in those insects exhibiting complete metamorphosis (holometaboly: butterflies, moths, flies, wasps, beetles, etc.), all the early stages are completely different from the adult. When the final larval instar moults, the adult is not revealed but, instead, a further distinct stage appears, the pupa, which is immobile and does not eat. Inside the pupal exoskeleton, the whole organism is transformed into an adult which, when the process is complete, emerges as a creature radically different from both the larval and pupal stages—not only in appearance but also in biological needs and functionality.

Differentiating between early stages and adults makes us aware that, in holometabolous species particularly, a single individual has in effect several lives, looking and behaving very differently, having different antagonists, often requiring different resources in its different stages (both abiotic and biotic: consider, e.g. the different lives of maggots and the flies they become or caterpillars and butterflies). Keeping insects in captivity therefore poses various welfare and husbandry challenges that are not only different from vertebrates but more complex in addition.

Individuality and insect life cycles René Descartes famously declared that animals are simply machines (Hatfield 2018, see also Hatfield 2008). However, few now consider organisms to be automata—neither are they robots nor, generally, clones. We thus need to consider the needs of individuals and, especially, *individual life cycles*, and not just base discussions of welfare on abstract concepts such as ‘species’ and ‘species-specific differences’. Species can be defined as reproductive communities. However, within a given species, there are heritable as well as environmentally induced differences between populations, and, within populations, differences at the level of the individual are universal. Most (although certainly not all) individual insects are the result of sexual reproduction, and almost all are genetically polymorphic; as a result, even with respect to genetics alone, we can safely assert that the great majority of individual insects are unique. This is to be considered in addition to the ‘individuality’ issue (e.g. Carere and Maestripieri 2013). Insects, when forming a superorganism, can respect other individuals: *Megaponera* ants after fights with termites rescue their injured sisters (Frank et al. 2017).

3.7 Living in the Wild vs Living in Confinement

Here we address the reality that the welfare of insects in captivity potentially involves a complex mix of issues including stress, loss of individual choice (denial of their agency), suffering, pain, sentience, even aesthetics—and the subjectivity of our individual moral views and how we collectively comprehend the ‘nature’ of nature itself. This opens a huge debate which can only be touched upon here. Discussion can be intense, emotional and non-objective. As an aid to thought, we offer a few facts or realities that are not well recognised, if recognised at all, in the current welfare and ethics literature.

Stress

Living inevitably involves stress, in whatever way the word is defined (e.g. ‘stimulation beyond the capacity for complete adaptation’: Broom and Johnson

1993). In the wild, animals experience stress related to abiotic factors, including light, temperature and moisture, in addition to biotic pressures such as obtaining food, defence against enemies and the struggle to reproduce (finding a partner and a suitable host(plant) or substrate)—all usually involving competition. Many of these unavoidable 'natural stresses' are more or less eliminated in well-managed captivity. Confinement can offer optimal abiotic conditions, abundant food and considerable protection from enemies, as well as meet many other basic needs—resulting in high survival rates right through to the adult stage. But such benign conditions are in fact *un*natural. Thus Hoffmann and Ross (2018) comment that 'Laboratory-adapted lines tend to be more sensitive to stress, likely reflecting relaxed selection for stress-related traits' (see also Garnas 2018).

Survival and Reproduction

In nature, biological fitness (contributing to the gene pool of a population through individual reproductive success) is only achieved by relatively few individuals. In an intact ecosystem, all populations are approximately balanced, i.e. they remain quite stable over time even though they undergo fluctuations. Looking at numbers demonstrates the challenge: of N eggs laid by a given female insect, on long-range average only two become parents, whether $N = 50$, 500, 5000 or any other number. Generally, the same is true for offspring of all animals and plants. This implies that, in untouched nature, the vast majority of individuals die or get killed long before they can achieve biological fitness, most serving as food within the ecosystem. In contrast, in captivity the vast majority of individuals survive to adulthood—but we often then deny all of them any chance to reproduce. Is this right, ethically? It is noteworthy that the insects living in human-made monocultures often do, at least for periods, mass reproduce—just as insects in households; an anthropogenic habitat reduces stresses they would naturally experience.

Animal Behaviour and Public 'Taste'

Not everything that animals do (in the wild or captivity) is appealing to us or readily meets our 'standards'. Many if not most of us consider certain hunting strategies or food preferences 'disgusting', even though it is species-appropriate. Infanticide among animals is widespread (Hrdy 1979). Does our tendency to be repulsed by such behaviours relate to welfare? Not directly, but such reactions almost certainly affect public opinion or 'taste'—and ethics, morals and thus welfare may seem as much 'emotional' as simply 'technical', 'scientific' or 'principled' issues.

Insect lifestyles are also more or less likeable/acceptable from a human perspective. Many fly maggots feed on faeces, dead bodies or rotting plant material; praying mantids consume their insect prey without prior killing; and pompilid and sphecid wasps provision their burrows with paralysed spiders or insects, respectively, as food for their developing larvae—this way, the hosts don't decay and their blocked nervous system prevents escape. The grubs of most parasitoid wasps (e.g. Chalcidoidea, Ichneumonidea) feed inside the still living larvae or pupae of butterflies, moths and other insects. What appears disgusting to us is, billion-fold, an everyday reality in nature all around us—many pages could be filled with further examples. Such realities have to be accepted as ‘natural’. But do they clash with measures for the welfare of animals held in captivity?

Suffering and Pain

Some of the more grisly insect examples above provoke the question *Does nature inevitably entail suffering and/or pain*? And if so, is this different or not from vertebrates (Elwood 2011)?

As insect physiologist Sir Vincent Wigglesworth (1980) now famously asked: Do insects feel pain? Since pain cannot be measured objectively, it is a matter of continuing debate if, e.g. physical injury, temperature extremes, noise, thirst, hunger, poisoning and/or irradiation cause pain or suffering in insects (e.g. Eisemann et al. 1984; Lockwood 1988; Smith 1991; Elwood 2011; Broom 2013; Sneddon et al. 2014; Tiffin 2016; Adamo 2016).

Many discussions appear human-centred—about what we do, as agents, to insects. The debate has rarely if ever touched on ‘natural cruelties’ like those briefly mentioned above. Is, for example, moulting of a larva which not only affects the exoskeleton but also internal structures (the fore- and hindgut and entire tracheal system) or the transformation of larval into adult organs inside a pupa which—like the moulting larva—definitely has a functioning, complex nervous system associated with pain and suffering? Should we feel sorry for this struggling, seemingly almost desperate creature—or is it really oblivious to this process and we unjustifiably apply human feelings? The new debates on insect consciousness (e.g. Barron and Klein 2016; Klein and Barron 2016) and emotions (Mendl and Paul 2016) suggest that the neural capacities of insects, and the affective states they may experience, could be far richer and more sophisticated than hitherto believed.

The European Food and Safety Authority (EFSA 2005) lists nonsocial insects in ‘Category 2—The scientific evidence clearly indicates, either directly or by analogy with animals in the same taxonomic groups that animals in those groups are NOT able to experience pain and distress’. In light of the above, this EFSA edict seems far from convincing.

Nature Does Not Respect Individuals

Summarising, on an ecosystem level all organisms (including plants) are ‘simply’ individual entities, potentially providing food for others, nothing less but nothing more. That’s how nature works and implies that nature in general does not respect individuals; individuals do not have intrinsic rights (even if we perceive them as having intrinsic value as ‘things-in-themselves’)—in sharp distinction to our ‘intellectualised’ values. We give them ‘rights’—or not—according to our convictions.

3.8 Peculiar Welfare Standards for Insects?

Living conditions In general, one might think for animal keepers it would be in their own interest to look after their livestock as well as possible—but this is not always the case, particularly when commercial mass breeding is involved. As we all know from the chicken farm debate, animals can be accidentally or even deliberately mistreated without precluding the goal of keeping them. Chickens may have wounds and no feathers and never see natural light—but they can still lay nutritious eggs; farmed mammals may receive inappropriate food but still provide meat.

A general rule seems to be that most vertebrates have a much higher tolerance of poor, species-inappropriate conditions than insects, at least for significant periods of time. In other words, there are fewer options for inappropriate keeping of insects compared to vertebrates; it is thus less likely that insects can be kept alive under 'inhumane' (species-inappropriate) conditions. While most vertebrates can survive—within relatively wide limits—on inadequate food and/or under poor space and unfavourable light and climatic conditions, with insects their plasticity limits are much narrower. Being ectotherms, thermoregulation is more difficult—notably in captivity. Humidity levels can be critical. Many phytophagous species can only eat a very small range of plant species (comparable in this way to, e.g. the giant panda) and so on.

Consequently, to keep insects in captivity, it is generally essential to practise more precise husbandry compared to that needed to maintain vertebrates. Vertebrates can be neglected for days, sometimes weeks, but insects will often die within hours or even minutes if conditions are not right. Good artificial diets for insects, essential for mass breeding (see Sect. 3.10), are notoriously difficult to create (Morales-Ramos et al. 2014a, b), requiring far more precise formulation than the pellets and biscuits produced for, e.g. chickens or dogs.

Veterinary practices Vertebrates face a variety of diseases that are similar or comparable to human illnesses, and a huge discipline (veterinary science) takes care of this with a great repertoire of prophylactic and curative medicines, surgical and other procedures. Medicinal means (vaccinations, etc.) are *always* necessary to keep vertebrates in captivity for prolonged periods, but for insects such means are not available.

Insects are also heavily affected by pathogens (viruses, bacteria, fungi; Rolff and Reynolds 2009; Vega and Kaya 2012; Eilenberg et al. 2015; Maciel-Vergara and Ros 2017). De Goede et al. (2013) conclude that it is 'practically infeasible to provide insects in rearing facilities the freedom from diseases'. Although insect pathology is a growing subject, including the ecology of naturally occurring diseases (e.g. Hajek and Shapiro-Ilan 2018), effective treatment remains almost impossible—prophylactic methods, including hygiene and culling, are the main means for control (Eilenberg et al. 2015). In a valuable review of the threat of viruses to mass-bred insects, Maciel-Vergara and Ros (2017) suggest the possibility of RNA interference and transgenic technologies.

Special manipulations and interventions For some purposes highly unnatural treatments of insects, which can and probably do add very unnatural stresses, are unavoidable to meet the goals of breeding them. To produce infertile individuals for release to control fruit flies, mosquitoes and screwworm, for example, billions of individual insects are subject to various manipulations, including sterilisation by exposure to x-rays (see Sect. 3.10).

These mass release cases present major issues if we wish to approach insect welfare from an extensionist position. From a utilitarian interpretation, we are clearly not taking account of the insects' interests, at the very least with respect to reproduction. From a deontological perspective, we are surely using the insects merely as a means to an end. However, given that we now have technologies to produce, e.g. 115 million screwworms and 5×10^9 medflies per week (Leppla et al. 2014; see Sect. 3.10) with the potential to greatly reduce suffering and save the lives of thousands if not millions of humans and other mammals (many of the latter already in our care), or make a major contribution to environmental health by reducing the need for insecticides, is it ethical *not* to use insects in this way? More generally, should we or should we not trade-off mistreatment and exploitation of one organism versus 'avoidable harm' to another—with or without considerations of human self-interest—representing yet another ethical dilemma?

There are other interventions to consider. For example, selective animal breeding has been practised for many hundreds of years and is generally accepted—but how and in what way does this differ from the production and release of genetically modified organisms (GMOs) using modern biotechnologies (Benedict 2014)—often the subject of heated debate? The existence of GMOs, produced in captivity and then accidentally or purposefully released into the wild, has unknown environmental consequences and raises many ethical issues, not least regarding the organisms themselves (Ormandy et al. 2011; GeneWatch UK 2015) and our relationships with them (Schicktanz 2006).

Context dependency A further complication concerns context-specific behaviour and human self-interest. For example, the green bottle fly *Lucilia sericata* lays its eggs on open wounds, where the larvae normally develop on the necrotic tissues. Sheep are a frequent 'target', and this can result in 'sheep strike'—if the available wound material becomes insufficient to feed all the growing fly larvae, they will invade the living flesh of the sheep and cause injuries so gruesome they can even lead to death (Hall et al. 2016).

Prevention of sheep strike by the use of insecticides is obviously an economic concern as well as a welfare issue for sheep farmers. Further, the same fly species is being used purposefully to heal humans by controlled 'maggot debridement therapy (MDT)'—which is dependent on both the local release of powerful antibiotics by the larvae and their effective 'microsurgery' in cleaning necrotic and infected wounds (Sherman et al. 2000; Fleischmann and Grassberger 2003; Hall et al. 2016). Thus the enemy of the sheep and the farmer can be the saviour of an ulcerated senior citizen. Many people are disgusted by maggots—but in this context they are of great help to us and are even bred industrially for such medicinal use and disease management (see Sect. 3.10). All these conflicting human values and interests affect our approach to the welfare of maggots bred in captivity.

Do the ends ever justify the means? Paradoxically, some ethical questions become even more acute where we can be confident that the overall environmental consequences are likely to be minimal. In reviewing the great potential for 'gene drive' genetically engineered mosquitoes (e.g. Carvalho et al. 2014; Hammond et al. 2016) in the 'war' against malaria, Eckhoff et al. (2017) call for 'the development of a robust governance framework codesigned by all interested parties'. However, it is not at all clear if the mosquitoes let alone the plasmodia they may carry are to be included as parties with interests (sensu Singer 2011).

What Standards Can Be Established?

The above suggests that setting general (or principled, ethical) standards for insect welfare in captivity is an impossible task. Due to the functional diversity of insects, and the wide range of purposes addressed by keeping them, generalised criteria cannot be formulated—simply there is no 'model insect' with respect to development, food, behaviour, ecology, etc. from which general conclusions could be drawn. More specialised husbandry is nearly always necessary to keep insects successfully—which means that the possibilities for 'chronic' mistreatment of captive insects are, fortunately and incidentally, reduced (although unintended killing is all too easy). Keeping insects in captivity depends on replication of the key conditions, requirements and processes they experience in the wild. To show proper respect, this means setting aside any idea about applying 'human standards' in favour of delivering species-appropriate and species-specific welfare. A rule 'keep insects under as natural conditions as possible', taking species-appropriateness into account, looks to be about the best guidance that can be offered—even though, as already discussed, perfectly 'natural' stresses are relieved and new stresses induced—and in some circumstances, 'mistreatment' may be justified if the ends arguably preclude terrible suffering or death for other organisms. This further underlines the conclusion that personal feelings about what might or might not seem 'natural' or desirable for us, or any feelings of disgust and repulsion, cannot play a role in setting welfare standards for insects.

The 'Five Freedoms': A Way Forward?

The 'Five Freedoms' approach to animal welfare developed in the 1960s as a result of growing concerns over farm animals such as chicken and veal calves being mass bred in intensive confinement (FAWC 2009). The Five Freedoms (FF) can be summarised as freedom from thirst, hunger and malnutrition; from thermal and physical discomfort; from pain, injury and disease; from fear and distress; and freedom to express normal behaviour (Webster 2016).

De Goede et al. (2013, see also Erens et al. 2012) recently considered extending the FF framework to the management of mass-bred insects. As they discussed, while several of these freedoms can be helpful in addressing care-standards for insects, freedom from contracted diseases, as we have already noted, is unrealistic due to the lack of veterinary procedures, and freedom to express normal behaviour is very difficult to implement—not least because so many mass produced insects are first subject to manipulation (e.g. sterilisation) and then released. Moreover, there is evidence that just the process of breeding or even simply rearing insects in confinement can alter behaviour (e.g. Jandt et al. 2015). De Goede et al. (2013) also point out that the Five Freedoms are essentially about ethical standards rather than science-based prescriptions for actual welfare—although there have been proposals to make the FF approach operational, such as the 2004–2009 EU WelfareQuality project (e.g. Botreau et al. 2007).

Others have called for more positive 'five domains' or 'quality of life' approaches to animal welfare (Green and Mellor 2011; Mellor and Webster 2014), or welfare founded on capacity for change (allostasis), leading to 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity' (Korte et al. 2007). Thus the concepts of animal welfare are becoming more diverse and more nuanced (Hagen et al. 2011; Maple and Perdue 2013; Plannthin 2016). Even so, Webster (2016) reaffirms FF principles as 'timeless' and 'a memorable set of signposts to right action'. Thus the Five Freedoms, as De Goede et al. (2013) have suggested, appear to offer the most appropriate framework to advance welfare principles and practices for the mass breeding of insects (cf. Taponen 2015). We next look at a specific example where the Five Freedoms could be used for welfare guidance in the future.

3.9 Butterflies in Captivity: What for and How?

Tropical butterflies are addressed as an example for insect welfare, being well-liked and the most widely known insects held in captivity. This involves all life stages, with the adults being used in various ways. Despite our reservations about generality, many of the issues are relevant for other insects—some of which are now mass produced in billions (see Sect. 3.10).

Although most consider adult butterflies beautiful, their early stages are often viewed as ugly 'worms'. But 'What the caterpillar calls the end, the rest of the world calls a butterfly' (Lao Tse). While caterpillars serve few if any human utilitarian needs (but see Sect. 3.10), the butterflies they become are used in live butterfly exhibits (butterfly houses, butterfly zoos) for edutainment, outdoor and indoor release at events, souvenirs and raw materials for artworks. These uses are partly conflicting with respect to the 'moral domain'.

Mass Production of Butterfly Pupae

Mass production of butterflies mainly happens in tropical countries. Annual global production of live butterfly pupae as a cash crop represents a multimillion dollar industry, with numerous stakeholders (Boppré and Vane-Wright 2012). Producers vary from single individuals to small- to medium-sized businesses ('butterfly farms'). Trading is usually done by dealers who ship pupae internationally.

Factors affecting welfare include food, water, temperature, light, humidity, density, antagonists and hygiene. Different butterflies require more or less different conditions and treatment at all life stages (eggs, each of the several larval stages, pupae, adults). While most butterfly larvae live individually and can become cannibalistic if crowded, some species have gregarious larvae, thriving and even pupating in groups. Some 200 (1%) of the 20,000 world butterfly species are bred commercially, although about only 30 account for the majority.

How the many hundred butterfly farmers handle their cultures is uninvestigated and unmonitored. Some raise larvae in cages or boxes on cut foodplants; others keep larvae under 'sleeves' on living trees. Several manuals provide information on the basic biology and keeping conditions of commonly bred species (e.g. Stone and Midwinter 1975; Venters and Rogers 2001; Harberd 2005; Aisi et al. 2007; Montero 2007), but they do not address welfare. Even so, it is reasonable to assume all early stages, and those adults needed to found the next generation, are generally well treated—otherwise the farmers would not get enough healthy pupae to sell (see Sect. 3.6). Butterflies (in contrast to many other insects) do not lay eggs in unsuitable conditions, in particular without having species-specific hostplants available. Larvae will fall ill and die if they do not get correct or sufficient food. The same is true if they are forced to live too densely, suffer inappropriate physical conditions or are exposed to predators and parasitoids.

However, species-inappropriate treatments almost certainly occur at least occasionally within the butterfly house industry, notably during the 'self-training' phase that most novice breeders go through—but this cannot be chronic; otherwise insufficient pupae would be produced for sale. Nevertheless, with continuous breeding diseases often build up, and so rigorous hygiene is essential (Lees 1989). Failure can lead to 100% mortality—but such events are not reported and escape analysis. If breeders are happy to get much less than 90% of eggs or larvae through to the pupal stage, then conditions for welfare are definitely wrong.

If butterfly producers consistently and repeatedly rear from gravid females taken from the wild instead of breeding from their captive stock, this is not sustainable, and there is thus environmental concern. However, with respect to the lives of the individual insects once in captivity, the welfare issues are the same.

Shipping of Butterfly Pupae

Shipping creates potential for mistreatment. However, perhaps even more than at the breeding stage, it is in the dealers economic interest that pupae reach customers in good condition. There is now much experience on shipping pupae successfully, and welfare does not appear to be a significant issue.

Live Butterfly Exhibits

Butterflies are generally seen as beautiful and harmless and thus have greater potential for creating environmental awareness than other insects. Butterfly houses have become popular because they delight and entertain people, young and old. They can then also make visitors aware of the ecological diversity of insects, and how invertebrates play key roles in the ecosystems on which we depend. In other words, butterfly houses can offer an entertaining yet revelatory introduction to ecological literacy and the needs for conservation (Boppré and Vane-Wright 2012).

For this 'edutainment' to be realised, butterfly houses need not only to be well organised with respect to education but also care for their live butterflies properly. Adult butterflies have quite different requirements to their larvae, with fundamental consequences for welfare. Several countries have established rules on how a butterfly house must be run and which species can be exhibited; however, these focus solely on ‘security’ (environmental safety) (USDA 2002) and do not address welfare.

As natural as a butterfly house might look and feel (tropical plants, high temperature and humidity), it is an artificial environment. When you want to entertain, educate and raise ecological awareness, you need a variety of species (small, large, colourful, cryptic, etc.), and these are typically derived, as are the plants, not only from different habitats but even different continents: they don’t occur together in nature or represent a natural community. This is practically unavoidable: few species can be bred reliably in large numbers, and many are unsuited for displays since they do not behave well in confinement (e.g. sitting in corners or flying incessantly against the glass).

Even so, each butterfly species will have its own requirements. Thus, a live butterfly exhibit must provide diverse structures with sunny and shady, warmer and cooler areas, to simulate different natural habitats (forests/open areas). Unavoidably, the light regime (day-length, light quality) is very different for tropical species brought to temperate latitudes, including artificial lights. Whether this is stressful is unknown and, as far as we are aware, uninvestigated. Diurnal variations in temperature and humidity are difficult to match with each species’ natural preferences, all with unknown consequences for stress and longevity.

Food is a critical issue. Of the species usually exhibited, some require nectar, others rotten fruit. For nectar feeders, suitable flowers (with relatively simple floral structure that allows butterflies to access the nectar easily, i.e. daisies or verbenas rather than exotic orchids) cannot be provided to offer sufficient nectar for numerous butterflies in confinement. The usual solution is ‘artificial feeders’ offering sugar water. These can supply the butterflies’ basic needs for water and energy. In nature, nectar feeders use a wide variety of flower species—in contrast to their larvae, which are much more specific. More important than the particular sugars offered is the structure of the feeder, because this determines accessibility. Viscosity can be a major problem. The butterfly proboscis is like a drinking straw—from personal experience everybody knows that a larger diameter straw is needed for a smoothie than a soda! Due to evaporation the viscosity of sugar solutions offered in exhibits can change quickly from being acceptable to lethal—death can ensue if the proboscis becomes clogged with crystallising sugars as a feeder dries up.

Fruit feeders rarely visit flowers or take artificial nectar, but in butterfly houses they often do, seemingly in desperation—because fruit is often not provided in an appropriate state. Fresh bananas look nice, but they do not feed a butterfly—rotten, fermenting bananas look bad, but they offer just what a fruit-feeding butterfly needs. Other fruit also has to be overripe and decaying—but citrus is not appropriate even though it may be visited for lack of anything better.

In summary, although a compromise, the abiotic conditions for butterflies in a butterfly house can be made ‘as natural as possible’. From an ecological perspective, the butterflies are, in effect, sacrificed. Keeping them under unnatural light, temperature and humidity regimes and food-supply conditions, almost certainly results in some kind of stress which they have no opportunity to avoid. Females need specific host plants for egg-laying which cannot be provided because it is too difficult or expensive, or resultant early stages have to be culled to stop them eating too much, starving, spoiling the plants and producing excrement—this last a potential source of infection. Is a gravid female unable to lay her eggs stressed, or even in pain? On the other hand, adult butterflies in well-managed captivity are not usually exposed to antagonists, and so have the chance to live longer than in nature.

In contrast to breeding, if due to poor welfare adult butterflies in an exhibit do not live as long as they could, they can easily be replaced by new individuals. Thus for butterfly exhibitors, there is less pressure than for breeders to exercise good husbandry because the additional cost of replacement is marginal. Indeed, it will often be more cost-effective to replace than practice good welfare. This can be to the financial benefit of the breeders and traders too—but not the interests of the insects themselves. The educational value could also be compromised if visitors see moribund butterflies.

Event Releases of Butterflies

To 'bring colour' to a party or celebration (birthday, wedding, funeral), the release of living butterflies has become fashionable (Pyle et al. 2010). These are normally produced by different breeders to those producing pupae for butterfly houses: they use far fewer species, and the insects are shipped not as pupae but as adults.

At first glance, release of butterflies appears benign. Suppliers even justify it on the grounds that it will increase local butterfly numbers—what could be nicer than that? However, much depends on which butterflies are released, when and where. Will they find food, partners and hostplants? Or do they cause environmental harm if released beyond their natural range, with the risk of introductions and detrimental interactions with the local fauna? Even when the same species occurs locally, rarely will the released butterflies be genetically the same as the native population, also with potentially negative consequences (Pyle 2010). The educational benefits appear minimal if non-existent, especially if we conclude that butterflies are simply being sacrificed for human vanity. Releases of living butterflies within meeting rooms, auditoriums, clubs and other such spaces are arguably unethical with respect to the plight of the insects.

Breeding Insects to Supply Adult Deadstock

This affects butterflies, various giant moths (Saturniidae), stick insects, beetles, etc., in the context of production for collectors, souvenirs and artwork. The welfare issues are comparable to those described for butterfly farmers, together with the problems of euthanasia. The ethical issues relate to extensionism—clearly these are purely utilitarian activities, lives taken as a means to an end (collecting and decorative arts).

3.10 Industrial Mass Production of Insects: What for?

Butterflies represent the tip of the iceberg on welfare and husbandry of insects in captivity. They are an interesting example because they are liked insects, but the vast majority of insects currently kept in captivity are (to most) unappealing creatures that the average person never encounters—yet they are produced in billions! This 'industrial entomology' can only be sketched here, but it has to be addressed because of a variety of potential welfare issues and its exceptional magnitude. If insects suffer from stress, the high density at which they are raised (e.g. crowding effect: Weaver and McFarlane 1990; Morales-Ramos and Rojas 2015), necessarily exceeding the highest densities that occur in the wild by far, would definitely be an issue. In addition there are major ethical conflicts between 'big business', benefits for humanity and ecosystem health, versus mass generation and use of insects with little or no regard for their lives, welfare or interests.

Insects for Release to Suppress Pest Populations

The greatest numbers of insects currently mass generated in captivity are produced to control pest populations affecting agriculture, forestry and farm animals or exotic species or vectors of human diseases. A large diversity of taxa are targets. More than 200 species of natural enemies are commercially available for biological control (van Lenteren 2012b) but 'data ... are very hard to obtain' (van Lenteren 2012a). To suppress Mediterranean fruit fly (*Ceratitis capitata*) populations, to give one example only, a single facility produced a maximum of 5,000,000,000 flies per week (Leppla et al. 2014). Some species are produced by private companies for sale and others by state agencies for 'eradication programmes'.

Schneider (2009) provides an overview on principles and procedures for breeding high-quality insects and discusses, inter alia, insectary design, genetics, abiotic conditions, nutrition, pathogens and quality control. Further overviews on mass breeding techniques/technologies (see 'entotechnology', Kok 2017) include Leppla and Ashley (1978), Parker (2005), Cáceres et al. (2012), Carvalho et al. (2014) and Cortes Ortiz et al. (2016). However, with respect to breeding technologies actually employed by commercial companies or public institutions, little is publicly known.

Generally, two approaches to pest management are served by mass-bred insects:

Release of bred parasites, predators and parasitoids: These are primarily targeted against exotic weeds (e.g. Moran et al. 2014) and early instars of mostly phytophagous insect pest populations affecting horticulture, agriculture and forestry. Predators commercially bred are, for example, ladybird beetles (Coleoptera) (Riddick and Chen 2014) or true bugs (Hemiptera: Heteroptera) (De Clercq et al. 2014). Parasitoids affect mostly insect eggs or larvae and belong to various families of wasps and flies (e.g. Braconidae, Aphelinidae, Trichogrammatidae, Tachinidae) (e.g. Boivin et al. 2012; Sithanantham et al. 2013; Dindo and Grenier 2014; Wang et al. 2014). Usually, 'augmentative biological control' (van Driesche et al. 2008) is practised: the beneficial organisms are necessarily released periodically since they do not usually establish in the field.

Release of bred sterile males: These are produced (Parker 2005) and released to reduce female fertility of target species (autocidal control; sterile insect technique; sterile insect release method, SIRM; Krafsur 1998; Wyss 2000; Dyck et al. 2005). Target species are numerous but mainly belong to four families of Diptera: fruit flies (Tephritidae), the maggots of which infest fruit preharvest, mosquitoes (Culicidae) carrying virulent human diseases such as malaria, and blow flies (Calliphoridae) and flesh flies (Sarcophagidae) the maggots of which (sometimes fatally) parasitise livestock, wild mammals and humans (flystrike and myiasis; Hall & Farkas 2000). In addition to the parasitoids, in the case of fruit fly control, use can also be made of irradiated hosts for breeding and for release in the field for monitoring purposes (Cancino et al. 2012).

Both approaches involve the release of tens of thousands to millions of individuals (King et al. 1985; Leppla et al. 2014; Skoda et al. 2018). Industrial production necessitates the establishment of ‘biofactories’ (Leppla et al. 2014) employing automated processes with complex engineering and mostly using factitious food or artificial media (‘diets’) as substitute food (e.g. Singh 1977; Grenier 2009; Riddick 2009; Panizzi and Parra 2012; Parra 2012; Morales-Ramos et al. 2014a, b; Cohen 2015). Nowadays, silkworms are also produced on artificial media (Hamamura 2001). Antibiotics are often added to insect diets to prevent diseases, but many insects depend, to varying degrees, on symbiotic bacteria (Duron and Hurst 2013), and artificial diets might have hidden (sublethal) effects for development and/or vitality (e.g. Thakur et al. 2016). From a welfare perspective, feeding captive insects on artificial diets saves lives of their natural prey or hosts, with which, however, mass production would neither be practical nor economic. SIRM typically requires x-ray irradiation of the living insects—does this involve stress, pain, discomfort and/or suffering?

A modification of SIRM involves transgenic technologies (Benedict 2014; Li et al. 2014) to create genetically modified insects, obviating the need for x-ray sterilisation. The mass-produced insects carry altered genes that will kill offspring or confer female sterility after mating with wild conspecifics. This has the potential to suppress target insect populations over the scale of years, but requires continuing release as dispersal and/or natural selection will overcome the induced extreme maladaptation (see, e.g. Carvalho et al. 2014; Hammond et al. 2016; Eckhoff et al. 2017). The ethical, welfare and environmental issues of such techniques do not appear to be addressed at all.

Insects as Food

Insects serve as basic food for innumerable (insectivorous, entomophagous) animals, from other insects to mammals, and provide supplementary food for people, mainly in tropical countries. Traditionally worldwide more than 1500 species (Ramos-Elorduy 2005; cf. Yen and Van Itterbeeck 2016), from caterpillars to grasshoppers, beetle grubs, wasp maggots and adult termites, are opportunistically harvested in the wild and used as food (‘anthro(po)entomophagy’) for millions of people, partly also taken into human custody (farming) and representing a significant food source for rural people including economic benefits (e.g. Ramos-Elorduy 1996, 2005, 2009;

Ramos-Elorduy et al. 2011; Thomas 2013; Makhado et al. 2014; Chakravorty 2014; Baiyegunhi and Oppong 2016; Feng et al. 2018). For most species, traditional extraction of insects as extra food for a small local community has a quantitative limit set by the need for sustainability, not overharvesting a target species and thus risking its local extinction (Yen 2009; see also Secretariat of the Convention on Biological Diversity 2001 and Münke-Svendsen et al. 2018). However, outbreaks providing huge numbers of edible insects can occur—e.g. at Lake Victoria tiny midges (Chaoboridae) appear in clouds stretching many miles and are harvested to produce 'kungu cake' (Capinera 2008: 2384). Unfortunately, such traditional foods can nowadays be quite unhealthy, such as mopane worms (Lepidoptera: Saturniidae—*Imbrasia belina*), recently found to have high concentrations of heavy metals (Greenfield et al. 2014).

In the West, although the idea of insects as food has long been given serious consideration (e.g. Bodenheimer 1951; Zumpt and Schimitschek 1968; DeFoliart 1989), it currently enjoys very active promotion by several NGOs—including the Food and Agriculture Organisation of the United Nations (FAO; van Huis et al. 2013)—as well as the media, and is advocated by an increasing number of authors (Ramos-Elorduy 1996, 2009; Paoletti 2004; van Huis 2013; van Huis et al. 2013; Shockley and Dossey 2014; Evans et al. 2015; van Huis et al. 2015; Vantomme 2015; Dossey et al. 2016; Payne et al. 2016; Mishra and Omkar 2017b; van Huis and Tomberlin 2017). They state that with insect protein, the impending food crisis can be combated, and since insects produce protein in an environmentally more friendly and sustainable way than traditional stock (cattle, pigs and chickens), insect protein would, in addition, help reduce the ever-increasing stress on our environment.

If a move from insects as *supplementary* food 'for some' to insects as *basic* food 'for many' is seen ethically as an excellent idea, too, we have to ask: which insect species would be suitable for being mass produced to feed millions of people more or less regularly/continuously, and how can mass production be done in an eco-friendly way at affordable prices? Then the question on welfare of insects in such breeding facilities comes even more sharply into focus, while similar to the mass breeding of wasps and flies (above), it brings an extra dimension: the tiny wasps and flies mass produced for pest management are generally considered as 'un-nice', while many of those under discussion as food are much larger and would be considered by many as 'nice'.

Currently, limited mass production of mealworm beetle larvae (*Tenebrio*) (Grau et al. 2017), crickets (*Acheta, Brachytrupes, Gryllus*) and grasshoppers (*Locusta*) for human consumption and non-local trade is practised. They serve to supply restaurants specialising in insect cuisine and the production of lollypops with 'worms'; 'real crickets and worms, dipped in a chocolate coating'; and insect powder, insect bars, insect snacks and insect hamburgers (see Internet for products and prices). This is a new business idea to make money with 'specialities', 'trendy food' or 'novelties' for people who can afford such extras. The current scale of operation is, however, far too small, and the effort and costs involved far too great to serve food security and/or reduce dependence on the admittedly unsustainable production of vertebrate protein—all of which would potentially be an ethical justification for human use of insects as food (facile arguments about environmental benefits, however, play a big role in marketing). Rather, the scale appears similar to breeding insects as food for pets and zoo animals—there the effort (and consequently the price) is high and only worthwhile because there is no practical substitute. Whether such breeding is always 'eco-friendly' or not is an unanswered question.

To market insects in the West for food as a way to educate people to overcome disgust towards them distracts from the importance of the subject, simply because insect protein-containing food can be produced like vegetarian 'hamburgers', where taste and structure can be engineered to simulate beef or other conventional meats. Then it can no longer strictly be called entomophagy—which in the future will not and cannot mean consuming 'entire insects'.

Critical assessments with respect to the practicability of real mass production (Leppla 2009; Sileshi and Kenis 2010; Maciel-Vergara and Ros 2017; Kok 2017) are scant although, from an entomological/natural history perspective, it is not surprising that mass production of insects for food and feed is not (yet?) properly established. In contrast to the examples of mass production of predators, parasitoids or sterile males (above), the biology of (most of) the wild species used for human consumption is generally different. The majority do not develop continuously (that is why harvesting from the wild is always a seasonal affair). To have sufficient food for the species to be mass produced, to manage disease risks (Eilenberg et al. 2015) and to maintain the necessarily complex technology require much effort, and probably huge investment and operating costs. Mass production should not have an ecological footprint greater than the savings gained by using insects instead of vertebrates. Also killing methods (see Sect. 3.11) for currently millions, eventually trillions of insects require consideration.

In conclusion, only a very limited number of non-saprophagous species are, perhaps, suited for continuous, eco-friendly industrial mass production of insect protein. Considering insect lifestyles, and taking into account what was said above about insect generally low tolerance of non-near-optimal conditions (see Sect. 3.6), mass production of insects for human food on an industrial scale is, positively expressed, a great challenge. That no 'big player' in the food industry has yet built or commissioned biofactories for producing and mass marketing insect protein (cf. Kok 2017: 171) seems to support our scepticism.

Many additional issues, some of which are now being debated, are in need of much further research. These include and range from food safety and hygiene (Belluco et al. 2013; EFSA 2015; Grabowski and Klein 2017) to potential health benefits from insect metabolites (Roos and van Huis 2017; Lee et al. 2017). Effects of harmful insect metabolites (Blum 1994) seem to gain little attention as do those related to long-term consumption of particular insect species as a primary food source. A basic complication, or even an obstacle, is the diversity of adaptations insects exhibit, which not only manifest in their visible appearance but—even more so—in hidden characters. There is no 'model species' stakeholders of entomophagy could study to gain reliable, transferable insights.

Insects as Feed and Decomposers

The supply of live insects as feed for pets or small animals in zoos is a long-established practice. Numerous zoos and households are supplied with mealworms, crickets, locusts and other insects for this purpose. There are no data on the magnitude of the industry, but, as it is possible to buy live insects off-the-shelf in almost any pet shop, and they are widely available on the Internet, it is clear that many millions of individual insects are involved annually. Sold by weight and generally transported in densely packed containers without food, the prices per gramme are high compared with human foodstuffs. This is economically sustainable because, to feed many captive insectivorous animals, as already noted, there are no practical alternatives.

Insects as feed for poultry or aquaculture to some extent parallels the issue of insects as human food but, although a more advanced practice, there are still problems (Rumpold and Schlüter 2013; Makkar et al. 2014; Henry et al. 2015; Lundy and Parrella 2015; Smetana et al. 2016). Despite all the brilliant advantages, the conclusion of Józefiak et al. (2016) '... legislation barriers in the European Union, as well as relatively high costs and limited quantity of produced insects are restrictions in the large-scale use of insect meals in poultry nutrition' likely holds true not only for insects as feed but also for insects as food. Even so, the French agro-industrial company Ynsect produces 'over one tonne of proteins and derivatives, lipids and chitin and derivatives per day' (PRO*te*INSECT 2016) using robotics and automation to farm mealworm.

The stock example is the black soldier fly larva (BSFL, *Hermetia illucens*), in many ways a unique animal (Müller et al. 2017). The larvae are bred on manure or organic waste and are already successfully used for aquaculture and animal feed (although there are limitations: Barragán-Fonseca et al. 2017), as well as for composting (e.g. Riddick 2014). Breeding BSFL for feed is in many ways comparable to what happens in a garden compost heap, just on a larger scale. With respect to welfare, the saprophagous lifestyle of BSFL makes a huge difference in comparison to insects with more demanding lifestyles. Does the gain from decomposing organic human waste also add to the 'ethical dimension'? Ideas for using BSFL go far beyond feed—'bioconversion of organic wastes into biodiesel via insect farming' is a proposal (Surendra et al. 2016; Leong et al. 2016).

If hygiene problems (see EFSA 2015) can be solved, BSFL might also be suited as food for humans. Several other Diptera with a saprophagous lifestyle (e.g. house flies, *Musca domestica*; Hussein et al. 2017) could play a big future role too. Intrinsic problems with keeping such species are much reduced compared with phytophagous and carnivorous insects. However, diseases and antagonists can still be a welfare as well as a practical problem: recently, an established BSFL production in West Africa was hampered by a pupal parasitoid, which reduced future broodstock by almost 72% (Devic and Maquart 2015). This clearly exemplifies the ever-present risks of operating large-scale breeding facilities—but such incidents are rarely reported.

Insects as Pollinators

Pollinators are now mass produced in captivity for targeted release. Originally bumblebees were bred and traded for pollination of plants in greenhouses (Velthuis and van Doorn 2006). Commercial bumblebee breeding farms now also produce insects for release into the wild for pollinator supplementation, and bumblebees have become subjects for domiciliation and domestication. 'Bombiculture' (Kwon 2008) is a growing issue because, with decline of the honeybee, pollination of crops by other insects needs to be assured. The emergent industry trades several *Bombus* species and subspecies globally, in increasingly large numbers. For example, in 2006 'approximately one million colonies [of *B. terrestris dalmatinus*] were transported across 57 countries . . . 16 of which [were] outside its native range' (from Ings 2006, not seen, as reported in Owen et al. 2016). In parallel, 'raising bumble bees at home' is also promoted (e.g. Strange 2015). Long before, the solitary alfalfa leafcutting bee (*Megachile rotundata*) was mass-produced and released specifically for pollination of alfalfa (Pitts-Singer and Cane 2011; Peterson and Artz 2014), a leguminous plant used for feed and fertilisation.

As in the cases of mass-bred insects for pest management, mass-bred pollinators are also released into the wild, with potential consequences for wild insects and our responsibilities towards them. The associated problems are multifold (see, e.g. Winter et al. 2006; Dafni et al. 2010; Goulson 2010; Graystock et al. 2013, 2016; Manley et al. 2015; Cameron et al. 2016; Gisder and Genersch 2017; Pirk et al. 2017; Tehel et al. 2016) and cannot be discussed here in detail, in particular because of the complexity and numerous gaps in knowledge.

3.11 Welfare and Ethics of Industrial Mass Production of Insects

Ethical concern for the insects themselves does not in general appear to be a significant matter in the literature on insect mass production. As Gjerris et al. (2016) note, in the context of mass producing insects for food and feed, ethical issues concerning insect welfare are hardly addressed. Not only 'the keeping' needs to be assessed ethically but also all the associated logistics (packing, shipping, methods of release, killing, etc.). As noted by Plannthin (2016), the stresses associated with the annual long-distance movements of huge numbers of honeybees across the USA by truck (Bond et al. 2014) can only be guessed at—but recent research suggests this may really be one of the factors responsible for honeybee colony failure (Simone-Finstrom et al. 2016; see also Perry et al. 2015). With respect to the ever-increasing trade in large numbers of living insects for feeding small animals, stress and welfare have never been addressed. It is not obvious how the practice could be improved practically—even though the shipping methods seem far from ideal and the fate of unsold, unused or uneaten individuals is unknown.

The welfare, breeding conditions and killing of insects currently produced for human consumption are not transparent, or the information available is too scant for serious comment; an example from the homepage of a commercial dealer of insect food is 'They [crickets] are fed a healthy diet of mixed grains and vegetables and raised in clean hygienic conditions. In addition they are also fed on a unique food blend specially developed for crickets'. Nevertheless one should ask: is mass production of animals for making novelty snacks marketed on the basis of amusement really justifiable? It does not show respect towards insects.

However, even details on how insects are kept and mass produced in biofactories cannot be analysed because, due to competition, suspicions, 'trade secrets' and so forth, methods and outcomes are not shared or published. As Erens et al. (2012) report, 'breeders [understandably] prove reluctant in sharing information on their techniques'. See also Dobermann (2017) for valuable insights into the tension between research and business in the development of insects for food and feed.

One of the major uses of mass-produced insects is biocontrol. We tend to believe that the use of supposedly species-specific parasitoids is more benign and 'better for the environment' than chemical control, because of the social and environmental costs of the latter (Pimentel et al. 1980). However, in a broad-ranging review, Lockwood (1996) stated 'With biological control there is the potential for a single, poorly conceived introduction to forever damage the well-being of an entire ecosystem. Perhaps no other human activity has the potential for a single individual [human] to undertake such a spatially, temporally, and ecologically devastating course of action'. Biocontrol thus represents a particularly powerful example of where we cannot consider the welfare of insects in captivity as an issue that ends at the biofactory door: our responsibilities if we resort to such powerful technologies are protean, extending even to the whole biosphere.

Are insects produced for insect restaurants and as expensive 'special' food (with no significant impact on overall human food supply) to be treated, ethically or with respect to welfare, in a different way to those (eventually) produced en masse to overcome hunger and/or to provide more eco-friendly protein? Are those to feed animals (e.g. pets, poultry, fish) to be treated differently from those for human consumption? Brando and Harfeld (2014) raise similar ethical questions and dilemmas about zoos and zoo animals: discrepancies between animal-friendly values for welfare of the animals exhibited *versus* disregard for welfare issues affecting the origins of protein (meat and fish) offered at zoo restaurants—and fed to the zoo animals themselves (including mass produced insects).

It does not need much imagination to realise that industrial mass production of insects cannot be done 'as natural as possible'. Do for insects artificial diets taste different than natural food?—and even if not, do they cause stress during digestion or modify quality/vitality? Dozens of such questions can be raised but they cannot be answered. Answers, actually, would not help much for ethical evaluation since there is no general foundation for insect welfare and, as already discussed, developing such a baseline is difficult to imagine or even impossible because of insect diversity.

Despite all of these uncertainties, our statement that insects hardly tolerate mistreatment (see Sect. 3.8) would appear to hold in particular for mass breeding.

This receives some support from the work of Portilla et al. (2014) who, in life-table studies of some mass-bred insects, found high reproductive rates on artificial diets, even including the case of specialised Colorado beetle predator *Perillus bioculatus* (Hemiptera: Pentatomidae) when fed on factitious prey. However, in a wide-ranging review of aphid parasitoids used in biological control, Boivin et al. (2012) comment on a variety of physiological, nutritional and natural selection problems associated with meeting the developmental needs of such parasitoids—which inter alia can lead to a loss of efficiency when released (due to inappropriate selection effects when bred at very high density). Artificial diets can reduce the quality of the adults (Grenier and DeClerq 2003), and genetic adaptation to captivity as well as loss of biological fitness and inbreeding depression can occur (see Gilligan and Frankham 2003; Boivin et al. 2012; Hoffmann and Ross 2018). Nevertheless, one can assume that when millions of individuals are being bred, the conditions must be species-appropriate or very close to it. Even so, during the research to find appropriate food mixtures/artificial diets and/or operational technologies for keeping the insects, likely mistreatments are unavoidable. Again, we face a lack of data for analysis. Although these problems do not directly affect issues of welfare in captivity, the possible effects on wild populations when potentially huge numbers of 'substandard' individuals are released are unknown. Again, if we choose to adopt these powerful technologies, our responsibilities cannot end at the biofactory gates.

Given the human misery caused by malaria, it seems unlikely that many of us would protest against the successful use of such technology on the grounds of insect welfare. Thus any exhortation to keep insects under 'as natural conditions as possible' could be set aside if the benefits of breeding in unnatural conditions or subject to genetic engineering are seen as ethically justifiable in their application—in other words, 'the ends do justify the means'. Even so, it is almost certainly the case that, in accordance with Albert Schweitzer's 'reverence for life' principle of ethics and the *Ahimsa* doctrine of Jainism, some people would not agree with such manipulations, even when the goal is to save human lives. Moreover, in many such cases, even the ends may be in conflict: releasing millions of insect parasitoids for biological control can cause ecological harm to nontarget species and whole ecosystems and thus become a threat to biosecurity and wider human interests.

Euthanasia

Despite their various uses, including ecological roles (e.g. pest control, pollination), in very many cases, keeping insects eventually means killing them. Emotionally, taking the life of a beautiful butterfly for many of us (but not all: Knutsson 2016) is quite a different issue than killing a tiny mosquito or the puparium of a fly. As said above, whether we like it or not, we inevitably destroy a lot of insects during our lives (see Sect. 3.2). But a difference remains between unintentional killing of some and intentional killing of many (notwithstanding that intensions and intentionality represent major issues in moral philosophy that we cannot address here).

In addition to mechanical killing of individuals, the main mass killing options are using a gas (e.g. hydrogen cyanide, ether, ethyl acetate; these 'anaesthetic' methods should be followed by, e.g. freezing: AVMA 2013), heat (e.g. boiling water, the traditional method for silkworm pupae) or cold (rapid or slow freezing). There is debate about which is ethically ‘best’. As insects are ectotherms and naturally become torpid at lower temperatures, freezing might seem more humane than gassing—but this relates to the question of feeling pain which remains unanswered (see Sect. 3.7). Freezing after cooling without prior anaesthesia, often advocated, is considered an unacceptable method for ectothermic vertebrates (AVMA 2013: 78), and this view has now been extended to invertebrates including insects (Pellett et al. 2013). Simply due to the very high numbers involved, killing insects for food and feed requires new ways.

However, guessing which is ‘best’ on the basis of human experience, even something akin to emotional transference, without underlying physiological and neurological knowledge is in the end inadequate and potentially misleading. As Cooper (2011) and others have pointed out, more research is needed to help ensure that, even at the point of death, insects in captivity ‘are handled with the respect due to any living creature’ (Murray 2012: 44).

3.12 Insect Welfare in Captivity vs in the Wild

While the majority of animals currently considered in the context of welfare are domesticated races of vertebrates not found in the wild, there are many (e.g. birds and reptiles kept as pets) that do have natural populations. But *all* insect species in human custody (other than the silkworm and honeybee) occur in the wild. Trying to keep insects in captivity in as ‘humane’, natural or species-appropriate way as possible has a logical consequence—we also have to pay respect and give attention to insects living in natural habitats: on the one hand to learn about their life in the wild and on the other because many wild populations are in effect in human custody due to our now major and relentless impacts on the biosphere.

Risks of Mass Releases

It follows that the issue of insect welfare cannot be restricted to husbandry in captivity only, but must include environmental issues too. When insecticides are sprayed against pest populations of insects, are we primarily concerned with our own welfare, insect welfare and/or the health of the environment as a whole? When herbicides are used, are we concerned that food for insects is destroyed? And insects as food for vertebrates? If we release pollinators, parasitoids and sterile flies—what about their individual fate and welfare, what if they mate with wild relatives? Thus from an ethical perspective, we also need to consider ecological harm.

While we are not able to address this additional dimension adequately here, we wish to make the point that ethical and welfare issues still arise even if we accidentally—let alone purposefully—release insects bred in captivity into the general environment. Even giving ‘harmless’ insects like butterflies their ‘freedom’ by opening the butterfly house doors would not be an unquestionably ‘noble’ act. Quite the opposite in most cases—more akin to the problems of captive mink release by animal rights activists (e.g. Macdonald and Burnham 2010). Risks of mass releases include aspects of biosecurity, nontarget risks, epigenetic effects, inbreeding depression, etc. GeneWatch UK (2015) published evidence that genetically modified (GM) insect factories could spread antibiotic-resistant bacteria into the environment, posing a risk not only to environmental but directly to human health.

3.13 Is There a Need for Legislative Regulations on the Welfare of Insects in Captivity?

More and more states including the European Union enact regulations in the context of animal welfare. There are laws prescribing conditions under which meat and eggs may be produced. How to keep vertebrates in zoos is regulated. How familiar pets such as dogs, cats and various birds are kept is usually seen as a private matter—but if cruelty is demonstrated, legal action can be taken. Because of various risks including the potential for poor husbandry, some countries have considered bans on keeping reptiles and other exotic pets in private (e.g. UK: CDP 2015).

For insects, there are hardly any regulations regarding their welfare (the recent Dutch Animal Act is a rare exception: Wet Dieren 2013; De Goede et al. 2013). Conservation laws restrict collecting certain species in the wild (e.g. the butterfly *Parnassius apollo* is legally protected in at least 19 European countries: Nakonieczny et al. 2007), and this normally includes prohibition of keeping early stages and adults in captivity. Sooner or later, however, the welfare of captive insects will come to the attention of legislators in more countries than just the Netherlands. What general criteria could or should be applied? As outlined above, from a purely scientific point of view, little general advice can be given. Perhaps legislation regarding the release of reared or bred insects, however produced, might be easier to address.

Even if not entirely logical or even enforceable, a law can act as a signal to make people think and might thereby contribute to awareness of the need to respect nature in general and individual organisms in particular. But if anything well-considered is to be done with regard to legislation, it will require an interdisciplinary approach involving the humanities as well as sciences.

3.14 Some Conclusions and Perspectives

In view of the worldwide general decline of insect species (Sánchez-Bayoa and Wyckhuys 2019) and numbers due to human destruction of natural habitats, land use change, water and air pollution, use of herbicides (reducing hostplant availability) and insecticides (which always kill many nontarget species) and even genetically engineered insects, when you take into account global climate change as well, discussing welfare and husbandry of the *relatively* few insect species humans keep in captivity may seem trifling, even irrelevant. Moreover, very large-scale uses of insects without defensible utilitarian justifications appear to be rare.

There are plenty of research deficits (cf. van Huis 2017), and some of the common general problems (inbreeding depression, diseases, environmental safety, etc.) of the mass breeding industries for edutainment, pest management, food, feed and pollination should be tackled cooperatively.

Be that as it may, faced with evaluating welfare of insects in captivity, we find that their vast diversity, uncertainties regarding susceptibility to stress and pain and widely divergent attitudes of people force us to conclude that any attempt to lay down general 'rules' is practically impossible. In the foreseeable future, there will be no consensus on how we should address insect welfare. We have focused here on the intrinsic peculiarities of insects which affect their keeping in captivity—in part because, for a critical assessment about how they should be kept, there are few if any data available for evidence-based analyses. Theoretical discussions can and should be pursued—but even if we had relevant data, we believe we would still face ethical dilemmas and have to go into value theory (axiology). As De Goede et al. (2013: 241) comment '. . . the idea that we are required to give insects moral concern by analogy with "higher animals" may be hard to accept. We therefore argue that not only scientific evidence, but also consensus on the moral status of insects is needed'. Such complexity requires holistic approaches capable of including such issues as integrity (Singer 1975; Regan 1983), consciousness, mind and even spirituality, as well as systems ecology (e.g. Capra and Luisi 2014). Some of these and other ethical challenges are well reviewed by Röcklinsberg et al. (2017).

Theoretical discussions aside, the reality is that with the massive and continuing growth in the 'human enterprise'—encouraged by global economic expansionism and many religious traditions alike—we now face a desperately urgent need to reassess our relationship with 'nature'. Pragmatism and education (Mather 2011) are probably now more effective than ethics in discovering or rediscovering that respect for nature and all life forms is almost certainly the only secure long-term 'solution'. For example, although Adamo (2016) recently concluded that the evidence that insects experience pain is 'weak' (but see also Klein and Barron 2016), Lockwood (1987, 1988) counselled that we 'refrain from actions which may be reasonably expected to kill or cause nontrivial pain in insects when avoiding these actions has no, or only trivial, costs to our own welfare'. Lockwood went on to quote Robert L. Rabb (in Perkins 1982): 'The use of [technological] power is a tremendous responsibility and must be done without arrogance and with a subtle sensitivity, if not a reverence, for the value of all life'.

We conclude that our general attitude towards insects as 'living things' is crucial, regardless of whether the insects are in captivity or not. Perhaps an excellent step would be to create an international Insect Welfare Charter—a framework that could be used to evaluate our current and future 'handling' of insects, based on species-appropriateness and respect towards all organisms and considering environmental issues, too. It could, at least, also generate more respect for insects and the ecosystem services they provide and would perfectly complement the EU's Charter of Invertebrates (Council of Europe 1986).

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Chapter 4
Welfare of Managed Honey Bees

Claudia Garrido and Antonio Nanetti

Abstract Honey bee (*Apis mellifera*) colonies are "superorganisms". Individual bees do not display the complete behavioural and ecological range of the species. With its caste structure and division of labour, the colony acts as a functional entity. These social insects are in tight relationship with the environment, which they exploit usually in a symbiotic food-for-pollination exchange. From plants, they draw nourishment for immediate use and to build stores. As a reared animal, *A. mellifera* has spread far beyond its areas of origin, now living in all inhabited continents. This dispersal made them confront novel stressors, like unsuitable environments and management practices or new pathogens and pests. The severity of these factors extensively obliterated the wild honey bee population in many areas of the world, where the species survives only thanks to domestication. Most of the present scientific knowledge on honey bees is based on managed colonies. This superorganism has high resilience against disturbances, which probably led beekeepers to overlook the colony welfare for long time. Nevertheless, increasing importance is now attributed to honey bee health, also for its economic impact on the honey crop and other productions. Multifaceted is the relationship between honey bees and agriculture. The latter is at the same time a source of food and of hazardous agrochemicals and a factor for the loss in floral biodiversity, which may be detrimental to colony fitness. Nutritional demands of honey bee colonies are a subject of increasing research interest. Global warming is potentially producing a mismatch between honey bee colony development and plant phenology. In addition, it changes the equilibrium with important honey bee pests. This stresses the need of a holistic perspective on the welfare issue.

Honey bees are eusocial insects. Their colonies typically consist of ten to thirty thousand adult worker bees, the queen, drones, brood and stores. A single member of

C. Garrido (✉)
BeeSafe, Leverkusen, Germany
e-mail: claudia.garrido@bee-safe.eu

A. Nanetti
CREA Research Centre for Agriculture and Environment, Bologna, Italy

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the colony does not express the whole range of behaviour and ecology of the species; it is the colony that gives the whole picture. Within the colony, the division of labour is an outstanding property of the adult individuals: the queen and drones are the reproductive animals, but they depend on the workers (i.e. sterile females) for food and defence.

Because of their sociality and this multilevel organisation, honey bee colonies are considered "superorganisms". A superorganism represents a reproductive, self-organised unit: reproductive animals (queens and drones) and workers depend on each other. Queens and drones monopolise reproduction, while the workers' tasks are brood care, defence and foraging (Cremer and Sixt 2009). Workers display an age-dependent polyethism: young workers stay within the nest and care for the brood and the queen, clean the nest or build combs. Older worker bees defend the nest entrance and eventually get out for foraging. Important physiological changes (as gland activity) accompany these different tasks (Winston 1987).

The life history of the superorganism honey bee colony differs from the individual members. The colony is perennial, even if the workers are not. Its "birth" is the moment when a swarm gets established at a novel nest. In successive generations of workers, the colony grows and survives several years. The workers have a polymorphism of ageing: due to physiological adaptations, they were estimated to live 14–50 days in summer and 154–170 days in winter (Moritz and Southwick 1992). The queen is the only individual persisting over the years. The genetic integrity of the colony depends on the queen: when she dies, it is disrupted, which represents the death of the colony. However, the maternal genes survive partly with daughter queens that may be reared from remaining eggs.

4.1 How the Concept of Superorganism Influences Honey Bee Welfare

The concept of the superorganism does not apply to all aspects of honey bee biology. Thus, it is helpful to understand the different levels considering aspects of honey bee welfare: the adult bees, the brood (i.e. preimaginal/juvenile individuals) and the colony as a whole. These different levels are interconnected and regulate each other.

Due to the complexity of the superorganism, honey bee colonies have a major capacity for buffering stressors (Straub et al. 2015). As long as the functionality of the colony and the reproductive female is not affected, the integrity of the colony remains (Fig. 4.1). Negative effects of management practices, parasites and diseases or agrochemicals are mitigated and often not immediately apparent. In this context, it is important to consider the different levels and the complexity of the colony. Due to the interconnectivity and self-regulation of the superorganism, small impacts like little pollen availability in spring may lead to carry-over effects and colony losses over winter (Requier et al. 2016). Single causes for disturbances or colony mortality

Fig. 4.1 The queen (in the center surrounded by workers) guarantees the genetic integrity of the honey bee colony. She is the only reproductive female but depends on the sterile workers for brood care, colony defence and foraging (Photo: Garrido)

are difficult to identify. Most probably, different factors act together and add up to the visible effect of colony death or weakening.

The Western honey bee, *Apis mellifera*, has spread beyond its areas of origin, and it is used for beekeeping worldwide. Though other bee species are used for honey production (the Eastern honey bee *A. cerana* or some stingless bees), *A. mellifera* is the most used bee species in human care. Management practices have been developed to increase productivity for honey and other hive products as well as pollination services. Due to the mentioned buffering capacities of the colony, adverse effects of these procedures are often neglected by beekeepers.

Despite the resilience of honey bee colonies, high colony mortality occurred in the past years (Genersch et al. 2010; Brodschneider et al. 2016; Traynor et al. 2016; Jacques et al. 2017). Different factors have been identified as causes, such as parasites (mainly the mite *Varroa destructor*), intense agriculture by means of lack of foraging plants and agrochemicals or the effects of global warming (Potts et al. 2010; Nanetti et al. 2014). The synergies of these factors are poorly understood until now. Some evidence exists that colony losses depend largely on control of the main honey bee parasite *V. destructor* and associated viruses (Genersch et al. 2010; McMahon et al. 2016), but also the wintering conditions, starvation and queen failure are reported as causes (Lee et al. 2015b). However, only recently efforts have been made for maintaining the welfare of the colony and the sustainability of beekeeping practices instead of avoiding the breakdown (Panel on Animal Health and Welfare (AHAW) 2016).

In this context the superorganism concept is very useful: the buffering capacity of a honey bee colony depends on different factors that can change depending on the season, the age, the size of the colony, etc. (Straub et al. 2015). Understanding the most prominent drivers for honey bee health and how they interact in the complex system superorganism is key for maintaining the welfare of managed honey bee colonies and, by this, their populations and productivity.

4.2 Domestication of the Superorganism Honey Bee Colony

Apis mellifera, the Western honey bee, is the most known and managed pollinator worldwide (Potts et al. 2010). Its distribution originally was limited to Europe, Africa, Central Asia and the Middle East. In this distribution range, 26 subspecies are deemed to occur. However, the most preferred by beekeepers are two of European origin: *A. mellifera carnica* and *A. m. ligustica*. These two subspecies are traded worldwide and have replaced the naturally occurring subspecies in some regions (Meixner et al. 2015).

Beekeeping as it is known today goes back to Lorenzo Langstroth, who discovered the distance between two combs that the bees do not close: the so-called bee space. This space is enough to let two bees on opposite combs pass each other. The discovery was the starting point for modern beehives with movable frames. By this, it was possible to perform a variety of management operations formerly not possible: inspections comb by comb, swarming control, honey harvest without destroying the nest, etc. A big step towards higher productivity was also the separation of honey supers and brood chambers: a grid between these spaces prevents the queen from laying eggs in the honey super. Therefore, this space remains without brood, which facilitates honey harvest and promotes qualitative honey production.

Honey bees are one of the most studied insects. Yet, most knowledge derives from managed honey bees. Differences to feral colonies without any management are largely unknown. There is only scarce knowledge about how much management techniques affect honey bee health. One of the major transitions for honey bee colonies is managing the nesting conditions. Feral colonies nest several hundreds of metres apart from each other, while beekeepers crowd hives in apiaries. The extent of this crowding depends on the local uses: from massed apiaries in agricultural landscapes for pollination purposes to a few colonies on hobbyists apiaries. Feral colonies, however, nest in much wider distances: recent data from Arnot Forest (NY, USA) show densities of ca. 1 colony/km^2 (Seeley et al. 2015). The nearest distance between two colonies was 850 m, instead of less than 1 m on apiaries (Seeley and Smith 2015).

This crowding affects colony behaviour and health: the risk of having honey stolen by bees from other colonies (robbing behaviour) during periods of low nectar flow, of drifting between colonies (i.e. homing errors) and of parasite transmission is increased on apiaries (Pfeiffer and Crailsheim 1998; Frey and Rosenkranz 2014; Seeley and Smith 2015). To avoid negative effects of crowding on honey bee welfare, already small measures like dispersing hives with about 30 m distance could help (Seeley and Smith 2015). For developing practicable measures for the beekeeping industry, procedures should be tested in smaller pilot studies. By this, a close interaction between beekeepers and scientists is possible. Adapting management practices to honey bee biology in an achievable manner could mean a large step towards higher welfare for managed honey bee colonies. Implementing these operations, however, the productivity has to be kept in mind to make the efforts sustainable.

In addition, the nest itself differs between feral and managed honey bees. The decision for a new nest site is the result of complex feedback mechanisms between individuals of the same colony in wild living honey bees (Seeley 1995). This self-regulation process is interrupted and superseded by beekeeping practices: the colony is transferred into hives according to the beekeeper's convenience and for maximising productivity. For example, honey bees prefer small cavities of 30–60 L (Loftus et al. 2016) in about 5 m elevation from the ground (hollow trees, rock cavities, etc.). In contrast to this, managed colonies are maintained in hives near to the ground for practicability. The hives' volume differs depending on local practices and the different hive systems existing worldwide. However, a common trait is the focus on high honey productivity: hives are enlarged with supers during the season, reaching volumes of 100 L or more.

Recently, the thermic differences between natural nesting cavities in trees and wooden hives have been studied (Mitchell 2016). The author concluded that some behavioural characteristics of managed honey bee colonies may be induced by the less insulating properties of man-made hives compared to tree cavities, which could have consequences for the overwintering performance of colonies. According to this study, honey bee colonies in artificial hives are forced to cluster earlier than they would under natural nesting conditions. This impairment of heat regulation could have a high energetic cost for the colonies and, therefore, affect its integrity as a superorganism in a critical period of the year (Fig. 4.2).

Winter is a major challenge for honey bee colonies, as they maintain activity throughout this period (Döke et al. 2015). Depending on the period and region, the bees may need to maintain the nest temperature tens of degrees higher than in the external environment. In this period they rely completely on their stores and the physiological changes of the winter bees. Excessive energy need for maintaining the thermal homoeostasis of the colony could affect also the physiological stage of the individual bees. However, this relation is still unclear and needs further verification. Mitchell (2016) concluded positive effects on colony survival and lower *Varroa* infestation through better insulation of the hive walls. If this would be confirmed, improving the heat transfer of hive walls should be a practicable measure for beekeepers to enhance colony welfare.

Colony size is discussed controversially as a factor for honey bee health: natural colonies tend to be smaller as they use smaller nesting cavities, leading to frequent swarming. Swarming and small colonies are therefore discussed together as factors for maintaining low parasite levels like *Varroa* infestation (Seeley et al. 2015; Loftus et al. 2016). On the other hand, there is evidence that larger colonies have better

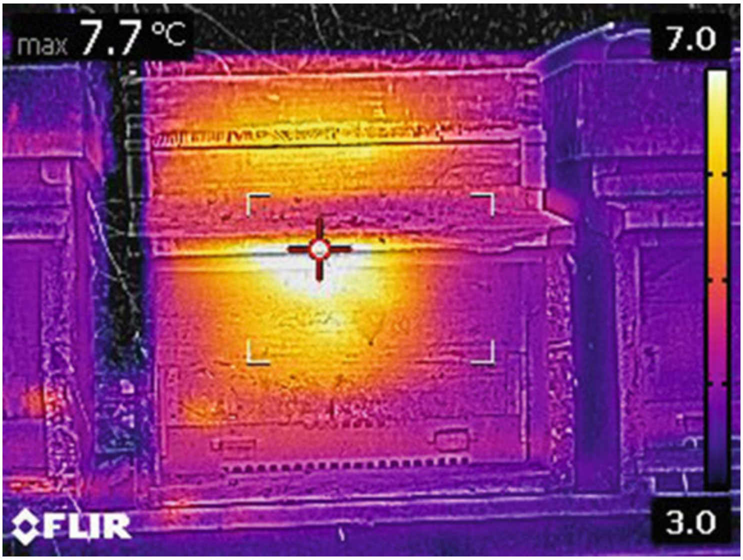

Fig. 4.2 Thermography of a hive in winter. The distribution of the bees in the hive is indicated by the yellow to orange colour. The lower insulation of hives in comparison to natural nesting sites could mean higher energetic for the overwintering colony

overwintering success (Büchler et al. 2010; Döke et al. 2015). However, it is difficult to establish absolute numbers for "small" or "large" colonies. There may be a trade-off between colony size, swarming frequency, colony homoeostasis and other factors that influence the complex regulation during overwintering (Döke et al. 2015).

4.3 Management Practices Impair Basic Traits of Honey Bee Colonies

Beekeeping also modifies another basic trait of honey bee biology: reproduction. Honey bee colonies reproduce by swarming, which reduces honey productivity. Because of this, beekeepers generally suppress swarming behaviour. This is done by breaking queen cells, giving more room and by selecting stocks with low swarming tendency. On the other hand, there is evidence that swarming has positive effects on colony health: Seeley and Smith (2015) found lower infestation levels with the parasitic mite *Varroa destructor* in colonies that swarmed compared to colonies that did not. Interestingly, this positive effect persisted only in colonies that were not crowded in apiaries, showing the interaction of these two management induced factors on colony welfare.

Indirect evidence on the positive effect of swarming on colony welfare comes from Brazil and South Africa: in these countries, there is a large population of feral colonies and less intensified management in apiculture. The management partly relies on captured wild swarms, and swarm control is not as intense as in Europe or North America (Locke 2015).

Beekeeping practice includes some measures to mimic the swarming in an artificial way: making nuclei (by taking out brood combs, some adult bees and a new queen) or bee packages (only adult bees and a new queen). This is partly done to anticipate swarming behaviour, but it is also accepted as a measure to maintain healthy and productive apiaries and is part of good beekeeping practices (Ritter 2012). On certain occasions though, the bee colonies do not accept the queen introduced during this process. In these cases, the introduced queen is killed by the workers, and they try to substitute this queen by rearing a new one. Understanding the factors influencing successful queen introduction by beekeepers could improve the efficacy of the managed reproduction of honey bee colonies. As the integrity of the superorganism depends on its queen (Moritz and Southwick 2012), this would be a step forward for increased welfare of managed honey bee colonies and maintain practicability as well as productivity for beekeepers.

Superorganisms are usually sessile (Moritz and Southwick 1992). Even if the individual adults are highly mobile, the colony itself stays at a defined place once it is established. The pursuit of high productivity from the beekeepers' side, however, makes them transport the hives to different places. Bee colonies are transported for two reasons: to mass-flowering crops, promising high honey yields, or for pollination purposes. The latter is especially required in the USA for almond pollination. More than a million hives are transported each year to California on large trucks crossing the country (Simone-Finstrom et al. 2016). Bees from migratory colonies have a shorter lifespan and higher levels of oxidative stress than workers at stationary apiaries. This effect is not clearly visible; the colonies in Simone-Finstrom et al.'s study had the same strength in both groups and did not differ in infestation levels with the *Varroa* mite. However, the physiological impact of migratory management had long-term consequences. Interestingly, sufficient floral resources compensated the impact of migration.

In a previous paper (Ahn et al. 2012), the authors found indications that worker bees that were transported over long distances did not fully develop their food glands. This has implications for the next generation of worker bees which would not be cared for properly. Finally, migrating colonies also means strong gene flow between populations, which prevents local adaptations of honey bees and may also increase the spread of pathogens (Seeley et al. 2015; Wilfert et al. 2016).

High productivity may impair honey bee welfare indirectly, via the above-mentioned discrepancies between the natural choices of honey bees and beekeeping practices. The strong focus on honey production and/or pollination services in mass-flowering crops (like fruit orchards, oilseed rape and others) made beekeepers often neglect the honey bee colony's needs. Because of its high resilience in front of stressors, the consequences of these practices are buffered as long as the colony functionality remains unharmed (Straub et al. 2015). However, the boundaries of this resilience seem to be reached: increasing colony losses are observed, mainly in Europe and North America (Genersch et al. 2010; Brodschneider et al. 2016; Traynor et al. 2016; Jacques et al. 2017). The phenomenon is multifactorial, and the factors discussed are pest and pathogens (e.g. the above-mentioned *V. destructor* and associated viruses), as well as environmental factors and genetic diversity and vitality, altogether interacting with each other (Potts et al. 2010).

Thus, little attention is given to the management techniques in general. Recommendations are given for good management practices (Ritter 2012) to keep bees healthy, but the main focus remains on keeping honey bee colonies productive. However, beekeepers would rapidly switch to more sustainable practices, if these stabilise their yields. Adequate dissemination and training are key in this aspect, to establish knowledge-based and practicable techniques. There already are several initiatives aiming at a better connection between science and beekeeping practice. To enhance their outreach, however, more local groups are necessary to build the direct connections. The local or regional connection while implementing improved management practices is important also because of the variability in beekeeping traditions and techniques in different areas.

4.4 Organic Beekeeping: Taking into Account the Superorganism?

Organic beekeepers seek a more sustainable management of their colonies, respecting honey bee biology to a higher extent than do conventional beekeepers. Longevity and health of the colonies are considered more important than honey yields, and they take into account the superorganism honey bee colony. Depending on country, association or guidance used, organic beekeepers have to follow different restrictions. For instance, they may be obliged to rear their own queens or buy them only from other certified organic beekeepers, to have their own wax recycling practice, using only defined materials for the hives or a narrow range of products for colony feeding. However, the differences between organic and conventional apiculture are often not clearly defined. Organic beekeepers pay special attention to the absence of residues in their products. They try to keep their bees in an uncontaminated environment and manage diseases without synthetic products. However, because of the large flight range of honey bees, it cannot be guaranteed that worker bees forage only on organic or natural crops. This flight range can be up to 5–6 km (Seeley 1995), but this full range is used only if floral resources are missing in the nearer surroundings of the hive. A smaller range of 1.5–2 km is assumed to be the average activity range in practice. For treatments against the *Varroa* mite, organic acids or essential oils are widespread also within conventional apiculture. However, organic beekeepers underlie several controls that they correspond to the guidelines of their associations. Therefore, colony welfare may get larger interest in this group.

Not all of the requirements for organic beekeeping are knowledge-based and oriented on colony welfare. An example is the use of queen excluders. Beekeepers use these to separate the honey supers from the brood nest of the colony. It is usually a metal or plastic grid, wide enough for workers to pass but too narrow for the queen. By this, the honey harvest gets easier: no brood combs must be sorted out during the procedure, and the quality of the honey is secured. Beekeepers following highly restrictive rules are not allowed to use queen excluders. Yet, there is no evidence that this disturbs the colony development or has any other effects.

Also organic beekeepers manage their colonies for honey production and pollination services. Therefore, the colonies are transported to sites with promising honey crops and are located in crowded apiaries. Both have been discussed above as possibly detrimental to colony health (Seeley et al. 2015; Simone-Finstrom et al. 2016). Requier et al. (2016) found that honey harvest in spring negatively affects colony development as it removes stores in a critical period of the year. These practices, however, are substantial for beekeepers to get their honey harvest, which often cannot be obtained at a single place. In addition, spring honey is often the most important crop. Mitigation measures like providing floral resources near the apiary after the harvest or leaving part of the honey in the colony could attenuate this impact. The aim should be to find a balance between the needs of beekeepers (i.e. productivity) and those of the honey bee colonies.

In summary, though organic beekeeping aims more for animal welfare than conventional beekeeping, there is urgent need of further knowledge-based development of the concept. Beekeeping is highly influenced by traded knowledge and experience. Though this often leads to good results, emergent stressors like habitat loss or global warming are only poorly addressed by current organic (or conventional) management practices. Approaching these issues could lead to better animal welfare for honey bee colonies independent of the type of management.

Another management attempt for increasing honey bee welfare is selection programmes. In Europe and North America, these programmes aim to increase the resistance against *Varroa* mites maintaining the productivity with some success (Büchler et al. 2010; Rinderer et al. 2010). Nevertheless, the question remains how these efforts will be transferable for the various different beekeeping realities worldwide. It seems more promising to take a step back and consider honey bee biology first and search for a new equilibrium between honey bee welfare and economic interest of beekeepers. Small changes in beekeeping practice may already bring good results:

- Spacing up apiaries to avoid crowding (Seeley and Smith 2015)
- Adapting the artificial hives to natural nesting properties (Mitchell 2016)
- Maintaining good apicultural practices for controlled colony reproduction
- Providing sufficient forage to counter the negative effects of migration practice (Simone-Finstrom et al. 2016)

Monitoring and adapting the measures under different beekeeping practices would improve the sustainability of this process. Most importantly, beekeepers should be integrated into these programmes at eye level to ensure the sustainable implementation of the measures.

4.5 Honey Bee Health

Animal welfare in managed honey bees is usually addressed as “bee health” in scientific and public discussions. The issue was raised mainly after an increase of winter colony losses in Europe and North America (Genersch et al. 2010; van der Zee et al. 2014; Traynor et al. 2016). Colony losses have multiple causes that interact with each other (Genersch et al. 2010; Potts et al. 2010) and may include different bias depending on the management system and the local uses. However, parasites and pathogens play a prominent role in the scientific debate.

Honey bees host a wide range of parasites and pathogens. Two of them stand out because of their major impact: the parasitic mite *Varroa destructor* and the endoparasitic fungus *Nosema ceranae*. Both parasites are new for European Honey Bees, originating from their Asian sister species *Apis cerana*.

The mite *V. destructor* is considered the most challenging health issue for *A. mellifera* populations. It is a major problem for beekeeping and is currently present in nearly all countries worldwide. An important effort is given in sustainable control methods. As an ectoparasite, the mite feeds on the haemolymph of its host. The life cycle is divided into two stages: reproduction takes place in sealed brood cells and is closely synchronised with the host development (Garrido and Rosenkranz 2004; Rosenkranz et al. 2010; Nazzi and Le Conte 2016). Within the brood cell, the female mite lays eggs onto the cell walls, which develop in one male and several female offspring. The male mates with its sisters, and eventually the invading mother mite and her fertilised female offspring leave the brood cell together with the hatching bee. Then they spend a phoretic phase on adult bees until entering new brood cells for another reproductive cycle.

For feeding, the invading female pierces a hole on the bee pupa’s cuticle. Both mother mite and offspring feed on the haemolymph at this place (Donze and Guerin 1994). In the wounds of the feeding site, several bacteria are found, and the mites often occur together with virus infections. The damage caused by *Varroa* mites is attributed both to the direct effect of the feeding activity and indirect effects by other parasites vectored by the mite (Genersch et al. 2010; Rosenkranz et al. 2010; Annoscia et al. 2012; McMenamin and Genersch 2015).

At colony level *Varroa* infestation affects honey bees mainly by reducing its reproductive capacity: drones infested during their development have a lower chance to mate, and infested colonies form fewer swarms (Rosenkranz et al. 2010). In managed populations, the economic damage may occur already at infestation levels when clinical symptoms are still not visible. Low infestation rates may already affect colony growth and, by this, the honey yield. Clinical symptoms of irreversible colony damage are scattered brood, crawling or crippled worker bees and sudden reduction of the bee population (Shimanuki et al. 1994). However, these symptoms may appear very quickly after a period in which colonies look apparently healthy. This is the case especially in late summer because the *Varroa* population still increases, while the colony strength is already decreasing before winter (Fries et al. 2003). The damage threshold differs depending on different factors like colony strength, brood activity of the colonies, the phenological stage of the colony and the presence of secondary infections like viruses (Rosenkranz et al. 2010). *Varroa* mites are a substantial driver of recent colony losses in the USA and several European countries (van Engelsdorp et al. 2008; Meixner et al. 2014).

Untreated colonies or the use of inappropriate treatments lead to breakdowns within a few years. Feral populations of honey bees have been mostly eradicated by this parasite because of lack of treatment. Therefore, it is important to control infestations with this parasite before reaching the irreversible damage level. There is a series of different substances that are used for control of *V. destructor* in managed honey bee colonies. Synthetic acaricides are usually easy to apply and can be used without knowledge of the parasites' biology. On the other hand, if not used correctly, there are several problems associated with these substances:

- They leave persistent residues in the hive products (Wallner 1999).
- They may harm the bees exposed to multiple compounds by these residues in the wax (Rosenkranz et al. 2010).
- *Varroa* mites may become resistant to these compounds (Milani 1999).

Due to these problems, substantial effort has been made to develop alternative treatments with natural substances. These include essential oils (Imdorf et al. 1999) and organic acids such as formic acid and oxalic acid (Nanetti et al. 2003). These substances have sufficient efficacy against the parasite paired with a low risk of resistance and residues in hive products. However, also with these treatments, a correct and integrated use is necessary; several recommendations have been suggested (Rosenkranz et al. 2010). In addition, the beekeeping industry, contrary to other areas of livestock production, is in the happy situation that organic treatments have higher efficacy than synthetic acaricides. As residues under correct use are not an issue, hive products are not falsified and correspond to high-quality standards. Organic beekeepers are obliged to use organic acids or essential oils against varroosis, and an increasing numbers of conventional beekeepers follow to maintain the quality of their products.

Feral populations of *A. mellifera* usually die within a few years without treatment. However, there are several naturally surviving populations worldwide (Locke 2015). Until now, no common resistance or tolerance factor for all these populations has been found. Some characteristics of these surviving populations (like small colony size or reduced *Varroa* reproduction) correlate significantly with survival in some populations, but they do not in others (Locke 2015). This may be due to differing environmental conditions that influence both the infestation level and the threshold a colony tolerates (Meixner et al. 2014).

Deformed wing virus (DWV) causes one of the symptoms of severe varroosis, the crippled wings (Fig. 4.3). In the absence of the mite, DWV only rarely leads to

Fig. 4.3 The *Varroa* mite transmits the deformed wing virus (DWV), which leads to wing malformation and shorter lifespan

symptomatic disease (de Miranda and Genersch 2010). Recently data show that *Varroa* mites are responsible for the dispersal of this virus and that migratory management of honey bee colonies fuels the epidemic (Wilfert et al. 2016).

The clinical symptoms are caused by the parasitic mites present during the pupal development. These bees usually die shortly after hatching (acute infection). When the parasitic mite transmits DWV to adult bees by feeding, the virus causes a chronic infection, which leads to sublethal symptoms (de Miranda and Genersch 2010). However, the presence of DWV in overwintering colonies has been shown to cause winter mortality even after *Varroa* treatments (Highfield et al. 2009). The interaction between mite and virus leads to immunosuppression of the host (Nazzi et al. 2012). DWV under these conditions replicates rapidly, leading to colony collapse in late summer.

Therefore, early control of *Varroa* mites, before high infestations and DWV loads build up, is necessary respecting the local conditions and beekeeping practices. Because of the common practice of crowding colonies, the danger of reinfestation must be considered (Greatti et al. 1992). In colonies with a high density of neighbouring colonies, *Varroa* infestation reaches high levels (Frey and Rosenkranz 2014). The authors suggest regionally coordinated treatment concepts for avoiding reinfestations. Assuming that DWV infections affect colonies with high *Varroa* infestations, these measures would provide protection from DWV and other secondary infections. For a more successful implementation of such procedures, beekeepers should be integrated into their development.

Also the endoparasitic microsporidian *Nosema ceranae* is thought to originate from the Asian honey bee *A. cerana*. Natural infections in *A. mellifera* are known only since 2005 (Higes et al. 2006), though it was suspected to be infective for this bee species earlier (Fries 2010). More recently, it was found that it must have been present in Italy since at least 1993 (Ferroglio et al. 2013).

The emergent disease with *N. ceranae* does not show clear clinical symptoms and is a widely underestimated health issue for managed honey bees. Apparently, it is better adapted to warmer climates where it produces bigger health issues for managed honey bees (vanEngelsdorp and Meixner 2010). It is widely believed that the emergent disease has superseded the former pathogen *N. apis* in most European countries (Meixner et al. 2014), though this point remains controversial (Martín-Hernández et al. 2012).

N. ceranae infections do not show any seasonality as the pathogen can be found throughout the year in adult bees (Martín-Hernández et al. 2007). The infection occurs by feeding, ingesting the mature spores. This probably happens via cleaning or trophallaxis (i.e. food exchange between bees). The spores germinate in the midgut and infect the epithelial cells. This occurs by injecting the sporoplasm into the host cell via the ejected polar tube. Within the cells, *N. ceranae* multiplies during a merogonic phase and finally forms new spores that leave the host cell.

Infected bees present degenerated ventricular epithelial cells; such lesions have been observed both in worker bees and queens. At colony level, the most evident symptoms are the depopulation of the combs and an unbalanced proportion between the number of bees and brood cells (Higes et al. 2008). The prevalence of *N. ceranae* in forager bees is the highest, as well as the infection intensity (Smart and Sheppard 2012). Therefore, in infected colonies, mainly young bees can be observed on the combs, due to the continuous loss of infected older bees. The colonies are weakened, and eventually they collapse, as recorded in Spain in 2004 and 2005 (Higes et al. 2008).

Until now, there is no registered treatment against *N. ceranae* in Europe. The antibiotic fumagillin was authorised in Spain and other EU countries as an emergency treatment after the severe colony losses in 2004 and 2005 (Higes et al. 2011). In North America, this substance is used to treat nosemosis and is found to have high efficacy (van den Heever et al. 2016). However, the use of antibiotic substances may leave residues in hive products and induce resistance. In a recent study, fumagillin was found to be effective against *N. ceranae* only for a short period of time, making regular treatments necessary (Little et al. 2016). In Europe, antibiotics are not allowed in apiculture.

Alternative treatments have been studied like thymol (van den Heever et al. 2016), garlic (Nanetti 2009), other plant extracts (Porrini et al. 2011) or oxalic acid (Nanetti et al. 2015). Control measures are urgently needed, though the disease is often neglected by beekeepers. However, *N. ceranae* causes energetic stress to infected workers, causing hunger (Mayack and Naug 2009). The infection decreases the lifespan of individual bees, and they begin to forage earlier. This goes together with atrophy of the hypopharyngeal gland (which produces larval food). Therefore, together with the decreasing number of adult bees, this leads to insufficient care of the brood. This affects the self-regulation mechanisms of the colony and may enhance the consequences of the disease. Recently, it has been found also to impair the homing ability of foragers (Wolf et al. 2014). All these factors together considerably interfere with the functionality of the colony and may be the cause for deficient colony development.

In addition, it has been found that infection with *N. ceranae* decreases the self-defence of honey bee colonies against *V. destructor* (Bahreini and Currie 2015). This interaction between these two important diseases is still poorly understood. *N. ceranae* may suppress deformed wing virus (DWV), a virus associated with *V. destructor* infestation (Doublet et al. 2015).

4.6 Further Factors Influencing Honey Bee Health

Honey bee health depends on a variety of factors and cannot be reduced to single parasites or pathogens. For instance, in Europe several populations exist that are not treated against *Varroa* mites. Interestingly, these populations lose their survival ability outside their native conditions, suggesting an interaction between genotype and environment (Meixner et al. 2015). Genetic variability of honey bees has been reduced due to beekeeping. In Western Europe, the native subspecies of *A. mellifera mellifera* has been mostly superseded by introduced stock with more convenient properties for beekeepers (vanEngelsdorp and Meixner 2010). These stocks could be gentle and have higher productivity or earlier timing of foraging activity in spring. In the Americas, honey bees are not native, and the stock descends from introduced colonies. It has been found that in the USA, only 500 breeder queens provided progeny for commercial apiculture. Low genetic diversity, on the other hand, could be responsible for higher susceptibility to pathogens (Potts et al. 2010; vanEngelsdorp and Meixner 2010). The high resilience of honey bee colonies as a superorganism (Straub et al. 2015) may have buffered for a long time the adverse effects of these practices. Recently, more effort is done to address problems in honey bee health with a more holistic approach to reflect the complex situation in honey bee colonies (Potts et al. 2010; Meixner et al. 2015).

The social structure of honey bee colonies adds a level of complexity to the response against parasites. As a superorganism, the colony as a whole has mechanisms to fight diseases, usually referred to as “social immunity”; using immunity in the general sense of combat parasites (Fig. 4.4, Cremer and Sixt 2009). This means that in addition to physiological, individual responses (e.g. humoral responses, wound healing, etc.), the workers cooperate to exclude or fight diseases in the colony. These mechanisms have been paralleled with those of long-lived vertebrates and can be considered at three levels (Cremer and Sixt 2009):

1. Border defences
2. Soma defences
3. Germ line defences

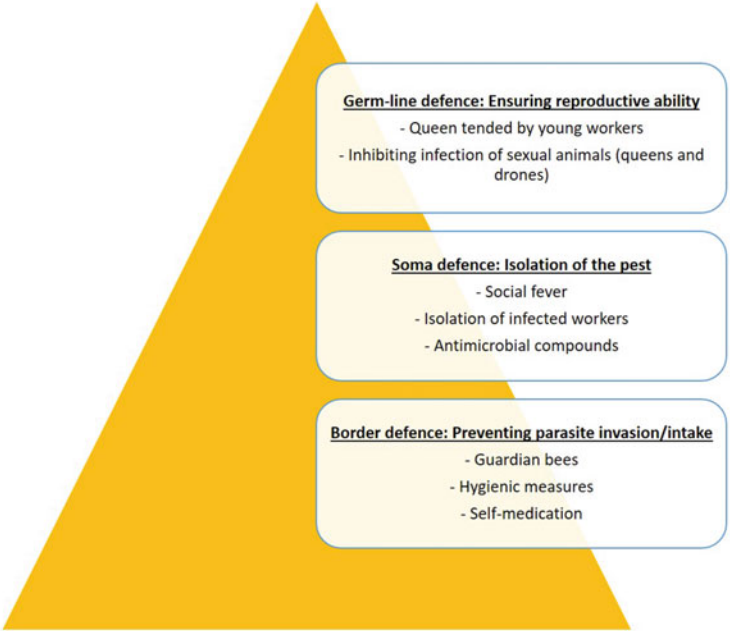

Fig. 4.4 The tiered defence mechanisms of superorganisms like the honey bee colony are usually referred to as "social immunity". More information and examples in the text

Border defences include preventive hygienic measures (cleaning of brood cells, carrying out dead individuals, etc.), hiding from parasites, self-medication [via self-produced gland secretions or foraged hive products as propolis (Simone-Finstrom and Spivak 2012; Erler and Moritz 2015)] and grooming behaviour. Honey bee colonies have guardians preventing predators and parasites from coming into the nest. These workers also recognise their nestmates and prevent foreign workers (that may bring in pathogens) to enter the colony. Honey bee colonies can discriminate between self and non-self (Cremer and Sixt 2009), which is based on the "colony odour". This pattern is maintained via continuous contact with the colony; foragers that deviate too much from this pattern will not pass the guardians at the nest entrance. Thereby, also the introduction of pathogens is prevented.

If parasites or pathogens manage to pass this border defence, soma defence begins. Immediate local actions help to isolate the pest from the colony. An example is the social encapsulation of small hive beetles (SHB) in colonies of the Cape honey bees, *A. m. capensis* (Neumann et al. 2001). This beetle species lives in honey bee colonies and damages them by feeding on brood, pollen and honey. Workers of *A. m. capensis* surround SHB with "cages" of propolis. This process takes several days, during which workers collaborate to guard the beetle until it is safely isolated.

Local responses are often followed by a systemic response like "social fever". By increasing their body temperature simultaneously, many workers cooperate to kill pathogens by heat. This has been found to be effective against chalkbrood, a fungal infection (Starks et al. 2000). Infected colonies increased the brood temperature about 0.6 °C, which represents about 20% of the normal range in which the brood is kept at. This prevents the disease outbreak, which would require chilled brood.

Other soma defence strategies are isolation of infected workers (Drum and Rothenbuhler 1985), recognising and removing parasitised brood, usually called "hygienic behaviour" (Gilliam et al. 1988; Gramacho and Spivak 2003), or antimicrobial compounds in the colonies (Erler and Moritz 2015).

Finally, germ line defence ensures the reproductive ability and, therefore, the fitness of honey bee colonies. The queen is the most long-lived individual of a honey bee colony and spends most of her life within the colony. She is tended by young workers, which have less probability to be infected with diseases. Interestingly, Wang and Moeller (1970) found that workers infected with *Nosema apis* were less likely to attend the queen. It is unknown if this is true also for the emergent *N. ceranae*. However, this behavioural change is attributed to the physiological ageing of infected bees, which is the case for both *Nosema* species (Wang and Moeller 1970).

Hardly any cases of sexually transmitted diseases were observed in social insects, though nearly all parasites could potentially infect reproductive individuals (Schmidt-Hempel 1998). Lately, DWV has been studied in this context (Yañez et al. 2012; Amiri et al. 2016). High DWV titres were found in the endophallus of drones either if collected at their colonies or in drone congregation areas where they mate with the queens. Apparently, infection with DWV does not impede drones from flying to these areas (Yañez et al. 2012). The transmission to the queens during mating flights has been demonstrated by Amiri et al. (2016). Queens develop high-level infections, which can lead to queen failure and, as a consequence, to the death of the superorganism.

The multiple factors discussed in this section support the view that health in honey bees does not equal the absence of disease. It is rather an interaction of parasites, responses of the colony and other factors like management and nutrition. Therefore, the way to increased honey bee health and reduced colony losses may be achieved via a compromise between honey bee biology and beekeeping interest in productivity. Surveillance programmes are gaining importance for identifying risks for honey bee health in practical conditions (Lee et al. 2015a). With these data, a more holistic and knowledge-based approach of management techniques could be achieved. In parallel, it is crucial to involve beekeepers in this development by knowledge dissemination and stewardship measures.

4.7 Nutritional Demands of Honey Bees

Freedom of hunger, malnutrition and thirst is one of the core principles of animal welfare as the World Organisation for Animal Health (OIE) defines it. Honey bees forage mainly on nectar and pollen. The nutritional needs of adult bees and larvae are mostly covered by these two resources (Winston 1987). Larvae and adults, as well as workers, queen and drones have somewhat different nutritional demands. In this aspect, the complexity of the superorganism becomes of additional importance: the different levels (whole colony, adults and larvae) interact with each other. Disorders at one level affect the others and may have long-term consequences (Brodschneider and Crailsheim 2010; Requier et al. 2016). Foraging and brood care are adapted according to the nutrient supply from foragers and the requirements of the colony by complex feedback mechanisms (Seeley 1995; Brodschneider and Crailsheim 2010). Therefore, nutrition gets an additional importance for colony welfare, maintaining the integrity of the superorganism.

Nectar is the main source of carbohydrates, which forager bees collect from flowers. But also honeydew (secretions of aphids and other plant feeding insects) or secretions from extrafloral nectaries are used as sources of sugar. Floral nectar contains 5–80% sugars. How attractive different sugar concentrations are for foraging honey bees depends on the resource abundance: with a rich availability of flowering plants, nectar with high sugar concentration is preferred. As soon as the quantity decreases, foragers will collect less concentrated nectar (Seeley 1995).

Nectar processing starts during the homing flight and continues in the nest. The water content is reduced during this process down to 16–20%, and different enzymes are added during converting nectar into honey. This hive product is the long-term reserve for the colony. In temperate regions, carbohydrates are needed in large amounts during overwintering: maintaining the temperature in the cluster consumes energy, which is provided by honey stores.

Both adults and larvae depend on the presence of honey stores (Brodschneider and Crailsheim 2010). Adult bees feed on the honey stores before foraging flights, and about 18% of worker brood food consists of sugars.

Breeding colonies consume about the double of honey during winter compared to broodless colonies (Seeley and Visscher 1985). Therefore, breeding activity in the colder months can be a risk for winter survival of the colonies if the stores are insufficient. As honey stores are mostly removed during the harvest, beekeepers integrate them with sugar syrup or candy before winter. However, starvation is still a common cause of winter colony losses (Brodschneider and Crailsheim 2010; vanEngelsdorp et al. 2010). This situation is usually avoided by beekeepers but may occur in long winters or if colonies lose the contact to the food stores.

Pollen is the only source of proteins of the honey bee colony and contains also lipids, minerals and vitamins. The pollen is processed to “bee bread” in the colony, adding enzymes and anti-germination compounds (Winston 1987). By this treatment, bee bread is protected from fermentation and is better digestible by the bees. Apparently, lactic acid bacteria are involved in this process, improving the nutritive value for bees (Vásquez and Olofsson 2009). Other essential compounds of pollen are lipids, minerals and vitamins; their ability influences both larval development and behaviour like the start of foraging (Winston 1987; Brodschneider and Crailsheim 2010).

Pollen consumption depends on the age of worker bees and is related to the division of labour (Haydak 1970). Nurse bees feed more on pollen than forager bees; this relates to their brood feeding activity and well-developed hypopharyngeal glands (which produce larval food) and proteolytic enzymes in the midgut. The development of these glands strongly depends on the quality of the pollen supply. Multifloral mixes outperform most monofloral supplies, producing highest gland volume, indicating the optimal development of the gland (Omar et al. 2016).

Larvae and adults have different protein requirements, as well as between workers, queens and drones. Larvae are fed with special brood food, built by the hypopharyngeal glands and mandibular glands of nurse bees. Worker, drone and queen larvae receive larval food with a different composition. Queen larvae receive high quality and high amounts of food (royal jelly) during the complete larval development. This determines their development into queens and as adults; they are fed by workers with brood food (Winston 1987). On the other hand, worker larvae receive brood food of a different composition ("worker jelly") in much smaller amounts, and starting from the third day, this food is mixed with pollen. Finally, drones are fed again with differently composed brood food in big amounts mixed with pollen for older larvae. Adult drones are fed by the workers for the first days and continue consuming pollen in the first days of their lives (Szolderits and Crailsheim 1993).

The principal needs for macro- and micronutrients of honey bees have been investigated mainly by deprivation experiments (Haydak 1970), and most research has been related to carbohydrates and proteins. Much less is known about the requirements for other nutrients such as lipids, minerals and vitamins (Brodschneider and Crailsheim 2010). Recently, the mineral foraging of honey bees has been studied (Bonoan et al. 2016). The authors found strong preferences for sodium-enriched water throughout the season, but seasonality in calcium, magnesium and potassium. The latter substances are common in pollen and were foraged for in water mainly in autumn, when pollen was scarce. Therefore, foraging for them in water happens mostly in autumn when pollen availability decreases. Honey bees need water for thermoregulation and brood rearing and have a strong preference for "dirty" over pure water. They take it from dung piles, puddles with rotting material and similar sources (Kiechle 1961). Bonoan et al. (2016) argue that honey bees are able to selectively forage for minerals required by the colony and by this compensate deficiencies in pollen and nectar through water foraging.

Hendriksma and Shafir (2016) have studied similar compensation for lacking nutrients: after feeding an amino acid deficient diet, they observed that bees preferred a complementary diet to balance the diet of the colony. Though specialised on a floral diet, honey bees are generalists concerning the flowers they visit and forage on a big range of plants. By this, they use the resources in their flight range and during the whole activity period most effectively. This behaviour is regulated by complex feedback mechanisms in the colony (Seeley 1995), according to needs of the colony (amount and type of brood, phenology of the colony, etc.) and resource availability (i.e. abundance and nutritive quality of flowers). In this context, honey and nectar stores are a buffer between the fluctuating resource availability and the more stable requirements of the colony. There is a constant information flow in honey bee colonies about supply and demand of nutrients, enabling foragers to switch accordingly (Kiechle 1961; Seeley 1995). This information transfer occurs by trophallaxis, a social exchange of food between the members of the colony. Food quality seems to influence the foraging preferences, though inexperienced foragers do not have innate preferences for certain flowering plants (Cook et al. 2003).

4.8 Malnutrition as a Risk to Colony Welfare

Due to colony losses and increased health issues, research interest in honey bee nutrition has grown (Brodschneider and Crailsheim 2010). Managed colonies are often brought to agricultural landscape both for honey harvest and pollination services. With the intensification of agriculture, the variety of flowering plants in the landscape has decreased substantially. It was suggested that nutritional stress by decreasing foraging resources could interact with emergent diseases, causing the increased frequency of colony losses in Europe and North America (Naug 2009). This is supported by different data showing the positive influence of floral variety on bee health and colony survival (Requier et al. 2015b, 2016; Vaudo et al. 2015; Alaux et al. 2017). Malnutrition, on the other hand, leads to shorter lifespans, development disorders, impaired learning and reduced immunocompetence with consequences for the integrity of the colony (Alaux et al. 2010; Brodschneider and Crailsheim 2010; Arien et al. 2015), affecting, therefore, the integrity of the superorganism.

Mass-flowering crops like oilseed rape or sunflower are very attractive to honey bee foragers. However, the nutritional quality of different flowering plants differs, and honey bees tend to forage on a large variety of different plants if possible (Requier et al. 2015b). Moreover, it was shown that the quality of food, not the caloric intake, influenced ageing and lifespan of honey bee workers (Paoli et al. 2014). Requier et al. (2015a, b) support this: in spring, oilseed rape pollen was under-represented in honey bee colonies in intensely managed agricultural landscape. Pollen foragers preferred plants from nearby seminatural habitats offering a bigger diversity of plants. The apicultural practice of transporting honey bee colonies to mass-flowering crops for honey or pollination purposes could therefore negatively impact the nutritional stage of the colonies if areas with bigger plant diversity are absent. This may lead to malnutrition and could further affect colony welfare without being noticed by the beekeeper.

Requier et al. (2016) give an indication for this assumption: they associated the seasonal disruption of foraging resources and colony health. The decline in pollen collection resulted not only in brood reduction but also in higher levels of *V. destructor* infestation and higher seasonal and winter losses. The relationship between nutrition and disease was already stated earlier for chalk brood, a fungal disease (Gilliam et al. 1988). A diet with high floral diversity enhances the immune responses of honey bees (Alaux et al. 2010), at different levels of physiological defence (hemocytes and enzymatic activity). On the other hand, diseases can cause energetic stress and precocious foraging as shown for *N. ceranae* infection (Mayack and Naug 2009). Increased hunger induces a higher consumption of sugar and makes infected workers beg for food more often than uninfected nest mates (Naug and

Fig. 4.5 Honey bee colonies are fed with sugar syrup or candy over winter to replace the honey harvested by the beekeeper

Gibbs 2009). Due to this influence of nutrition on the health status and development of honey bee colonies, nutrition can be considered a key factor for maintaining the overall welfare of the superorganism.

In managed colonies, a nutritional shortage is often met with supplements (Fig. 4.5). Sufficient feeding of sugar syrup before winter secures overwinter survival. On the other hand, proteic supplements are often insufficient. This is due to lacking knowledge about nutritional demands and essential nutrients for honey bee colonies at different stages of their development. In addition, supplements may harm bees: soybeans are used in some supplements as pollen substitutes, but 40% of soybean sugars are toxic to bees (Brodschneider and Crailsheim 2010). Commercial prebiotics and probiotics could also increase the susceptibility for *N. ceranae* (Ptaszyńska et al. 2015), having the opposite effect as intended. Until now, natural forage seems to be the only resource guaranteeing adequate nutrient supply and colony health. This was confirmed also in another study (DeGrandi-Hoffman et al. 2015a): colonies provided with natural pollen had higher overwintering survival, lower pathogen loads or queen losses than colonies fed with pollen supplements. In addition, sufficient natural forage seems to mitigate negative management effects like migration over long distances (Simone-Finstrom et al. 2016). Variate and high-quality nutrition, therefore, may also buffer negative effects of apicultural practices.

The available information suggests a strong impact on different levels concerning the health of managed colonies. Nutrition may be a neglected factor in recent colony losses. To support honey bee colonies in their defence against biotic and abiotic stressors, provision of high-quality (i.e. variate) natural foraging resources is necessary. Increasing floral resources in the landscape, however, may be achieved only by involving different stakeholders such as beekeepers, farmers, municipalities, etc. Therefore, a concerted effort is necessary to improve honey bee nutrition.

4.9 Agriculture: A Double-Edged Sword

Honey bees are the best-known pollinators and managed by humans both for honey and pollination services. The relationship between beekeeping and agriculture is traditionally close, due to the dependence of both fields on the other. On the one hand, agriculture relies for many crops on the pollination services by bees. On the other hand, beekeepers rely on agricultural, mass-flowering crops for honey harvest (e.g. oilseed rape or sunflower). To other crops like fruit orchards, honey bee colonies are brought mainly for their pollination services. Pome and stone fruit mostly are highly dependent on insect pollination, but beekeepers usually harvest no honey from these crops. However, the colonies use pollen and nectar for their spring development.

Despite this interdependence between agriculture and beekeeping, the relationship has become conflicting in the past two decades. The reason for this was increased colony losses that were attributed to pesticide use, mainly to neonicotinoids. Neonicotinoids are systemic insecticides, i.e. they are distributed throughout the plant in contrast to topic applications. They are often used as seed coatings, to protect the seedling during its growth. The developing plant absorbs the substance, which then disperses through stem, leaves and flowers. This way of application has created problems for apiculture: abrasion from qualitatively insufficient coatings created contaminated dust, which was blown on neighbouring flowering plants. This happened in several European countries: during maize sowing, dust with high concentrations of clothianidin (a neonicotinoid) were released and deposited on nearby oilseed rape fields. This and similar incidents created high pressure to reassess the use of these substances in flowering crops.

Another point of discussion was guttation water. These are active excretions from plants to maintain the water flow within their body, mainly under cool and humid conditions. These droplets are sometimes used by honey bees as a water source. If the seeds were treated with systemic insecticides, these end up in the guttation droplets in toxic concentrations for honey bees. This was first brought up in lab experiments (Girolami et al. 2009), followed by an intense discussion about the risks for honey bee health. In the following, some cases of intoxication apparently caused by the uptake of these droplets. For winter oilseed rape, this was assessed in the field (Reetz et al. 2016). The main guttation activity in this crop occurred in autumn when the water uptake of honey bee colonies decreases due to lower brood activity. In exposure studies in different crops, the uptake of guttation water was dependent on the distance to the fields: foraging bees usually searched for water only in the next vicinity of the hives. In addition, there was no difference in the overwintering success of exposed and nonexposed colonies. Despite rapid technical solutions for avoiding dust drift and inconclusive results for the risk from guttation water at this point, the discussions led to a ban of three neonicotinoids (imidacloprid, clothianidin and thiamethoxam) and another substance with a similar mode of action and use (fipronil) in Europe. After a moratorium to revaluate these substances in 2013, outdoor uses of the three neonicotinoids were banned in the EU in 2018 (European Commission 2018).

After these incidents, much research has been done on acute and sublethal effects of neonicotinoids. The latter relate to the welfare of honey bee colonies: effects that do not kill colonies but weaken them by disrupting physiological and/or behavioural mechanisms. For instance, there is a negative modulation of an immune transcription factor that controls antiviral defence in honey bees with sublethal doses of clothianidin and imidacloprid (Di Prisco et al. 2013). This led to a replication of DWV, which is discussed as a major factor in colony losses (see above in “Honey Bee Health”). This connection is discussed also in the context of a wider interaction between neonicotinoids and *Varroa* mites (Sánchez-Bayo et al. 2016) which both undermine immunity and, therefore, enhance the susceptibility against virus infections. These authors discuss also the influence of fungicides that inhibit detoxification mechanisms and, by this, increase the toxicity of neonicotinoids to bees.

Other sublethal effects include the reduction of reproductive abilities of drones (Straub et al. 2016), reduced specificity of memory formation after long-term exposure (Williamson and Wright 2013) or physiological changes the queen metabolism (Dussaubat et al. 2016). However, the interactions between these factors and colony welfare remain unclear. In a 2-year study, there was no significant response of honey bee colonies with realistic exposure to neonicotinoids at a landscape level, while there was a substantial risk for wild pollinators (Rundlöf et al. 2015). Recent studies (Tsvetkov et al. 2017; Woodcock et al. 2017) have approached the issue on a landscape scale and under realistic agronomic conditions. One study (Woodcock et al. 2017) assessed the impact of honey bees in three European countries (UK, Germany and Hungary) over 2 years in oilseed rape fields. Interestingly, the results were country-specific: while in Germany no negative effects on colony survival were recorded, both in the UK and Hungary, neonicotinoids (clothianidin and thiamethoxam) had a negative impact. In Hungary, colonies exposed to clothianidin after winter had a mean of 24% fewer workers after winter than unexposed colonies. With thiamethoxam, no such effect was observed. In the UK, high colony mortality did not allow statistical analysis. The authors state that the country-specific impact suggests that the effects of neonicotinoids are the product of an interaction with other factors. They point out that low levels may reduce colony fitness and impact by interaction with environmental factors. In the second study (Tsvetkov et al. 2017), the exposure to neonicotinoids and other agrochemicals of honey bee colonies in maize fields in Canada were studied. The exposure proved to last up to 4 months, which is a substantial part of the active season of honey bee colonies. In addition, the presence of a common fungicide (boscalid) doubled the toxicity of the neonicotinoid clothianidin.

Similar data from other crops and world regions may help to clarify the often controversial results found in different studies.

In consequence of the discussion around neonicotinoids and their impact on honey bee health, the risk from pesticides for honey bees and other pollinators is getting increased attention. Besides neonicotinoids, also other substances have been tested. For instance, bees feeding on fungicide-contaminated pollen consume less pollen and have lower ATP levels and higher virus titres than bees foraging on uncontaminated feed (DeGrandi-Hoffman et al. 2015b). Currently, the registration process, necessary studies and data evaluation are under review to improve the risk assessment for honey bees and other pollinators.

There are different possibilities to decrease risks coming from agricultural management to bees. The fastest may be technical solutions to adapt to concrete problems. For instance, after the incidents with dust drift when sowing maize, sowing machines were adapted to redirect the exhaust air to the soil. By this, dust drift could be reduced substantially. Another method, recently under development, is dropleg nozzles. This technique lowers spraying nozzles so that blossoms are not hit by pesticides in flowering crops like oilseed rape. However, for reducing risks from agricultural management for bees, the risk assessment and management after registration has to be modified and improved.

A frequent objection on current risk assessments is that they do not take consideration of the complexity of the superorganism honey bee colony. Recent data (Woodcock et al. 2017) suggest that the picture is far more complex than represented in the current approach. Intense monitoring studies would fill this data gap and supplement the data from the current assessment that apparently does not meet the complex regulations in the superorganism honey bee colony and different agronomic situations. Real-case scenarios might not reach the statistical power of the current tiered approach but would give more information about the biological relevance under defined conditions. Linking both approaches would also help to create practical and more efficient risk management strategies under the premise of conserving colony welfare. This is especially important in areas with both intensive beekeeping and agriculture like Southern Europe or Turkey.

4.10 Foraging in Agricultural Landscape

The influence of agriculture on honey bee welfare goes farther than possible intoxications and sublethal effects from pesticides. The agricultural landscape has changed considerably from a heterogeneous space with high structural and biological diversity to homogeneous areas with low diversity (Naug 2009). In some areas, beekeepers complain that their bees do not find enough forage anymore over large parts of the season, and there is a need for supplementary nutrition due to agricultural monotony (Brodschneider and Crailsheim 2010). As mentioned above, there is evidence that the nutritional state of honey bee colonies affects both its state of health and its ability to cope with stressors (vanEngelsdorp and Meixner 2010; DeGrandi-Hoffman et al. 2015a, b; Simone-Finstrom et al. 2016).

Honey bees naturally forage on a big variety of flowers, as discussed above. However, they show distinctive behaviour in nectar and pollen collection. For nectar, they prefer mass-flowering resources, like oilseed rape or sunflower fields. On the other hand, pollen sources are very diverse and not limited to mass-flowering crops. In a study over 5 years in France (Requier et al. 2015b), honey bees were found to use a large variety of plants (mainly weeds and trees) as pollen source. These plants grew in seminatural habitats within the agricultural landscape and constituted up to 40% of the pollen diet. The composition of the pollen diet was influenced by the nutritional value and the landscape composition. The latter results in a critical importance of heterogeneous agricultural landscape to maintain the food supply for honey bees, both for nectar and pollen.

These results are confirmed by a study comparing the densities of pollinators in mass-flowering crops and seminatural habitats in agricultural landscapes (Holzschuh et al. 2016). With increasing covers, honey bee densities decreased within the crop while increasing in neighbouring seminatural habitats. The authors observed a redistribution of honey bees from the mass-flowering crops (with high floral density but little diversity) to the seminatural habitat (with less floral density but higher diversity). This distinct distribution of honey bees between the two habitat types depends on the distance between them and the phenological state of the honey bee colony.

Pollen collection throughout the year shows two periods with especially high demand (Requier et al. 2015b). These periods indicate the time of highest brood presence in spring and the raising of longer-lived winter bees in late summer. In intense agricultural areas, crop flowering does not necessarily coincide with these periods of high nutritional demands, leading to an important protein lack. Declining pollen resources in spring produced a carry-over effect for colony health, leading to smaller colony size later in the season, higher *Varroa* infestation and colony losses (Requier et al. 2016). Similar results were found when exposing honey bee colonies to different landscapes in the pre-wintering period (Alaux et al. 2017). These effects can be demonstrated also at physiological level: fat body mass and vitellogenin levels were increased when the colonies had access to flowering catch crops and seminatural habitat. Both parameters positively influence honey bee health and are linked to winter survival of the colony.

All these data suggest that landscape degradation, availability of diverse foraging plants and habitat structure influence the recent higher honey bee colony losses. By focussing on pesticides (mainly neonicotinoids), the issue for honey bees in agricultural landscape may be oversimplified. Both issues should be addressed in parallel to maintain honey bee welfare in agricultural landscape. Engaging both beekeepers and farmers to find practicable solutions may give more sustainable solutions to this conflict. A first step for improving honey bee welfare may be the implementation of agro-environmental schemes considering both agronomic operations (plant protection, crop rotation, etc.) and the dietary needs of honey bees and other pollinators like flowering strips, habitat restoration, catch crops, etc. Finally, the implementation of these measures should be monitored to prove their efficacy and adapted to the single agronomic situations to attain sustainable solutions for bee health and farming.

4.11 The Challenge of Global Warming

Climate change is affecting living systems (Parmesan and Yohe 2003), and there are several aspects in which the welfare of honey bees can be altered by it. There is a big data gap on the impact of global warming on honey bees. However, climate change is considered one of the major threats for pollinators (Brown and Paxton 2009; Potts et al. 2010; Brown et al. 2016) in which honey bees are included but seldom directly addressed.

Honey bees are highly dependent on environmental conditions, such as photoperiod, temperature and precipitations. They directly affect the individual behaviour and physiology as well as the development and phenology of the colony. On the other hand, extreme weather events like droughts or floods could change the distribution and phenology of flowering plants. By this, a mismatch between honey bee activity and the flowering of their food plants may occur (Le Conte and Navajas 2008; Hegland et al. 2009; Bartomeus et al. 2013; Reddy et al. 2013).

The ability of honey bee colonies to "buffer" adverse effects and the complex interactions regulating its development and activity hampers the analysis of a possible impact. However, global warming itself is well-documented, and these data help to understand possible impacts on honey bee welfare. For instance, there is a trend in temperate regions to spring anticipation after mild winters. This leads to earlier blooms of important food resources, while honey bee colonies are still inactive or show low activity.

Indicators for such changes are reported by beekeepers. For instance, goat willow (*Salix caprea*) is an important early nectar and pollen resource for honey bee colonies in Germany. In 2008, after a mild winter, this plant bloomed about 15 days earlier than usual (DWD 2008). In central Germany, beekeepers complained that their colonies were not able to exploit this crop due to only low honey bee activity in this period. As a consequence, the spring development of the affected colonies was slowed down.

On the other hand, bee colonies start their brood activity earlier or never stop it during mild winters. By this, the colonies require food resources in periods in which no or few flowering plants are available. The consequences for colony welfare of this changed phenology are still unknown.

Changed plant phenology affects also management practices and productivity: *Robinia pseudoacacia* (false acacia) is an important honey crop in Northern Italy. Notably, the flowering period of this tree starts earlier, and, by this, beekeepers lost the indicators for preparing the colonies for this important crop. Once, there were indicators for the approaching false acacia bloom, like wild cherry flowering some weeks earlier. Beekeepers then knew when to begin preparing the colonies for the false acacia. Nowadays, due to changed plant phenology, this rule is obsolete, and beekeepers lost this important indicator for their management.

In addition to early spring, honey bee colonies remain active for a longer time in autumn due to mild temperatures. In these periods they use new resources like ivy (*Hedera helix*). The nectar of this plant crystallises very fast (Greenway et al. 1975, 1978), leading to hard honey stores in the colonies which are not accessible to the bees during winter. Therefore, colonies can starve to death despite abundant honey stores.

The aforementioned examples rely on anecdotal evidence from beekeepers and are not studied systematically yet. For understanding the connection between climate change, synchrony with important food resources and its impact on colony development, experiences and reports from beekeepers are important indicators. Research should address both the biological impacts and mitigation for practical management. In fact, honey bees are sometimes not addressed in studies because it is assumed that the human custody mitigates any impact of global warming (Bartomeus et al. 2013).

As discussed above, honey bee health is closely linked to the nutritional state of individuals and the colony as a whole. For spring development and overwintering success, pollen—i.e. protein—availability is extremely important (Requier et al. 2016; Alaux et al. 2017). Therefore, the finding of reduced protein content in pollen due to climatic changes is quite alarming (Ziska et al. 2016). The authors studied the protein content of pollen in goldenrod (*Solidago* spp.), which is an important pollen source for honey bees in autumn. The data are especially informative because also historical samples of the same plant were analysed. By this, a clear link was found between rising atmospheric carbon and decreasing protein in goldenrod pollen. Similar data from other important food plants are needed, as well as on the effect on overwintering survival and colony development in the following spring. Based on this, both management techniques for beekeepers and mitigation measures (increasing floral diversity, etc.) could be developed. An important aspect would be also evaluating the possible compensation by seed mixes and similar mitigation measures.

In temperate regions, honey bee colonies did not breed during winter for several weeks or even months. With global warming, queens continue egg laying and the colonies care for brood in some cases through the whole winter. These brood care activities have an impact on the consumption of honey stores: colonies with brood consume the double in honey during winter than broodless colonies (Seeley and Visscher 1985). This has to be considered when preparing colonies for overwintering: the amount of sugar supplements provided by the beekeeper must be adapted to these new conditions. Otherwise, the colonies could be starved to death because of this condition.

Another aspect in which global warming may affect—and in some areas is already affecting—honey bee welfare is its influence on diseases and parasites. The infestation development of *Varroa destructor* might be the most important example of this. Usually, in temperate regions honey bee colonies stop breeding for some time in winter. As *Varroa* mites reproduce in sealed brood, this means that the infestation in this period does not increase. In the presence of brood, on the other hand, *V. destructor* might continue to reproduce and infestation levels go up. This means a higher starting infestation at the beginning of the productive season. During spring and summer, the infestation doubles every month; starting with higher levels, therefore, means also reaching the economic and survival thresholds earlier in the season (Nanetti et al. 2014).

In addition, the presence of brood during winter reduces the efficacy of winter treatments. These usually act only on phoretic mites. Therefore, depending on the proportion of *Varroa* females in the brood during winter, the efficacy of the treatments may be reduced considerably (van Dooremalen et al. 2012; Nanetti et al. 2014). Stewardship in beekeeping often already takes this phenomenon into account, advising more thorough treatments and observations to meet this risk. In addition, adapted treatment concepts are tested.

Nosema ceranae is currently perceived as a problem in warmer areas. This might be due to the sensibility of the parasite's spores against lower temperature (Fries 2010). With increasing temperatures, it could become an emergent disease also in formerly colder areas. This increasing range of possible distribution could be true also for two newly introduced honey bee pests in Europe: the small hive beetle (*Aethina tumida*) and the Asiatic hornet (*Vespa velutina*), which both originate from tropical climate.

Further risks could come due to global warming by emergent pests and parasites. An example of this is *Tropilaelaps* mites, which until now infest European honey bee colonies only in tropical Asia (Buawangpong et al. 2015). These obligate parasites need brood for survival and, therefore, until now do not affect honey bee colonies in temperate regions. However, with climate change and breeding also in winter, the risk of wider spreading of the parasite is higher.

The interaction between global warming, the internal state of honey bee colonies, changed flowering phenology and pests and parasites is becoming an increasingly urgent issue. Adapted management techniques together with the resilience of the superorganism honey bee colony may help to mitigate this risk to honey bee welfare.

4.12 Conclusions and Recommendations

Honey bee colonies are superorganisms and, therefore, consideration on their welfare must include this complexity (Fig. 4.6). The high resilience against external stressors led to management practices oriented mostly versus productivity. Some of them, like transports over large distances, crowding colonies on apiaries or unnatural nesting conditions, can negatively affect the colony welfare. Adapting management practices, however, must take into account different apicultural traditions and practices. Changed management must maintain the productivity of the colonies or offer other advantages (fewer material costs, facilitated management, etc.) to motivate the beekeepers to implement the measures on a sustainable level. Stewardship and training measures, as well as knowledge transfer between beekeepers and apicultural science, gain importance in this context. The recently increased colony losses may

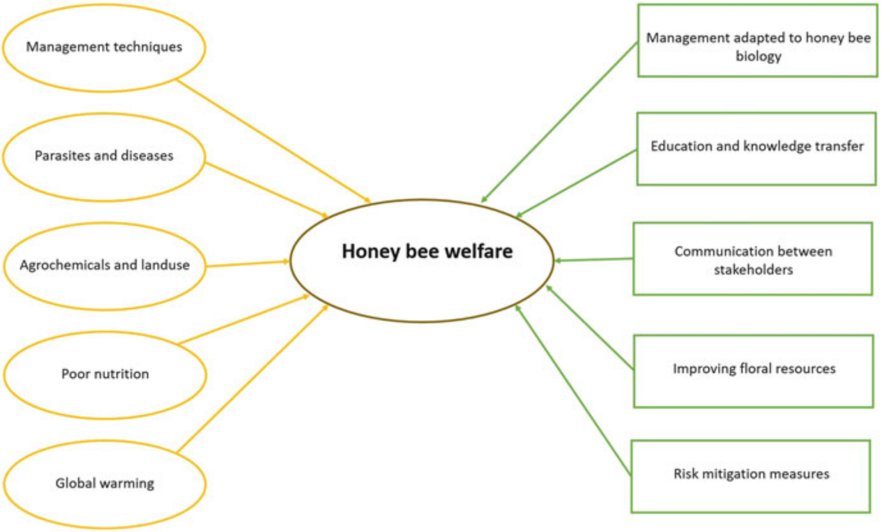

Fig. 4.6 Multiple factors have the potential to negatively impact honey bee welfare (left side). Measures to improve it need an interdisciplinary effort between beekeepers, scientists, farmers and decision-makers

have also increased the motivation to change procedures according to honey bee biology if they result in more sustainable populations.

Beekeeping industry and apicultural research are often closely interconnected. However, the implementation of research results into beekeeping practice is difficult. Communication at eye level helps to address the problems experienced by beekeepers with the focus on colony welfare and leads to more sustainable results.

Nutrition and global warming seem to be overarching factors for honey bee welfare, influencing others like pests and parasites. Beekeepers trained in treatment methods and taking knowledge-based decisions, in general, have fewer problems with *Varroa destructor*. However, existing strategies do not reach all beekeepers, and the outreach of existing knowledge has to be improved. Better structures for successful knowledge dissemination could mean a large step forward a better, more welfare-oriented management in apiculture. Most importantly, it has to be considered that management techniques are different throughout the world. Therefore, a holistic view of apicultural traditions must be taken into account. This is especially important facing emergent diseases like *Nosema ceranae* that may impact honey bee colonies more in certain regions.

Rules for “good apicultural practices” to improve colony welfare should cover only the basic management techniques. An additional toolbox that considers different conditions in beekeeping could then step by step increase the focus on colony welfare and more sustainable management. Stewardship structures and practices differ as well in different countries. In regions where beekeepers have the possibility to work and communicate closely with “their” institutions, new methods are easily implemented. Therefore, improving these structures is an important step for increased honey bee welfare.

There is evidence that intense agriculture, particularly pesticide use, affects honey bee health. However, the effects are not always clearly assessable or unequivocal. Communication between stakeholders often prevents acute incidents, as well as risk mitigation measures. However, it became clear that the current risk assessment procedure for agrochemicals does not consider the complexity of the superorganism honey bee colony. Risk assessors and decision-makers seldom are honey bee experts and need clear and standardised procedures for their decisions. Therefore, apicultural scientists should be involved in the development of risk assessment criteria. Monitoring studies and models tested with their data could help to answer complex "what if" questions and give a more realistic base for risk assessment than current test systems.

The impact of current agriculture on honey bee colonies cannot be reduced to agrochemicals. Agricultural landscape does not offer heterogeneous structures that offered plenty and diverse food sources before intensification. These considerations lead to the following recommendations:

- A worldwide net of stewardship and training structures for beekeepers to improve knowledge transfer from bee science
- Integrating veterinary services, agronomic stewardship structures, etc. for better dissemination of knowledge
- Development of basic rules for good apicultural practice and a adaptable toolbox for different beekeeping conditions
- Training farmers and beekeepers to increase communication between these stakeholder groups
- Adaptation of risk assessment procedures to complex honey bee biology including bee scientists in the procedure
- Monitoring studies and further development of risk mitigation measures according to knowledge on bee nutrition
- Adapting management techniques to global warming

Glossary

Apiary Site on which beekeepers place their colonies. The size of an apiary can vary between a few colonies from a hobbyist beekeeper until several dozens or hundreds in professional beekeeping

Bee packages Artificially assembled groups of a queen and worker bees. Used for starting a new colony

Brood Total of immature stages (eggs, larvae and pupae) in the colony

Colony losses General term for describing mortality of whole colonies due to different causes (overwintering, diseases, parasites, pesticides, etc.)

Colony strength Sum of adult bees and number of brood cells

Comb Build by bees with wax, consists of hexangular cells in which brood is reared and honey and pollen stores are deposited

Drifting Homing error of foraging bees; they return to other colonies than their own. Especially frequent in crowded apiaries

Drones Male honey bees

Hive Box in which the bee colony is kept by beekeepers

Honey super Additional story of the hive reserved for honey storage. Usually separated by queen excluder

Nuclei Artificial colony formed by the beekeeper with a new queen, worker bees and some brood combs. Used for starting a new colony

Polyethism Display of different, age-dependent behavioural patterns during the lifetime of worker bees

Propolis Mix of plant resin with secretions of worker bees. Closes small spaces in the bee colony and plays a role in the defence of honey bee colonies

Queen Only reproductive female in the honey bee colony

Queen excluder Grid separating the nest from the honey super. The spaces are large enough for the worker bees but not for the queens. By this, the combs in the honey super stay without brood

Robbing Foraging workers from strong colonies enter weak colonies and take their honey provisions. Occurs mainly when floral resources are low

Super Box added as additional level to the hive for honey storage

Swarming Division of the honey bee colony in two. Occurs after rearing of young queens. The mother queen leaves with a part of the colony and establishes at a new site

Treatment concept Framework for treatments against Varroa. Defines when and how to apply the treatments

Varroa—phoretic phase Nonreproductive time of *Varroa destructor*, a main parasite of honey bees, on adult bees

Varroa—reproductive phase *V. destructor* reproduces only in brood cells of honey bees, during the pupal development

Workers Infertile females in the honey bee colony. They provide all nonreproductive tasks (nursing, foraging, defence of the colony, etc.)

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Chapter 5
Spider Welfare

Simona Kralj-Fišer and Matjaž Gregorič

Abstract Spiders with around 48,000 recorded species are major terrestrial predators and thus crucially important for ecosystem functioning. They are widely used as research models and for biodiversity displays and sometimes also kept as pets. Nevertheless, we are not aware of any legal ethical rules bound to spider welfare during rearing or research. To set ethical standards, we first need to detect and assess how spiders "perceive" the external world. Based on the current knowledge of spiders' sensory and nervous system, it is difficult to judge whether spiders feel pain, distress and suffering, although their behaviours like thanatosis, "bailing out", autotomy and associative avoidance learning imply so. As is now known, arthropods are not simply mini-robots as traditionally believed. Thus, spider welfare deserves more research effort, and the ethical standards for rearing or using spiders in research need to be set. Here, we describe the variety of spider physiological and behavioural characteristics and how they apply to their rearing, housing, handling and experimental use. We hope reporting these methods will help ensuring welfare and well-being of spiders in captivity.

5.1 Introduction

Spiders (order Araneae, class Arachnida) are one of the most diverse animal groups on the planet, currently with more than 48,000 described species (World Spider Catalog 2018). They rank seventh in global diversity, only surpassed by mites and ticks (Acari) among arachnids (Coddington and Levi 1991). Spiders have occupied all terrestrial ecosystems (Foelix 2011), are the most important predators in prey biomass consumption (Nyffeler and Birkhofer 2017) and thus have a crucial role in ecosystem functioning. Among their most characteristic features are hunting using venom and the production of silk, nature's toughest fibre (Foelix 2011). For millennia, spiders have been a part of human imagination, mythology and art,

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S. Kralj-Fišer · M. Gregorič (✉)
Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia
e-mail: MatjazGr@zrc-sazu.si

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symbolizing patience, mischief and malice. They are widely used as research models in diverse fields, for biodiversity displays, and sometimes also kept as pets. Yet, arthropods including spiders are traditionally considered mini-robots that lack flexibility (Herberstein 2011). Consequently, professional standards securing spider welfare in captivity are not clearly established.

To set such standards, we should first address issues of the ability of spiders to feel pain, distress and suffering. In the aim of comprehending the "inner world" of spiders, we describe below their sensory and notably complex nervous systems, which are needed to receive and perceive external stimuli. While spiders lack higher nerve centres and thus should only be capable of reflexive behavioural responses to dangerous stimuli (nociception), their behavioural and physiological responses imply their potential to feel pain and stress. Spiders, for example, exhibit behaviours like thanatosis, "bailing out" and autotomy when in danger. The results of several studies further imply that spiders thereby activate their stress (octopamine) system (Punzo and Punzo 2001; Jones et al. 2011). Several species are also able to modify their behaviour depending on their previous experiences and exhibit associative avoidance learning in response to previous experiences with predators.

There is thus plenty of evidence that invertebrates, including spiders, are not just instinct driven and inflexible in their behaviour; rather they show behavioural plasticity and cognitive abilities, such as attentional priming and memory (Jakob et al. 2011). This evidence should not be ignored and needs to be used while establishing the guidelines for securing their welfare. The responsibilities of researchers to take care for study animals include the experimental procedures and also providing suitable conditions at which spiders are bred or kept when not being studied. To maximize welfare, the housing of spiders should incorporate as many of the important natural living conditions as possible. Also, some research fields can hardly avoid sacrificing individuals. Many scientists use ethanol, freezing or CO_2 as a method of spider euthanasia, but it is has not been tested whether these methods indeed induce analgesia. Yet the instant death at −60 °C might be more humane than several minutes long drowning in ethanol—the method still widely used in research. In the following chapter, we aim at presenting an overview of welfare considerations when keeping and experimenting on spiders in the laboratory.

5.2 Sensory and Nervous System

The spiders' main sensory organs are eyes, lyriform organs, trichobothria and chemosensory organs (Barth 2013). The sensitivity and distribution of the sensory organs vary among taxa and largely reflect a spider's life style. For example, the visual ability is much better developed in cursorial spiders compared to web-building spiders, which mainly rely on their vibratory senses. While most web-building spiders can only detect the direction of light and motion, cursorial spiders are capable of forming images. Jumping spiders have exceptional eye sight adapted for colour vision and high spatial acuity (Blest et al. 1981).

The mechanical senses of spiders involve the specialized hairs—trichobothria and slit sensillae (slits in the exoskeleton) that detect acoustic, vibratory and tactile cues. Vibratory cues transmitted through environmental surfaces, including silk threads, are among the most important information sources for spiders. Vibrations inform them about the presence of prey, mates, parasites and predators. Furthermore, spiders commonly use vibrations as an intraspecies communication channel, which is particularly important in mate recognition and mate assessment (Uetz and Roberts 2002). Trichobothria that detect airborne vibrations cover the legs and pedipalps, slit sensillae that detect substrate-borne vibration are distributed over most of the body surface, and are most common on legs (Barth 2013). Legs and pedipalps are also covered by chemosensitive hairs enabling them to recognize conspecifics, prey and predators. Besides the sensory functions of thousands of innervated hairs covering the spider body, some serve other functions, such as adhesion to the substrate, combing out silk and cleaning (Foelix 2011).

Sensory organs are innervated. Their axons form small bundles and join into sensory nerves that conduct the sensory information to the central nervous system (CNS). The CNS of spiders is highly compacted and consists of two ganglia with exiting efferent nerves. The syncerebrum, also supraesophageal ganglion, consists of cheliceral ganglia and the brain. The brain receives optic nerves and contains visual and association centres (Foelix 2011). Despite these relatively simple and small CNS, some spiders exhibit remarkably complex behaviours. For example, some species are able to improve their prey capture technique with experience (e.g. Edwards and Jackson 1994), learn to avoid “dangerous” prey (e.g. Higgins 2007), adjust their fighting behaviour according to their previous experiences (e.g. Whitehouse 1997), etc.

Sensory hairs are extremely sensitive; touching a single hair causes a spider to escape or counterattack (Foelix 2011). Given the sensitivity and importance of their sensory organs, spiders should always be handled with care in order to prevent damaging sensory organs and overstimulating the animals. The overstimulation of mechanoreceptors can be avoided by keeping spiders in rooms with minimal vibrational stimuli, either airborne (wind, music) or substrate borne (machines causing vibrations). Similarly, routine tasks like cleaning the enclosures and feeding should be done quickly and with minimal disturbance. Also, spiders should not be handled manually, rather, we recommend using a soft brush.

5.3 The Ability to Feel Pain, Distress and Suffering

Researchers are commonly concerned about the welfare of their model animals. An animal’s ability to feel pain, distress and suffering is often judged by the size and complexity of its nervous system and/or complexity of its behaviour (Mather 2011). Almost all animals with a nervous system can detect dangerous stimuli and will withdraw when stimulated. In other words, they exhibit nociception, the capacity to respond to aversive stimuli with activation of sensory and motor pathways.

Activation of the latter usually results in a reflexive behavioural response. Reflexive withdrawal may be mediated by simple sensory-motor pathways without involvement of higher processing centres. In this view, spiders, lacking higher nerve centres, should only be capable of reflexive behavioural responses to dangerous stimuli.

Thereby, it is more difficult to judge whether spiders experience pain, i.e. "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk 1994). While nociception occurs with or without conscious sensation, the definition of pain requires an emotional experience and thus consciousness. In vertebrates, the cognitive-emotional component of pain, or simply feeling and awareness of pain, involves higher brain structures, e.g. the limbic system, and processes. In this view, we could conclude that the spiders' nervous system precludes them to experience any kind of mental state. However, the spiders' neural architecture is not fully understood (Jakob et al. 2011). Also, arthropods in general might possess different neurobiological mechanisms for experiencing pain than vertebrates. In any way, the lack of knowledge should not be an excuse to handle spiders in a way that activates their nociceptive pathways.

Recently, additional criteria that should be fulfilled to accept potential pain experience have been proposed (Elwood 2011; Sneddon et al. 2014). Sneddon et al. (2014) stated that responses to noxious, potentially painful events should affect neurobiology, physiology and behaviour in a different manner to innocuous stimuli, and subsequent behaviour should be modified including avoidance learning and protective responses. In addition, animals should show a change in motivational state after experiencing a painful event such that future behavioural decision-making is altered and can be measured as a change in conditioned place preference, self-administration of analgesia, paying a cost to access analgesia or avoidance of painful stimuli and reduced performance in concurrent events.

Following these criteria, we will examine the below features indicating the potential for experiencing pain in spiders:

1. Nociceptors and central nervous system
2. Physiological changes to noxious stimuli
3. Protective motor reactions that might include reduced use of an affected area such as limping, rubbing, holding or autotomy
4. Avoidance learning
5. Cognitive abilities

5.3.1 Nociceptors and Central Nervous System

There is not much known about the presence of nociceptors in spiders and how nociceptive information is processed within their central nervous system. Nevertheless, spiders exhibit withdrawal or escape behaviours when exposed to noxious stimuli. They may also exhibit behaviours that may not simply be nociceptive reflexes; e.g. jumping (Suter and Gruenwald 2000), shaking web (Jackson et al. 1992; Kralj-Fišer et al. 2011) and autotomy (Eisner and Camazine 1983; Punzo 1997). Their antipredator behaviour includes thanatosis (feigning death—this behaviour is characterized by curling legs and freezing, resulting in a body posture very similar to that of a dead spider) (Bilde et al. 2006; Kralj-Fišer and Schneider 2012) and "bailing out", in which the spider drops from the web and hangs motionless from a dragline with huddled legs (Uetz et al. 2002).

5.3.2 Physiological Changes to Noxious Stimuli

Spiders detect predators by airborne vibrations stimulating their trichobothria (Foelix 2011). The increased sensitivity of trichobothria to air movements relates to increased octopamine (OA) levels (Widmer 2005). Octopamine in arthropods is considered analogous to the vertebrate norepinephrine, indicating its role in the stress system (Roeder 1999). Jones et al. (2011) experimentally elevated levels of OA and serotonin (5-HT) in the orb web spider, *Larinioides cornutus*, and observed that increased OA levels relate to decreased durations of thanatosis, while elevated 5-HT had the opposite effect (Jones et al. 2011). The elevated 5-HT likely makes the spiders more fearful and less aggressive, whereas elevated OA likely relates to increased arousal (Jones et al. 2011). The reductions of 5-HT and OA levels after agonistic encounters have been observed in the brain of male bird spiders, with subordinate males exhibiting lower levels than dominant ones (Punzo and Punzo 2001). These findings suggest that the activation of the stress system in response to aversive stimuli may be comparable to the one in vertebrates.

5.3.3 Protective Motor Reactions

Spiders regularly autotomize their legs when in dangerous situation, e.g. grasped by a predator or a conspecific during fight, in order to escape more easily (Punzo 1997; Foelix 2011). Furthermore, they self-amputate injured appendages (Kralj-Fišer et al. 2011; Kralj-Fišer and Kuntner 2012; Kuntner et al. 2014), and they "lick" or rub their wounds. Missing appendages may negatively affect development, web building, foraging success, competitive abilities and mating success in some species, whereas in several species it has no apparent costs (reviewed in Fleming et al. 2007).

Eisner and Camazine (1983) conducted an experiment that suggests a concordance between pain effects in humans and autotomy in spiders. They observed *Argiope* spiders that were stung in the leg by bees and wasps to undergo leg autotomy. They experimentally penetrated the leg-joint with a sterile pin and injected spiders with several venom components known to elicit pain in humans. Spiders employed autotomy when injected with histamine, serotonin, phospholipase and melittin, all of which induce pain in humans. Acetylcholine and bradykinin, which also induce pain in humans, did not cause the autotomy in spiders. However, injection of hyaluridase, adrenaline and dopamine, which do not induce pain in humans, also failed to be effective in causing autotomy in *Argiope*.

5.3.4 Avoidance Learning

Avoidance learning is the process by which an individual learns to avoid unpleasant situations on the basis of prior experience. There is abundant evidence for avoidance learning in spiders. For example, the wolf spider *Schizocosa avida* exhibits associative learning in response to previous experience with a predator (Punzo 1997). Individuals of the same species that have survived a scorpion attack by leg autotomy learn to avoid scorpion-scented substrates (Punzo 1997).

Several spiders are able to develop aversion to toxic and/or unpalatable prey (e.g. Edwards and Jackson 1994; Toft and Wise 1999; Skow and Jakob 2006; Hénaut et al. 2014). Furthermore, jumping spiders exhibit avoidance of visual stimuli associated with heat (Nakamura and Yamashita 2000), electric shock (Bednarski et al. 2012; Peckmezian and Taylor 2015) or vibration (Long et al. 2015). For example, jumping spiders of the species *Hasarius adansoni* were trained to associate heat with coloured papers. These spiders learned to avoid colours which were associated with high temperature, suggesting that they are not just able to learn to avoid heat but also to associate it with colour (Nakamura and Yamashita 2000). Thus, spiders are likely able to learn and take the appropriate action to avoid or reduce potential damage on the basis of prior experience with noxious stimuli; such a response is not the fixed, reflexive action of nociceptive avoidance.

5.3.5 Cognitive Abilities

There is ample of evidence indicating spiders' ability to learn. Several species are able to (adaptively) modify their behaviour in the contexts of foraging (e.g. Wilcox and Jackson 1993) and web building (e.g. Heiling and Herberstein 1999; Venner et al. 2000), intraspecific interactions (e.g. Taylor and Jackson 2003; Kasumovic et al. 2009), spatial learning and navigation (Tarsitano and Jackson 1997; Jakob et al. 2007). Some spiders even exhibit reversal learning (Liedtke and Schneider 2014). For a more exhausted literature on evidence for behavioural plasticity, learning, memory and other cognitive abilities in spiders, see reviews by Jackson and Cross (2011), Jakob et al. (2011), Nelson and Jackson (2011) and Japyassú and Laland (2017).

5.4 Keeping Spiders in Captivity

To secure the well-being of animals in captivity, we have to satisfy their general needs, e.g. ready access to fresh water; a balanced diet; appropriate housing; prevention from pain, injury or disease; and enabling an environment to express normal behaviours and ensure conditions and treatments causing no/little fear and stress. Ethical guidelines for research in vertebrates follow the "three Rs" (3Rs) principle, i.e. the replacement, reduction and refinement criteria (Russell and Burch 1959). While the latter two should be applied to spiders, the first likely does not.

Replacement generally refers to replacing animals with "non-sentient material" (Russell and Burch 1959), e.g. plants, microorganisms, animals with limited nervous and sensory systems, tissue cultures and computer models (Tannenbaum and Bennett 2015). Similarly, "lower" animals like arthropods are considered a replacement for "higher" ones, e.g. mammals, with the justification of evolutionary conservation of physiological processes allowing for application across animal groups (Horvath et al. 2013). Replacing arthropods for vertebrates might be argued for in some contexts, e.g. when experimental procedures involve trauma, and keeping a large number of experimental animals. However, arthropods and thus spiders can hardly be replaced by other animals, especially considering the gaps in our knowledge in these "lower" animal groups. These same knowledge gaps currently render replacing spiders with other invertebrates, tissue cultures and computer models impossible. The reduction and refinement principles apply to animal experiments in general and thus have to be taken into account in spider research. Reduction refers to using the minimal number of animals required to perform a study, where careful experimental and statistical planning is crucial. Refinement generally refers to finding experimental designs that maximize the scientific output while minimizing negative effects on animals, e.g. by planning for potential experimental problems, choosing the least invasive techniques and ensuring proper housing (Animal Behaviour 2018).

Until recently, the welfare of spiders and most other invertebrates in captivity has been neglected. Several invertebrate species have long been used as model organisms in research, to fulfil the "replacement" criterion in animal welfare guidelines for vertebrates (Carere et al. 2011). Do spiders and other invertebrates indeed suffer less and are they more suitable for life in captivity than vertebrates? Can we further ignore the fact that spiders fulfil several criteria that indicate their potential for experiencing pain? Instead, researchers should use the existing knowledge to establish standards for securing their welfare.

The responsibilities of researchers to take care for study animals do not only include the experimental procedures themselves but extend to providing suitable conditions at which spiders are bred or kept when not being studied. To maximize welfare, the housing of spiders should incorporate as many of the important natural living conditions as possible. While the general seasonal parameters, e.g. the day/night regime, temperature and relative humidity, can be mimicked by knowing a spider's habitat and diurnal cycle, other maintenance parameters are important. Researchers should consider the importance of the size of enclosure, providing

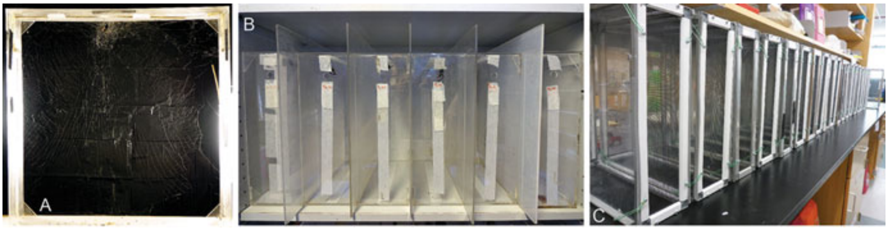

Fig. 5.1 Frame-shaped enclosures allow the construction of two-dimensional orb webs (**a**), can be made from Perspex (**b**), plastic mesh (**c**) or any other suitable material and are suitable for maximizing laboratory space (**b, c**)

biologically relevant enrichment and social interactions among individuals, as well as proper nutrition and euthanasia, all of which are discussed in the following chapters.

5.4.1 Husbandry

Spiders in general can be divided into two basic life style groups, the cursorial and web-building spiders, and within both of these several subcategories can be identified. For example, some cursorial species are completely ground dwelling, while others are arboreal, but most inhabit diverse habitats that cannot be classified into such extremes. Thus, maximizing the enclosure's floor space and climbing space or providing other enrichments is crucial. Similarly, some web-building spiders employ two-dimensional orb webs, while others employ three-dimensional webs. Thus, orb weavers can be efficiently kept in frames, usually piled like books on shelves, maximizing laboratory space and allowing the spiders to build webs (Fig. 5.1). On the other hand, three-dimensional webs demand cubic-shaped enclosures (Fig. 5.2), sometimes with supporting structures allowing web construction (Zschokke and Herberstein 2005).

While it is straightforward that larger spider species need to be housed in larger enclosures, it is sometimes difficult to identify biologically meaningful enrichment features for certain species, especially if their natural history is little known or they have not been previously kept in the laboratory. Web building spiders almost exclusively live on their webs. They either sit in the web itself or construct retreats in or at the side of the web. Thus, it is relatively easy to recognize such biologically meaningful enrichment features while collecting them in the field (e.g. providing shelters or materials for shelters). On the other hand, cursorial spiders are usually housed in simple enclosures, out of practicality often without enrichment features (e.g. easier observation, cleanliness (Jackson 1974). However, it is almost unknown how housing conditions affect cursorial spiders. There is evidence that environmental stimuli can influence behavioural syndromes, and thus laboratory-bred individuals differ from field-caught ones (Sweeney et al. 2013). This might be especially

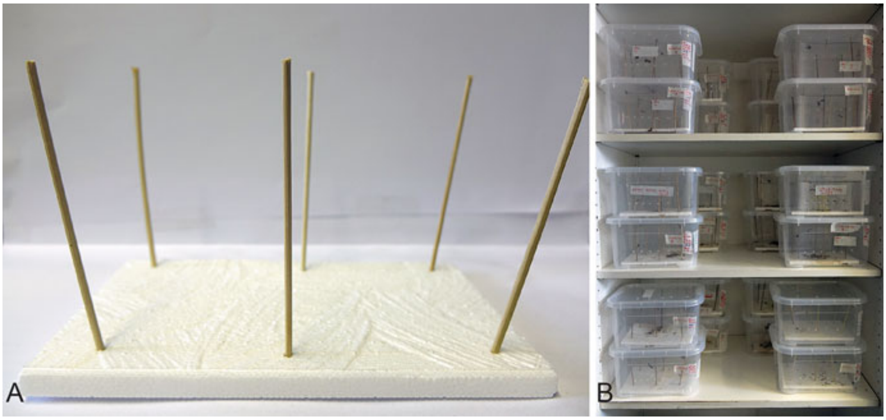

Fig. 5.2 Cubic-shaped enclosures (**b**) with supporting structures (**a**) that allow the construction of three-dimensional cobwebs

important in taxa with well-developed cognitive abilities, e.g. jumping spiders, although little is known about cognitive abilities of most other spider groups. In fact, laboratory raising jumping spiders of the species *Marpissa muscosa* in environmentally deprived, socially enriched or physically enriched enclosures consistently affects their personality traits (Liedtke et al. 2015; see Carere and Maestripieri 2013). Furthermore, recent research demonstrates neuroplasticity in jumping spiders by showing that *M. muscosa* spiders growing up solitary and in physically deprived enclosures have smaller volumes of higher order integrating brain centres compared to spiders growing up with siblings or in physically enriched enclosures (Steinhoff et al. 2018). These brain centres, composed of mushroom bodies and the arcuate body, receive visual and maybe also locomotory information and process it (Steinhoff et al. 2017). Both in vertebrates and arthropods, the variation in cognitive ability, and thus behaviour, seems to be directly influenced by brain size (Amador-Vargas et al. 2015; Benson-Amram et al. 2016; Corral-López et al. 2017; Feinerman and Traniello 2016). This highlights the need for further studies of how laboratory conditions affect the spider nervous system. Moreover, while spiders are generally considered solitary and intolerant of conspecifics, several species do live in aggregations, and some are even (sub)social (Foelix 2011).

Proper housing, be it through providing biologically meaningful enclosure enrichments or social interactions, can thus not only assure the animals' well-being but also minimizes potential factors affecting their behaviour in experiments. Furthermore, spiders are often kept permanently as laboratory model animals. Maintaining a healthy laboratory population can reduce the amount of needed wild-caught animals and allows the researchers to comply with the "reduction" and "refinement" principles of the 3Rs guiding principles for appropriate animal care. Namely, it is critical to choose correct experimental and statistical procedures, as well as planning and conducting experiments in a way to maximize the scientific benefit. Through keeping a predictable laboratory population of model taxa, one can overall reduce the number of spiders in experiments to the lowest number necessary to achieve experimental goals while ensuring the highest quality standards for the kept animals.

5.4.2 Nutrition

Nutrition importantly influences nearly all aspects of an individual's life history. Starvation and low food quality (i.e. nutrient imbalance and/or deficiency) are independent stressful conditions, and spiders can suffer from one or both (Toft 2013). The amount of food affects the rate of growing, adult size, lifespan and fecundity (Yip and Lubin 2016). Generally, smaller amounts of food increase developmental time and sometimes the number of instars, often leading to smaller adult body size, which in females is positively correlated with the number and/or biomass of produced eggs (Jakob and Dingle 1990; Vollrath 1987). Both prolonged developmental time and smaller and fewer eggs can mean heavy fitness penalties (Higgins 1995; Higgins and Goodnight 2011). Unless the experimental procedure demands otherwise, laboratory-kept spiders are generally fed ad libitum because assuring enough prey is not problematic. When providing laboratory-bred insect food, its diversity is usually lower than most natural conditions. Small instar spiders are typically fed with fruit flies or springtails, and larger spiders with house flies, mealworms and crickets. However, although a small food diversity could imply poor nutrition, it is not necessarily so. Inappropriate prey taxa can have an imbalanced nutrient composition or might even contain toxins and deterrents in too high amounts. Furthermore, an imbalanced nutrient composition and toxicity are not intrinsic to food items but properties of the consumer's physiology, i.e. the specific spider species (Toft 2013).

Food quality is best defined with its potential contribution to the fitness of the consumer. Because a certain prey taxon can be appropriate for some, but not other spider species, food quality cannot easily be determined using a chemical analysis but is best determined in fitness performance experiments. In these, the effect of single-prey diets is compared to high-quality control food and starvation (Toft 2013). Tests of multiple prey taxa have shown a continuum of prey qualities, classified into "high-quality", "intermediate-quality", "low-quality", "poor-quality" and "toxic" prey taxa. Only prey classified as high quality allows spiders on a monotypic diet a full life cycle with low mortality and successful reproduction (Toft 2013). Some prey taxa, e.g. aphids, seem to generally be of very low food quality to spiders, while others, e.g. Diptera and Collembola, seem to cover the whole spectrum of the needed nutritional composition and may be classified as high quality for most spider species (Schmidt et al. 2012; Toft and Wise 1999). A logical advice for proper spider nutrition is thus introducing known high-quality prey taxa even when keeping spiders that have not been nutritionally investigated before. Additional food enrichment can be achieved by keeping spiders on a multi-prey diet or by enriching the food of prey. For example, growth media of prey like fruit flies can be enriched with multiple or single nutrients, e.g. protein, lipids and vitamins. Similarly, adding dog food to the growth medium of prey like springtails, fruit flies, house flies and crickets proved successful (Toft 2013).

In recent years, the effects of nutrient composition on physiology and behaviour of spiders are getting better documented. For example, protein addition increases growth and the building of stabilimenta in the orb weaver *Argiope keyserlingi* (Blamires et al. 2009), while it decreases glue stickiness in the orb weaver *Nephila clavipes* and cob web *Latrodectus hesperus* (Blamires et al. 2014). Also, the protein content in food is positively correlated with growth rate in *Pardosa prativaga* (Jensen et al. 2011), but not in *Stegodyphus lineatus*, where lipids promote growth, but protein addition enhances juvenile survival (Salomon et al. 2008; Salomon et al. 2011). Unfortunately, we currently do not fully understand how nutrient demands differ among spider species and how ontogenetic stage and seasonality affect them intraspecifically. As mentioned above, starvation and nutrient imbalance represent independent stress factors (Toft 2013). In the laboratory, one can easily prevent starvation. However, well-fed spiders, i.e. such with a high body condition index (body mass regressed to body size), are not necessarily also receiving a balanced diet (Lomborg and Toft 2009; Wilder and Rypstra 2008), and there is currently no quick and easy way to assess possible nutrient imbalance. Thus, to ensure the functional definition of high-quality nutrition, laboratory-kept spiders need to be offered food that allows a full life cycle with low mortality and successful reproduction.

5.4.3 *Disease Monitoring and Disposal*

Like all arthropods and most invertebrates, spiders have an open circulatory system, where the haemolymph flows via a closed arterial system and an open venous system (Foelix 2011). Physical injuries are followed by haemolymph loss and expose spiders to microbial infections. Thus, the spider's immune system is composed of haemolymph coagulation and pathogen-defence that is localized in haemocytes. The immune system rapidly reacts to invasion, releasing several compounds into the haemolymph in a matter of minutes to hours, and comprises a clotting cascade to stop haemolymph loss, phagocytosis, the regulation of pathogen-destroying melanin production and the production of antimicrobial peptides (Kuhn-Nentwig and Nentwig 2013). To the best of our knowledge, the effects of diverse laboratory conditions on spider immune system appear unknown. Similarly, disease treatment is not developed, likely in large part because identification of a disease is mostly possible only in its latest stages of conspicuous symptoms or when spider behaviour changes drastically. Researchers thus mostly have to do their best to prevent disease. When choosing enclosure enrichment, it is advisable to clean, dry out or otherwise disinfect any material from nature. Prey should consist of laboratory-bred insects to prevent introducing pathogens or parasites. The enclosures should be kept as clean as possible, and especially when high humidity is necessary, air circulation needs to be ensured to minimize mould. However, the frequency of enclosure cleaning should represent a compromise between the level of required cleanliness and the amount of disturbance imposed to the spider.

Another important consideration is what happens to spiders after they die or after the end of experiments. Spiders that clearly died of disease should be disposed according to local waste handling procedures. On the other hand, it is likely safe to dispose spiders that died naturally as usual biological waste, as research on spiders usually does not include hazards occurring in other fields, e.g. dangerous chemicals (sterilants, disinfectants) and microorganisms, allergens and radiologic agents. If spiders are released after the experiments, they should be released where caught, and in the correct part of season based on their developmental stage. Released spiders should also not be in a state that will harm the natural population (e.g. because of disease or genetic modification).

5.4.4 Anaesthesia and Euthanasia

Due to a lack of research on invertebrate anaesthesia, analgesia and euthanasia, these fields are getting increasingly debated (Bennie et al. 2012; Cooper 2011) and are tightly linked to the issue of how animals perceive stress and pain (see "Spiders' ability to feel pain, distress and suffering"). Anaesthesia in spiders is usually employed to immobilize the animals, in order to facilitate tagging (e.g. for the need of individual recognition in experiments), examination (e.g. to determine the species, check for genital details) and to perform different procedures (e.g. obtain silk or venom). In terrestrial invertebrates, isoflurane (5–10%), sevoflurane, halothane (5–10%) or carbon dioxide (CO_2, 10–20%) are the suggested agents for anaesthesia (Cooper 2011). In our experience, the most common agent in spider laboratories is CO_2. While the suggested concentration of CO_2 is 10–20%, spider laboratories use a wide variety of concentration, always with fast recovery, and without mortality and visible long-term consequences. However, if anaesthetized spiders are used for potentially painful procedures, CO_2 is not advised as it is unknown to what extent, if, any at all, it induces analgesia. In such cases, using isoflurane, halothane or sevoflurane is advised, with isoflurane being the preferred in terrestrial arthropods in general (Cooper 2011) and also successfully employed in spiders (Pizzi 2006). Hypothermia is a traditional anaesthesia method in invertebrate studies but generally of limited use (Cooper 2011) and not advised in spider research. While it might be of use for some noninvasive procedures, it should not be used for invasive ones, and we generally advise against it in arachnids as many seem to not tolerate chilling well and can even die (Pizzi 2006). To avoid complications during anaesthesia, one should avoid employing it in individuals of visibly poor body conditions or otherwise unhealthy.

Some research fields and methods cannot avoid sacrificing a certain number of individuals. If spiders get euthanized, researchers have to state why and describe the method. A "good" death is an inseparable part of a "good" life for all captive animals. A variety of methods of invertebrate euthanasia are described in the literature, ranging from decapitation and injection of chemicals to freezing and immersion in ethanol, but most have not been properly studied (Cooper 2011; Lewbart 2011). Suitable methods of euthanasia need to be effective and simple to perform and cause the least distress to the animal, and they have to be compatible with given research method if performed for that reason (Bennie et al. 2012). For example, Bennie et al. (2012) suggest terrestrial arthropod euthanasia through “targeted hyperkalosis”, i.e. injection of potassium chloride to depolarize the thoracic ganglia, causing rapid death. While this method has been successfully tested on a variety of terrestrial arthropods, including a scorpion, it will likely not be widely used in spiders as many spider species are too small in size, many research fields operate with too many animals for individual injections, or the field conditions would make such a method inconvenient. For spiders, Pizzi (2006) recommends immersion in 70% ethanol and warns against freezing, as the latter can compromise subsequent histological examination. In fact, the most common euthanasia method in spiders is immersion in ethanol as it is compatible with most research fields. In some fields, especially the ones relying on molecular data, freezing is necessary, be it without a medium or submerged in ethanol or buffer. Other fields (e.g. systematics and taxonomy, behavioural sciences) sometimes necessitate the euthanasia of a number of individuals to store as vouchers, without the need for subsequent experiments. In such cases, one should choose the more humane method, even if compromising tissues. For example, when euthanizing spiders to store in ethanol, it might be more humane to first anaesthetize them using CO_2, low percent ethanol or instant deep-freezing (−60 °C to −80 °C) and subsequently transfer them into ethanol for preservation. In fact, a recent study shows a two-step method of first anaesthetizing gastropods in 5% ethanol for later preservation in 70–95% ethanol, to be the most appropriate (Gilbertson and Wyatt 2016). Unfortunately, as is true for most invertebrates in general, euthanasia methods for spiders are inadequately researched and necessitate much more attention.

5.5 Conclusions

Spiders are often used as research models and for biodiversity displays while also kept as pets. Due to anthropocentric views on invertebrates, spiders are historically considered as *automata*, but the growing evidence shows that spiders are not purely instinctive but exhibit behavioural plasticity including learning. This evidence must not be further ignored, and setting the standards for securing spiders’ welfare in captivity is needed, in order to comprehend (1) if/when spider feel pain and distress and (2) what we can do to avoid or minimize it.

1. Humans commonly regard spiders as animals without the capacity to experience pain, and their responses are considered as purely nociceptive reflexes. This reasoning is based on the fact that spiders lack the brain regions implicated in pain processing found in higher vertebrates. They might, however, possess

neurobiological mechanisms for experiencing pain different than vertebrates. Recent definitions of a possible pain experience include criteria such as appropriate nociceptors and a central nervous system, physiological changes to noxious stimuli, protective motor reactions that might include reduced use of an affected area (e.g. limping, rubbing, holding, autotomy), avoidance learning and cognitive abilities (Elwood 2011; Sneddon et al. 2014). We present here what criteria for potential experience of pain have been found in spiders.

In spiders, little is known about the presence of nociceptors and how nociceptive information is processed within their central nervous system. Besides exhibiting withdrawal or escape behaviours when exposed to noxious stimuli, spider avoidance includes behaviours that may not simply be nociceptive reflexes, e.g. jumping, shaking web, autotomy and thanatosis. Spiders detect predators or other potential aversive stimuli by airborne vibrations stimulating their trichobothria. Increased trichobothria stimulation relates to increased levels of octopamine, which is considered analogous to the vertebrate norepinephrine, indicating its role in the stress system in spiders. Spiders also exhibit protective motor reactions. They commonly autotomize their legs when in dangerous situation, e.g. grasped by a predator, in order to escape. Furthermore, they self-amputate injured appendages, and they "lick" or rub their wounds. An interesting experiment by Eisner and Camazine (1983) even suggests a concordance between pain effects in humans and autotomy in spiders. There is also abundant evidence for avoidance learning in spiders. Studies show that spiders are able to learn and take the appropriate action to avoid or reduce potential damage on the basis of prior experience with noxious stimuli. Such a response is not the fixed, reflexive action of nociceptive avoidance. Furthermore, there is ample evidence of the ability of spiders to learn. Several species are able to modify their behaviour in contexts of foraging and web building, intraspecific interactions, spatial learning and navigation.

2. To secure animal well-being in captivity, we have to satisfy their general needs. Thus, spiders need access to fresh water and a balanced nutrition and appropriate housing with biologically meaningful enrichments, and keeping should prevent pain, injury and disease, all of which enable an environment to express normal behaviours and ensure that conditions and treatments cause no/little fear and stress. In spider research, we need to follow the general ethical guidelines for animal care, i.e. the "3Rs principle". However, researchers using spiders as experimental subject need to follow the general reduction and refinement guidelines, while the replacement guideline (i.e. replacing animals with tissue cultures and computer models) is currently likely not applicable.

Additionally, some research fields cannot avoid anaesthetizing and/or sacrificing spiders. In spiders, using CO_2 for anaesthesia generally seems to enable fast recovery and no visible long-term consequences, while for potentially painful procedures, isoflurane, halothane or sevoflurane is preferred. The most common euthanasia method in spiders is immersion in ethanol as it is compatible with most research fields, but when the research protocol permits it, one should choose a more humane method. For example, when euthanizing spiders to store in ethanol, it might be more humane to first anaesthetize them using CO_2, low percent ethanol or instant deep-freezing and subsequently transfer them into ethanol for preservation.

While there are well-defined ethical guidelines for using vertebrates in research, welfare in invertebrates is minimally regulated and generally neglected by researchers. To address the numerous knowledge gaps in our understanding of welfare in invertebrates, we first need to set clearly defined criteria of how to assess experiences of pain and suffering in a given invertebrate group. Such definitions will allow us to set clearly defined hypotheses that can be experimentally tested. Despite this need for research, by following the above guidelines, we can avoid at least the known sources of distress in spiders.

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Chapter 6
Coral and Cnidarian Welfare in a Changing Sea

Ernesto Weil, Adriana Weil-Allen, and Alejandro Weil

Abstract Coral reefs worldwide are currently threatened by anthropogenic Global Climate Change (GCC) and local environmental degradation and, unequivocally, need protection. Coral reefs constitute one of the oldest, most diverse, and important marine communities. They are mainly formed by tiny, primitive, calcifying, Cnidarian invertebrates, the scleractinian corals, and provide substantial ecological services to other marine communities, coastal protection, food, and economic and social benefits to humans. Cnidarians and other reef invertebrates are exploited by the marine aquarium trade, but their capture, transport, and maintenance in captivity (for research or exhibition) are not regulated by any welfare provisions. Traditional principles of animal welfare are not easily applicable to wildlife, much less to simpler organisms such as cnidarians, but arguments could be made since scleractinian corals, as most invertebrates, are highly sensitive to changes in environmental conditions and display stressful physiological and/or behavioral responses. Higher than normal temperatures, for example, elicit the expulsion of their algal symbionts (i.e., bleaching), increase mucus production, and/or adjust metabolic pathways and physiological functions, to enhance survivorship. Global Climate Change is stressing marine animals and is threatening the health of the oceans. Welfare considerations to at least those cnidarians that function as foundation or keystone species could add up and help protect these communities from further decline. How we approach the solutions to the problems generated by the increasing human needs must include a change in attitude, from being mostly "reactive," which is costly and difficult, to being more preventive/proactive. We believe that approaches combining both conservation and welfare principles could be developed and implemented to increase the survivorship and good health of ecologically and economically important marine invertebrates. Besides convincing scientists, and mostly animal welfare scientists, that corals should be included in our "circle of compassion," the most essential

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E. Weil (✉) · A. Weil-Allen
Department of Marine Science, University of Puerto Rico, Mayaguez, PR, USA
e-mail: ernesto.weil@upr.edu

A. Weil
Center for Biospecimen Research and Development, NYU Langone Health, New York, NY, USA

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component for this to work is education. An educated population who understand the importance of our interaction with the natural world will help to institutionalize welfare principles to increase protection and to reduce, or stop, the current declining trends of coral reefs and other marine communities. This would enhance the possibilities of a successful recovery of these important communities so we can continue using them in a sustainable way and, more importantly, preserve them for future generations.

6.1 Introduction

Since the appearance of humans, we have been interacting with other animal and plant species in many different ways (parasite-host, predator-prey, competition, harvesters and domestication, etc.). Animal welfare has been a concern for thousands of years, for example, during the long process of domestication, different cultures and religions developed their own regulations for the animals they deemed important (Adams and Larson 2011). The ethical aspect about the quality of life (their well-being) of animals, however, has only been emphasized within the past century and has become the subject of public scrutiny and controversy. The investigation of animal welfare using rigorous scientific methods is a relatively recent development. The Animal Protection Act (1822) in the United Kingdom was the first national law developed to protect farm animals. It was followed by the Cruelty to Animals Act (1866), the first national bill to regulate animal experimentation (Fraser et al. 1997). Numerous countries in Europe followed with regulations regarding research with animals. The United States reacted almost 100 years later with the Animal Welfare Act in 1966, which is the only Federal law that regulates the treatment of animals in farms, research, exhibition, transport, and by dealers (Stevens 1990; Harvey-Clark 2007).

There is no universal definition for animal welfare, as it can vary depending on cultural, religious, social, or scientific context. However, three main aspects and ethical concerns are commonly expressed regarding the quality of life of an animal: (1) their physical health, (2) their subjective state, and (3) their natural life. At least two of these are applicable to invertebrates, but there are limitations on how to assess them and interpret the results and their validity. How to differentiate between fear vs. excitement and pain vs. stress, for example, or establish the animal's mental state can be harder in vertebrates and may not apply to invertebrates. The third state considered is their natural life, which assesses the ability of animals to perform their natural functions, behaviors, and capabilities in captivity and in their natural habitat (Fraser et al. 1997). The American Veterinary Medical Association (AVMA) regards animal welfare as a human responsibility that requires analyzing how animals cope with their living conditions, which includes and considers all aspects of animal well-being (housing, management, nutrition, disease prevention and treatment, responsible care, humane handling, and, when necessary, humane euthanasia). The AVMA defines animal welfare as: "An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear, and distress" (https://www.avma.org/public/AnimalWelfare/Pages/default.aspx).

Most of the abovementioned considerations, however, are exclusively applied to domesticated animals, which include mostly mammals and birds. When dealing with wildlife species and invertebrates in particular, applying the AVMA principles is difficult. There are several limitations and information gaps; for example, information on their biology, ecology, physiology, behavior, and even geographic distribution is usually limited at best, and direct observations and/or sample collection are difficult and costly. Wildlife welfare is mostly based and categorized within ecological and conservation measures rather than ethical/humanitarian considerations, and legislation efforts are usually in response to species' drastic population declines due to environmental degradation, habitat destruction, and/or overharvesting and aimed at the conservation and survival of the species (Tables 6.1 and 6.2, Fig. 6.1)

Table 6.1 Marine invertebrates (25 scleractinian corals and two abalones) that are either threatened (T) or endangered (E) according to ESA, their geographic distribution, and drivers responsible for the significant population declines (signs)

Phylum	Species	Dis	Status	Drivers
Cnidaria	*Acropora palmata*	CA	T	High temp./disease/habitat loss
Cnidaria	*Acropora cervicornis*	CA	T	High temp./disease/habitat loss
Cnidaria	*Orbicella annularis*	CA	T	High temp./disease
Cnidaria	*Orbicella faveolata*	CA	T	High temp./disease
Cnidaria	*Orbicella franksi*	CA	T	High temp./disease
Cnidaria	*Dendrogyra cylindrus*	CA	T	High temp./disease
Cnidaria	*Mycetophyllia ferox*	CA	T	High temp./disease
Cnidaria	*Acropora globiceps*	PA	T	High temp./disease/predation
Cnidaria	*Acropora jacquelineae*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Acropora lokani*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Acropora pharaonis*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Acropora rudis*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Acropora speciosa*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Acropora retusa*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Acropora tenella*	PA	T	High temp./disease/predation/habitat loss
Cnidaria	*Anacropora spinosa*	PA	T	High temp./disease
Cnidaria	*Cantharellus noumeae*	PA	E	Mining/sedimentation/habitat loss
Cnidaria	*Euphyllia paradivisa*	PA	T	Harvesting/ High temp/disease
Cnidaria	*Montipora australiensis*	PA	T	High temp./predation/disease
Cnidaria	*Pavona diffluens*	PA	T	High temp./disease
Cnidaria	*Porites napopora*	PA	T	Harvesting/disease
Cnidaria	*Seriatopora aculeata*	PA	T	High temp./disease
Cnidaria	*Siderastrea glynni*	PA	E	High temp./disease/coastal development
Cnidaria	*Tubastraea floreana*	PA	E	Possibly high temperatures
Cnidaria	*Isopora crateriformis*	PA	T	High temp./predation/disease
Mollusca	*Haliotis cracherodii*	NPA	E	Overharvesting
Mollusca	*Haliotis sorenseni*	NPA	E	Overharvesting

CA Caribbean, *PA* Pacific, *NPA* Northern Pacific

Table 6.2 Invertebrates "protected" by the CITES agreement

Phylum	Class	Number of species on list
Mollusca	Bivalvia	31
Mollusca	Cephalopoda	1
Mollusca	Gastropoda	4
Cnidaria	Anthozoa	All (over 6000)
Cnidaria	Hydrozoa	All (over 3800)
Echinodermata	Holothuroidea	1

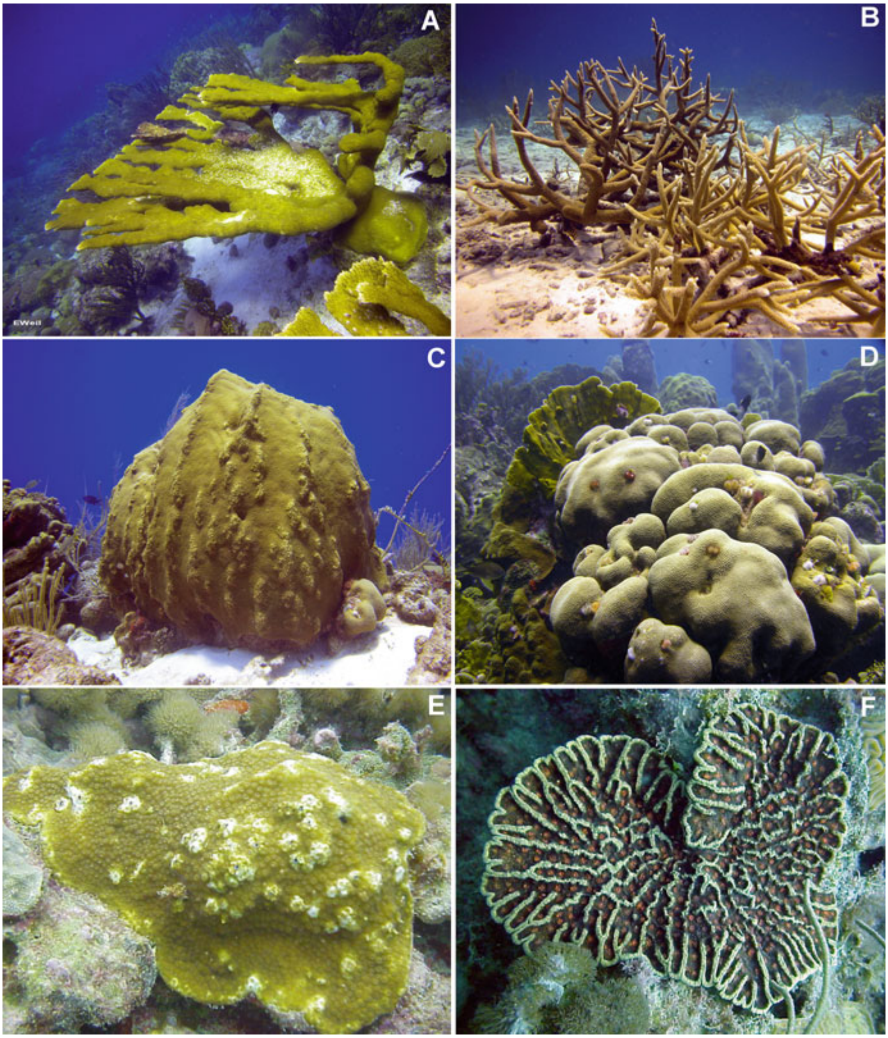

Fig. 6.1 Caribbean scleractinian corals listed as threatened and/or endangered by ESA. (**a**) *Acropora palmata*; (**b**) *A. cervicornis*; (**c**) *Orbicella faveolata*; (**d**) *O. annularis*; (**e**) *O. franksi*; (**f**) *Mycetophyllia ferox* (Photos E. Weil). Caribbean coral *Dendrogyra cylindrus* (**g**) and abalone species from the Northeastern Pacific, *Haliotis cracherodii* (**h**) and *Haliotis sorenseni* (**i**) listed as threatened species by ESA (Photos a to g by e. Weil; photos h and i from CITES webpage)

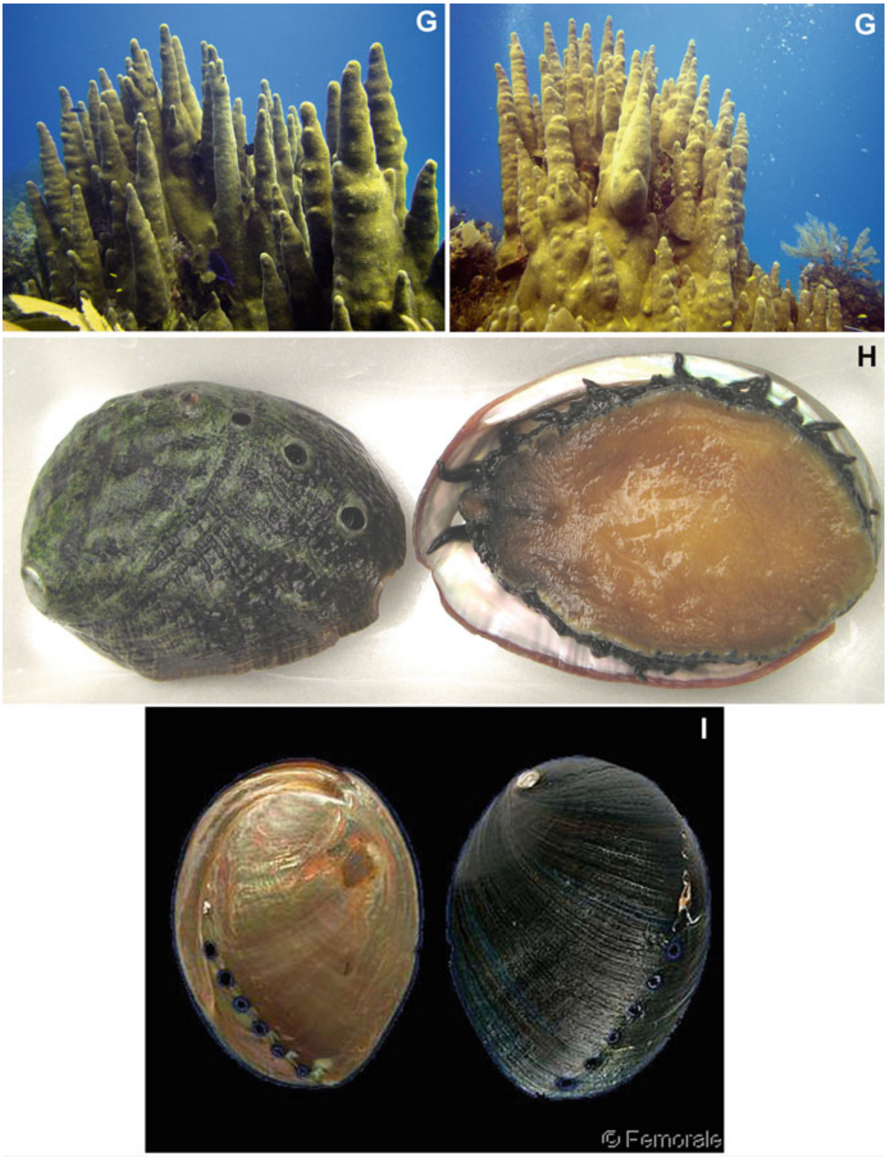

Fig. 6.1 (continued)

(Paquet and Darimont 2010). This gap between ecology and ethical/humanitarian principles should be eliminated or at least narrowed as to include welfare principles into any wildlife conservation and management plans, which will at least improve survivorship of the species involved.

Most conservation scientists now agree that human-induced destruction and deterioration of wildlife habitats and environments are characterized by a general disregard for the affected organisms living in those habitats, more so if they are invertebrates, which mostly result from lack of education and inadequate scientific and ethical guidance. Current anthropogenic-induced environmental and habitat deterioration is causing physiological stress and possibly "pain" to many important marine invertebrate species through thermal anomalies, floods, chemical imbalances (i.e., insecticides, fertilizers, hormones, untreated sewage, ocean acidification), displacement, starvation, physical injury, and disease, all traits included in the AVMA principles for animal welfare (Harvell et al. 2002; Bekoff 2002; Goodall and Bekoff 2002; Bradshaw et al. 2005; Bruno et al. 2007; Hoegh-Guldberg et al. 2007; van Oppen and Lough 2009; Paquet and Darimont 2010; Dubinsky and Stambler 2011; Horvath et al. 2013; Woodley et al. 2016; Weil et al. 2017).

The great majority of multicellular animals living on the planet are invertebrates. Many are parasites or pests, but a wide variety provide important ecological services at many different levels, and humans use them for food, agriculture, and commercial products, as pets and in research. However, contrary to their vertebrate counterparts, there are hardly any specific welfare considerations or laws to protect at least those beneficial invertebrates. With the exception of cephalopods, which are extensively used in research, marine invertebrates are only "protected" by local/international, ecologically based conservation regulations (i.e., marine protected areas, the Endangered Species Act, the IUCN Global Red List and Species Program, etc.), which mostly protect threatened/endangered species and their critical habitats to enhance survivorship. The Convention on International Trade in Endangered Species (CITES) is supposed to control collection, movement, and trade of wild species across international borders.

6.2 Cnidarian Welfare

Coral reefs constitute one of the oldest, most diverse, and important marine communities. They are mainly formed by the scleractinian corals, a group of tiny, primitive, simple, calcifying invertebrate organisms in the phylum Cnidaria (Coelenterates), which provide substantial ecological services to other marine communities, coastal protection, food, and economic and social benefits to humans. The colorful Cnidarians are known as the "flowers of the sea" because of their shapes and bright colors. One of the oldest animal groups on the planet (at least 490 million years) survived four of the major mass extinction events in the history of life (Wood 1999; Park et al. 2012). It is the only metazoan group with true radial symmetry and the first with organized tissue layers, nerve networks, and a gastrovascular cavity (coelenteron), an adaptation that allows internal digestion of large prey. Capture of large prey is possible due to their unique, large stinging cells called cnidocytes (nematocysts), a diagnostic trait for the phylum that packs a coiled thread with a harpoon-like tip and potent neurotoxins under hydrostatic pressure. They are also used for defense and protection. Cnidaria is considered the sister group to the bilateria (bilateral symmetry) and comprises two reciprocally monophyletic clades with six classes including over 10,000 of mostly marine species distributed across all oceans and depths. They are solitary or colonial (modular), sessile, and/or free-living animals (alternant generations), reproduce both asexually and sexually, and are important members of most marine communities. Several groups within the Cnidaria are ecologically very important: the hard corals (scleractinian), milleporids (hydrocorals), and octocorals (Alcionacea, octocorallia), for example, comprise foundation species that build complex, stable, three-dimensional, hard structures of calcium carbonate and/or protein (gorgonin) that provide habitat, energy, and resources to thousands of other species across tropical and temperate marine habitats and down to 6000 m deep. They protect coastal areas and are important touristic, research, and educational assets (Kellert 1993; Veron et al. 2009; Dubinsky and Stambler 2011; Horvath et al. 2013; Birkeland 2015; Hubbard et al. 2016).

Despite their ecological/economic importance, no specific welfare regulations are in place for cnidarians or any other important invertebrates. They are under some level of protection by the Endangered Species Act (ESA) and the Convention on International Trade in Endangered Species (CITES), which are based on ecological and economic rather than ethical or humanitarian arguments (Jones et al. 2017). Most of the American Veterinary Medical Association (AVMA) principles do not apply to the great majority of marine invertebrates, and assumptions that invertebrates do not experience pain and/or stress (Elwood 2019), while lacking the capacity for higher order cognitive functions, are usually used as justification for the lack of welfare consideration for invertebrates in general (Horvath et al. 2013). This is somehow reinforced by the some negative view and perception of many invertebrates by the public. Many people express feelings of aversion or fear toward most invertebrates due to concerns of disease (carriers, vectors), poisonous and painful stings, pests that eat people's food, or by being "unattractive" animals among others (Horvath et al. 2013). Even the scientific community has minimal ethical concerns for invertebrates they use in research, making them easier to use as lab models for many experiments instead of vertebrate subjects, which receive far greater ethical considerations (Vitale and Pollo 2018, Chap. 2; Kellert 1993; Mather 2001; Manev and Dimitrijevic 2004; Mather and Anderson 2007; Andrews et al. 2011; Horvath et al. 2013). This is slowly changing but unfortunately only for a few species, like cephalopods and some crustaceans. After extensive research in physiological, cellular processes, neuronal and behavioral responses, and stress resistance to environmental changes, results indicate that these invertebrates may be just as able as vertebrates to experience pain and stress and display comparable cognitive capacities (Horvath et al. 2013; Mather and Anderson 2007; Mather et al. 2010; Elwood et al. 2009; Horvath et al. 2013). To this day, cephalopods are the only invertebrates that have been included in welfare legislations related to the protection of animals used for scientific purposes (Ponte et al. 2018, Chap. 9); European Union Directive 2010/63/UE; Australian Code for Research Animals; Andrews et al. 2011). The remaining invertebrates used in research not included or not having been studied for adequate protection or welfare policies remain unprotected and to the discretion of the collectors, transporters, and scientists involved. On the other hand, thousands of Cnidarian species and other marine invertebrates are captured, transported, and sold at pet shops every year or used in large aquariums and exhibitions, with no ethical considerations for how these animals must be treated in each one of these steps, with a high mortality rate (Jones et al. 2017).

Scientific-based wildlife conservation seems to be a good approach to draw attention to invertebrate welfare, especially to those species and groups that are foundation species (such as scleractinian corals, octocorals, oysters, sponges, etc.) providing habitat, refuge, food, and other important ecological services and benefits to humans. There is no one global organization/association or law that protects all aspects of wild animals and plants, but there are a few local government regulations aimed at the protection of individual species and/or their essential habitats and ecosystems. In the United States, a significant piece of legislation to protect wild habitats and species is The Endangered Species Act (ESA) of 1973 (NOAA). It provides for "the conservation of species that are endangered or threatened throughout all or a significant portion of their distribution range, and for the conservation of the ecosystems on which they depend." The key signs include significant population declines over their geographic distribution and loss of critical habitat. Listing species is a complicated and long task, requiring the participation of scientists and managers who have to justify the request with actual quantitative data on top of extensive qualitative observations, which requires long temporal observations and data gathering. Unfortunately temporal scales generally work against invertebrate species that are short-lived (short generation times) and have small sizes and/or small population sizes, one reason why we are losing so many species nowadays. Only 2 commercial abalones (mollusks) and 25 scleractinian corals (8 from the Caribbean and 17 from the Indo-Pacific) are listed as either threatened or endangered under the ESA (Tables 6.1 and 6.2, Fig. 6.1).

Other countries have similar legislations that have helped to establish some sort of a protection "network" with minimal "welfare" policies for wildlife (Great Britain, the European Union, China). One of the very few global approaches to conservation/protection of wildlife is the IUCN Global Species Program and the IUCN Species Survival Commission, sponsored by the United Nations. Their goal is "to assess the conservation status of species, subspecies, varieties, and even selected subpopulations on a global scale" to provide information on conservation status and population distribution (densities, health conditions, etc.) in order to highlight taxa that are endangered and/or threatened with extinction and thereby promote their protection and conservation to enhance their survivorship (The IUCN Red List of Threatened Species. IUCN) (Table 6.2). In some way this program provides valuable information that is used by ESA to list species. It seems logical that principles of "animal welfare" could be included for both the IUCN and ESA to complement the other protection/conservation aspects and insure the welfare and survivorship of threatened and/or endangered species at least. Unfortunately they seem to act more as witnesses rather than guardians to animal welfare and conservation status and are more reactionary rather than advocating preventive/proactive actions.

The annual trade in wildlife animals, including invertebrates, is increasing constantly (Table 6.2 and Fig. 6.2), representing a major threat to wildlife populations, even without considering the major problem of illegal trade. The Convention on International Trade in Endangered Species (CITES) works with ESA and the IUCN

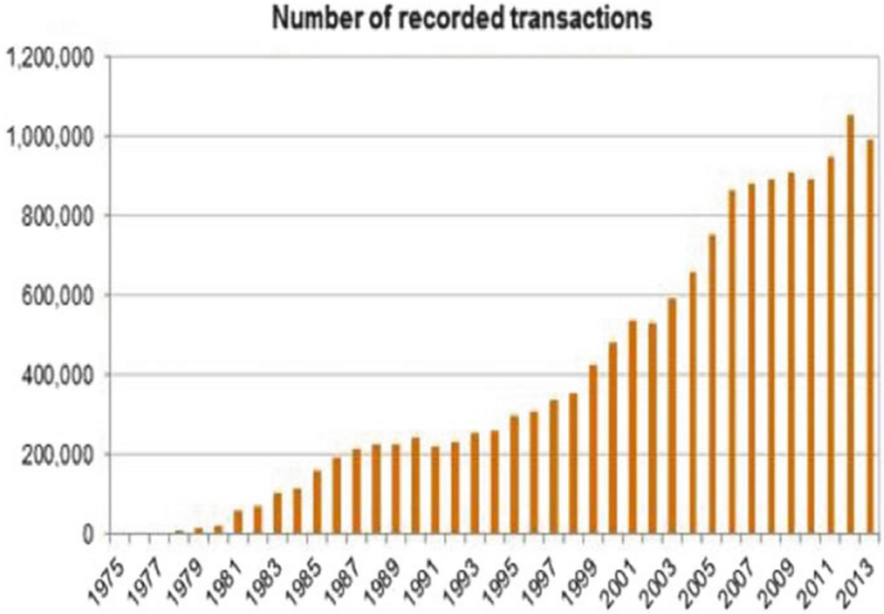

Fig. 6.2 Increment in the total number of recorded CITES transactions per year until 2013. (https://www.cites.org/)

to regulate movement and trade of wildlife. The marine aquarium trade, for example, moves thousands of marine invertebrates every year, most of these under stressful conditions that end up with high mortalities (Bruckner 2000; Tissot et al. 2010; Mason 2010; Rhyne et al. 2013). The only regulations on protection are restricted to species that are deemed threatened or endangered by the ESA and the IUCN red list or other agencies in other countries. There are roughly 5600 species of animals and 30,000 species of plants "protected" by CITES that establishes a global regulatory framework for the prevention of trade in endangered species and for the effective monitoring and regulation of trade in species that are not necessarily threatened with extinction but may become so unless trade is strictly controlled. Today, there are 183 country members that are bound by the provisions of CITES, but there is little information about enforcement of these provisions.

The CITES list includes whole groups of organisms (i.e., primates, cetaceans, sea turtles, Anthozoa), individual species or subspecies, that have regulations over their international trade only, but not their local welfare or conservation, which should be the responsibility of each member country. In principle, the CITES requirements are designed to ensure sustainable harvest; in practice, countries may be unable to make a science-based finding of no detriment because of limited resources and expertise. Therefore, CITES regulations allow an importing country to implement additional restrictions or to require additional documentation to enhance conservation of wildlife populations (Bruckner 2000; Shuman et al. 2005). However, there are no ethical considerations on how the harvest should be done with minimal distress and pain, how the invertebrates must be cared for during transportation and in confinement, or how to treat them during research activities. In general, the IUCN Global Species Program Red List, ESA, and CITES are a good start to providing a foundation for future wildlife protection efforts for invertebrates and marine invertebrates in particular. Ideally, this should be global and supported by all countries given the connectivity across marine ecosystems and not restricted to geopolitical borders since the geographic distribution of species and/or ecosystems transcends these borders. Even though protecting habitats and species is beneficial for everybody, agreements are difficult to reach in most situations due to cultural, religious, and social/political or economic differences.

These are just a few examples of how countries can agree on animal and plant welfare principles without the need for international laws and courts. Figure 6.2 shows why organizations and agreements such as CITES are needed. It shows the documented trade of flora and fauna between countries over time. The number of transactions has increased significantly over time, and while there is no guarantee that this will continue to be the case, with over a million recorded transactions in 2012, the need for clear establishment of specific animal welfare regulations is apparent. Ensuring a sustainable trade in coral reef organisms, for example, will require long-term international commitment to a policy that protects them from overharvesting and completely bans destructive harvest practices. The situation, however, is probably more critical when the illegal trade is taken into consideration.

A key first step is for exporting and importing countries to establish accurate data gathering and monitoring systems so that species-specific information is reliable. This would include the numbers of organisms captured and traded and the extent of their survival from harvest to consumer (Bruckner 2000). The only group of marine invertebrates included in the CITES list are the scleractinian corals because of their ecological and economic importance and their recent drastic decline at local and geographic scales. This is mostly due to disease outbreaks and bleaching linked to Global Climate Change, overfishing, and local anthropogenic-induced environmental deterioration (Harvell et al. 1999, 2002; Bruno and Selig 2007; Rosenberg and Loya 2004; Wilkinson 2004; Wilkinson and Souter 2008; Hoegh-Guldberg et al. 2007; Hoegh-Guldberg and Bruno 2010; Carpenter et al. 2008; Aronson et al. 2008a, b; Miller et al. 2009; Weil et al. 2009; Weil and Rogers 2011; Jackson et al. 2014; Mumby and Van Woesik 2014).

A common concern difficult to interpret when discussing invertebrate welfare is that of the cause (stimulus) of pain and suffering and the anthropomorphic interpretation of the "sensory" mechanisms and the physiological and/or behavioral response. Deciding how to interpret an invertebrate's response to noxious, stressful, or "painful" stimulus can be difficult, speculative, and highly variable across species, especially when differentiating between a nociceptive (reflexive) and pain-related (sensory and emotional) responses (Elwood 2019; Kellert 1993; Fraser et al. 1997; Elwood et al. 2009; Elwood 2011; Adamo 2012; Horvath et al. 2013). For example, interpreting the response to a "painful" stimulus when presented to an octopus will contrast significantly when applied to a coral or a sea anemone, since their sensory cells, "nerve" networks, and stimuli transduction are different. Just because the octopus can meet the criteria of sensing the stimuli, removing itself away from it and potentially learning to avoid it, does not mean that other invertebrates have the same capacity, or could not respond to it in different ways (Carere et al. 2011; Elwood 2011; Crook 2013). Evolutionarily speaking, it is logical to assume that most organisms must have the necessary receptors to "sense" changes in their surroundings (environment, predators, competitors) that could threaten their survival in order for an appropriate response to be elicited. Sensory cells and/or mechanisms associated with this are highly adaptive and surely evolved early in the history of life.

Most cnidarians (i.e., Anthozoa and Hydrozoa, 9800 species) are modular (colonial) and sessile. Modular sessile organisms had a different evolutionary history and life history traits compared to the free-living cnidarians and to non-modular, motile invertebrates. They live attached to the substratum and do not have the option of moving away from any stressful, noxious stimuli (i.e., high temperature), retracting polyps to avoid predators or expanding them to compete for substrate (Fig. 6.3). The individual modules, the polyps, have a limited number of responses to prevent/reduce injury (mortality) when threatened or when under stress by changing environmental conditions (Goffredo and Dubinsky 2017).

Can the capacity to sense and respond to stress in their way be used as arguments to provide them with welfare considerations? Cnidarians have rudimentary sensory cells capable of responding to stressful and noxious stimuli and an efficient nerve network that can transmit the stimuli bidirectionally very fast. These cells are naked (no myelin) and do not form any central nervous system. The structural array varies across classes with some showing nerve concentration that look like "ganglia." Sensory and motor "neurons" are spread throughout the individual polyps and colonies allowing for quick muscular contractions and expansions or a cascade of other adaptive responses in corals and other modular cnidarians. These colonies are formed by aggregations of thousands of individual polyps (modules) that are physiologically connected (tissues). Communication between nerve cells occurs by

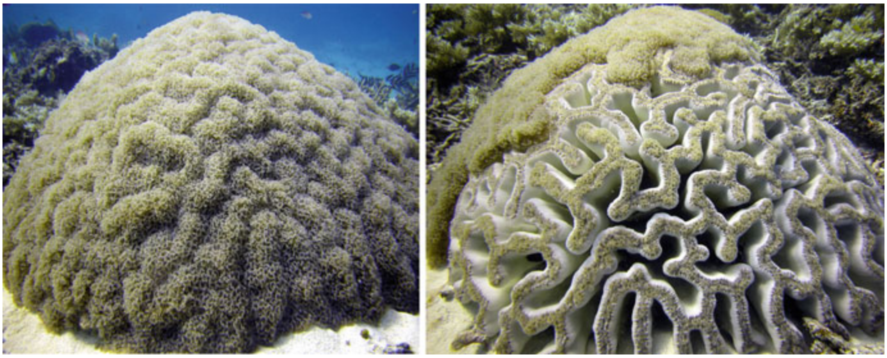

Fig. 6.3 Protection response by a coral colony. Polyps were fully exposed to gather sunlight (left), but they quickly retracted into their calices in a continuous fashion across the colony revealing a curious structure after the diver touched the lower right side. The whole process lasted less than 30 s (Photos E. Weil)

chemical synapses or gap junctions in hydrozoans, though gap junctions are not present in all groups (Galliot et al. 2009). Cnidarians have many of the same neurotransmitters that most of the more advanced metazoans have, including glutamate, GABA, and acetylcholine (Kass-Simon and Pierobon 2007).

Beside fast responses to stimulus like touch (pressure) (Fig. 6.3), Cnidarians show quick responses to changes in environmental stimuli (i.e., changing temperatures and light conditions, chemical imbalances, pH, salinity, sedimentation, etc.) that may threaten their survivorship. Some of the common visible adaptive responses include modular retraction, nematocysts discharge, polyp swelling, hyper-production of mucus, expulsion of zooxanthellae symbionts (bleaching), and immune responses like melanization (Fig. 6.4). They also experience changes in metabolic pathways and/or physiological functions when under stress (slow growth, reduced immune responses, decline in reproductive output, etc.) to distribute energy and resources to maintain basic functioning and increase survivorship (Szmant and Gassman 1990; Petes et al. 2003; Flynn and Weil 2009; Mydlarz et al. 2006, 2008; Galliot et al. 2009; Couch et al. 2013; Morgan et al. 2015; Fuess et al. 2017). The question then becomes whether this level of physiological response to environmental or anthropogenic-induced stressors is enough to consider ethical arguments to protect these organisms? Conservation measures do seem to provide some level of protection at the population and/or habitat levels, but they are only applied when there are strong indications (quantitative evidence) that population densities have declined significantly or the habitat is being destroyed.

A different situation is that of commercially important species that suffer from overharvesting (i.e., aquarium trade), and protection measures are imposed as a consequence of the lack of profitability rather than to any of the population, ecological or “welfare” principles (Horvath et al. 2013, Lafferty et al. 2015). Because natural habitat deterioration is increasing as a consequence of human activities and Global Climate Change (GCC), the scope of species conservation/protection nowadays includes the habitat(s) where the endangered/threatened species live to safeguard the very resources they need for survival. In most cases, however, the protective, conservation regulations have been applied only after drastic ecological consequences (reactive rather than preventive), such as significant population declines of foundation or keystone species, habitat quality degradation, pollution, or disease outbreaks (Gardner et al. 2003; Wilkinson 2004; Wilkinson and Souter 2008; Jackson et al. 2014; Jones et al. 2017).

Approximately 75% of the world’s coral reefs are considered threatened when local threats are combined with GCC threats. One common instrument to protect marine habitats and species is the designation of Marine Protected Areas (MPAs), where humans are not allowed or their activities strictly managed to keep the communities and their environment as undisturbed as possible. There are at least 400 MPAs that include coral reefs in more than 65 countries and territories. This would be encouraging if not for the fact that only a small percentage of these (23%) are well managed with sound conservation and usage regulations and enforcement (Burke et al. 2011; Jones et al. 2017). Besides the large number of countries and important reef regions with no formal protection for their coral reef communities,

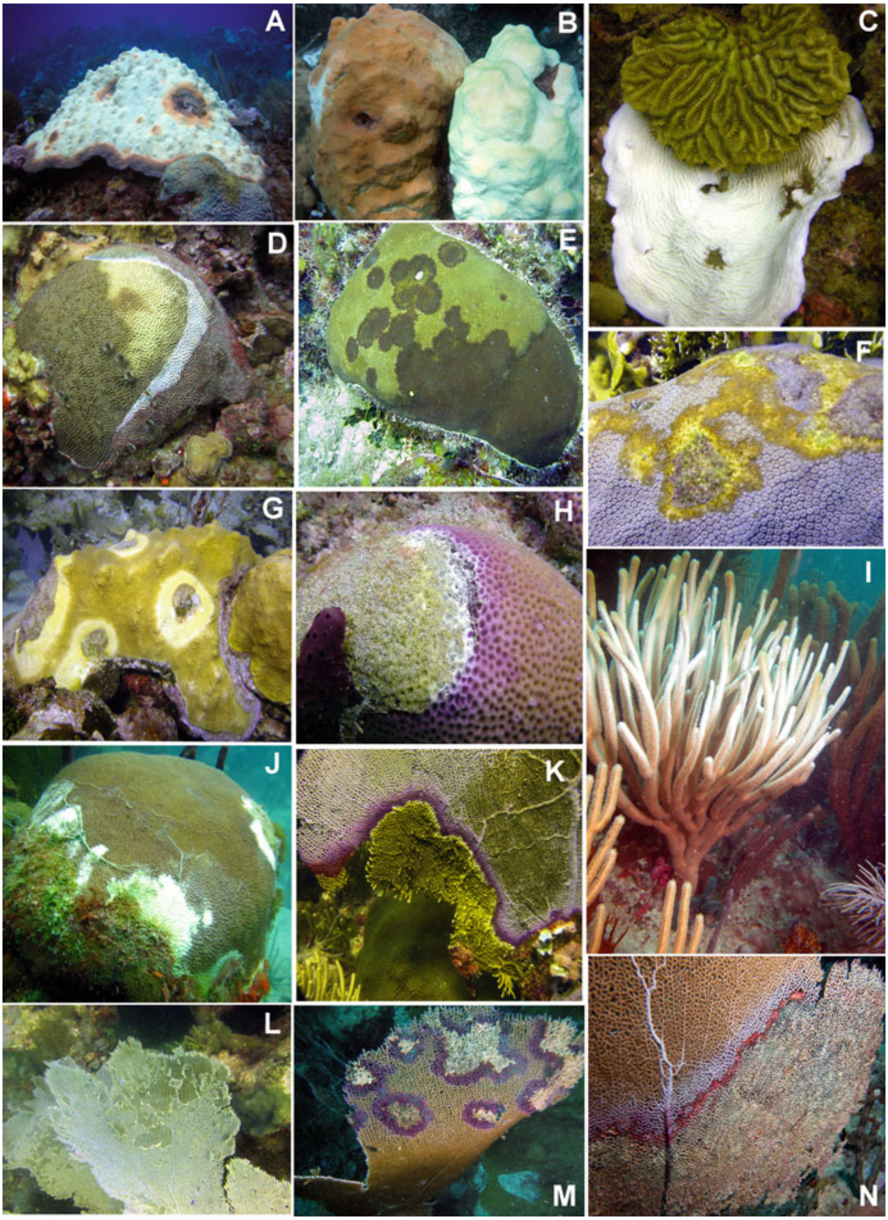

Fig. 6.4 Examples of stress and nociceptive (“pain”) responses in Cnidarians. High temperatures induce the expulsion of zooxanthellae in corals and octocorals (i.e., bleaching) (**a–c, i**), with some colonies more susceptible than others (**b, c**); bacterial and fungal diseases produce immune responses that are visible at the edge of the advancing dying tissue as in white plague (**d, j**), dark spots (**e, f**), Caribbean yellow band (**g**), and black band disease (**h**). The sea fan *G. ventalina* first response is usually melanization (purple band) as in aspergillosis (**m**) and red band disease (**n**). Increased production of mucus is another protective response to noxious stimuli (**j, l**) (Photos E. Weil)

there are geographic and regional gaps and underrepresented areas that must be included in a global network of coral reef MPAs that enhances genetic connectivity. Such a network could provide the needed framework for the inclusion of welfare principles into the conservation/protection regulations for scleractinian corals and other important invertebrate groups on a local, regional, and global scale.

6.3 Cnidarian Conservation and Welfare in a Changing Sea

Observed trends of climate change and modeling predictions have shown that anthropogenic-driven (increasing CO_2 and other greenhouse concentrations) fast climate change will have unprecedented impacts on terrestrial and marine biodiversity in the near future with significant negative socioeconomic consequences across local and regional scales (Michener et al. 1997; Still et al. 1999; Mimura 1999; Stern 2006; Nicholls et al. 2008; Bonan 2008; Hoegh-Guldberg et al. 2007; Veron et al. 2009; Nicholls and Cazenave 2010; Bellard et al. 2012; Chindarkar 2012; Lane et al. 2013; NRC 2010; IPCC 2014; Scarponi et al. 2017). Human disturbances, inadvertent and intended, continue to threaten the survival of species and the maintenance of natural ecological and evolutionary processes worldwide (Parmesan 2006; Wilson 2006; Smith and Bernatchez 2008; Darimont et al. 2009; Jones et al. 2017). One of the most significant impacts of thermal anomalies associated with GCC is the alteration of organismal body temperatures, a stressful condition, which ultimately drives almost all physiological processes and responses, including growth and reproduction. Prolonged high and long thermal anomalies over weeks or months could cause corals to die from "heat stroke" (i.e., metabolic failure), starvation, infectious diseases, and/or other causes related to high temperature stress and low energy availability. The compromised-host hypothesis (sensu Rosenberg and Ben-Haim 2002) suggests that rising ocean temperatures may increase the number and prevalence (proportion of diseased individuals in a population) of diseases by making marine invertebrates more susceptible to ubiquitous pathogens or by causing shifts in resident microbial communities turning some of them pathogenic or more virulent. Increasing demands for colorful coral species and other coral reef invertebrates for the aquarium trade add more pressure on natural populations since captive coral cultures do not produce enough to satisfy the demand. This trade, as mentioned above, has no particular regulations on the welfare of the taxa involved during capture, transportation, and sales.

Coral reefs are one of the oldest ecosystems on Earth. Following the Permo-Triassic mass extinction event (251 MYA) and the evolution of the symbiosis with zooxanthellae, scleractinian corals have been the major builders of these impressive structures, the largest living structures on Earth (Goffredo and Dubinsky 2016; Hubbard et al. 2016; Rossi et al. 2017). Coral reefs have the highest biodiversity of all marine ecosystems and provide important ecological goods and services to other important tropical coastal communities, to the oceans in general, and to at least one billion humans around the world. Humans exploit these communities for food,

Table 6.3 Ecological/economic services provided by coral reefs

- Form 1/3 of the tropical coasts.
- Deposit up to 2000 ton/ha/year of $CaCO_3$ (carbon and calcium sink) and influence chemical balance of oceans.
  - Absorb 1/2 of calcium entering oceans
  - CO_2 sink (700 billion kg/year)
- Generate essential living habitat for important commercial species.
- Highest marine biodiversity and genetic reservoir.
- 20–35% of marine species depend on coral reefs.
- High primary productivity maintains a 9–15 million tons/year of fisheries.
- Direct source of proteins and income for >100 million humans and indirect services for probably over 1 billion.
- Source of active chemical compounds for medical/pharmaceutical applications.
- Supports a multibillion dollar tourism industry.
- Protect coastal communities from hurricanes and storms and replenish sandy beaches.
- Source of building material and limestone.
- Stabilize human social structures and provide areas for recreation and education.

Sources: Dubinsky and Stambler (2011), Bertness et al. (2014), Goffredo and Dubinsky (2016), Hubbard et al. (2016), and Rossi et al. (2017)

building materials, active pharmacological compounds, tourism, and other commercial products (Table 6.3). Unfortunately, their Cnidarian builders have been declining rapidly all over the world in the last 30–40 years. Disease emergence and disease outbreaks with extensive mortalities have exploded in marine communities in the last 30 years (Weil and Rogers 2011; Burge et al. 2014; Woodley et al. 2016). The highest and more widespread mortalities of Cnidarians and other important marine invertebrates in recent times in the Caribbean, Indo-Pacific, and Red Sea have been associated with high thermal anomalies linked to GCC and compounded by local/regional anthropogenic stressors such as pollution, coastal development, and overharvesting (Lessios et al. 1984; Hughes 1994; Hughes et al. 2004, 2010, 2017a,b, 2019; Aronson and Precht 2001; Gardner et al. 2003; Harvell et al. 2002, 2004, 2007, 2009; Bruno et al. 2003; Weil 2004; Ward and Lafferty 2004; Bellwood et al. 2004; Bruno and Selig 2007; Wilkinson and Souter 2008; Hoegh-Guldberg et al. 2007; Carpenter et al. 2008; McClanahan et al. 2009; Hoegh-Guldberg 2010; Dubinsky and Stambler 2011; Mumby and Van Woesik 2014; Jackson et al. 2014; Fuess et al. 2017; Lafferty et al. 2015; Lafferty and Hofmann 2016; Randal and van Woesik 2017; Weil et al. 2017 and references therein). Cnidarian populations and coral reefs are rapidly declining worldwide, mostly as a consequence of these mass mortalities with significant changes in the composition, structure, and function of these communities, and impacting the ecological services they provide.

High temperatures are also affecting the composition and structure of microbial communities associated with organisms and/or the environment, with unknown consequences. Geographic, latitudinal, altitudinal, and depth distributions of tropical terrestrial and marine pathogens, for example, are expected to increase in the near future as the planet warms up, with the potential of deadly outbreaks in susceptible species (Harvell et al. 2002, 2009; Stephens 2016; Weil et al. 2017). Intensive thermal anomalies have also affected foundation and keystone species all the way into temperate environments. Some recent examples include the thermally induced disease outbreaks that produced extensive mass mortalities of many species of sea stars along the northwest and northeast coasts of the United States (Fuess et al. 2017), oysters, lobsters, crabs, and other important economic invertebrate species (Burge et al. 2014; Groner et al. 2016).

The common denominator and most widespread problem of environmental deterioration is the uncontrolled growth of human populations, their industrial activities, and the exponential demands for natural resources and space, which have resulted in significant alteration of natural habitats, the overharvesting of many wildlife species, greater dependence on domesticated animals or cultured wildlife species, and changes in the functioning of most ecosystems. The current rate of environmental change is so fast that many indigenous wildlife populations cannot cope with the increasing demands and the synergistic impact of stressful conditions and are having trouble responding to their changing environments (Stockwell et al. 2003; Parmesan 2006; Hendry et al. 2008; Kolbert 2014; Jones et al. 2017). The consequence is an unprecedented environmental impact and a reduction in the effectiveness of affected habitats to support important species and biodiversity, with many species going extinct (Czech 2000). Common sense approaches to reduce these impacts such as habitat and species protection, sustainable use of resources, and welfare considerations for at least all the foundation and keystone species that build and support susceptible marine and terrestrial communities seem to scape the interest of leaders and policy makers.

Coral reefs are perhaps one of the most susceptible and impacted marine communities; therefore, there is an urgent need to protect the main cnidarians that build coral reef structures, as well as other important invertebrate species that build other essential marine communities or have important ecological functions, from the poles to the tropics and from shallow to abyssal habitats. The recent inclusion of the Caribbean acroporids coral species (*Acropora palmata* and *A. cervicornis*) in the ESA list is a good example of protection for two individual species. These foundation species are the fastest-growing taxa in the Caribbean and build three-dimensional structures that become essential fish habitats in short periods of time. They also provided refuge, habitat, and resources to thousands of other species, including commercially important ones. The IUCN list of threatened and endangered species includes 92 species of reef-building scleractinian species, but their level of protection varies significantly across countries. There are international conventions between nations dedicated to protecting endangered flora and fauna, but it is difficult to evaluate how efficient these are across the member countries. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is one example of these. Their goal is to ensure that international trade in specimens of wild animals and plants does not threaten their survival in their natural habitat, but does not include specific regulations on the welfare of the living organisms that are being traded, including capture, transport, and maintenance. Conservation/protection measurements usually include the creation of Marine Protected Areas (MPAs) with specific rules on the use and exploitation of the resources. These regulations should be complemented with welfare principles to increase conservation and survivorship of important taxa.

Human impacts and the general lack of concern for the protection of the very resources we need raise important questions about the ethical natural foundation of our contemporary society and the responsibility of what should we do to reduce/eliminate the environmental stress for our own future survival (Wilson 2006; United Nations Environment Programme 2007).

6.4 Final Remarks

Although most people and Federal Protection agencies show little concern or interest to the welfare of the majority of invertebrates, including Cnidarians, it is the increasing environmental deterioration and loss of taxa and ecological services provided to other important marine communities and to humans what is forcing some conservation actions to be established. Mounting evidence is suggesting that some, if not most, invertebrates could "feel" and "suffer" through current capture, transportation, research, or captivity practices that do not take into consideration their sensory capabilities to stress and "pain." These considerations should not stop with the manipulation of live specimens by humans, but should also be expanded to the natural populations that "suffer" when humans impact their environments and living conditions. Cnidarians, with their simple nerve networks, probably do not have cognitive responses to noxious and stressful stimuli, but their nociceptive responses should not disqualify them from considerations for welfare provisions. The fact that the animals show stress responses in general indicate some sort of "suffering" in their cnidarian "language," and this should be taken into consideration when ethical arguments are used to develop welfare provisions.

The increasing trade of marine organisms (coral reefs cnidarians and fish mostly) over the last decades is now considered a threat to the world's coral reefs (Rhyne et al. 2012; Jones et al. 2017). Obviously, there is an urgent need to implement welfare provisions to protect the species most commonly harvested or cultured for this trade. The question is how do we go about doing this. A potential approach to institute some welfare provision for cnidarians (and other keystone invertebrates) could be through the use of a combination of conservation arguments given their biological/ecological importance and the inclusion of ethical/humanitarian arguments given their capacity to "feel and suffer." In today's world of high tech, indoor entertainment and reduced contact with nature, it seems unlikely that humans will develop affinities for many invertebrate species. The access to technology and social media could (and should) be used to change this by spreading information and educate the public about the importance of and the high contributions that invertebrates make to natural communities and to humans in general (Schuldt et al. 2015). Hopefully this tactic could help improve the understanding and sympathy for most of the foundation, keystone and economically important species, and pressure government agencies to implement the needed welfare regulations.

Ocean health is deeply intertwined with the health and well-being of human societies because of all the valuable ecosystem services the important marine communities provide. Most of these communities are built or formed by invertebrates, which are key in carbon sequestration, heat absorption, and coastal erosion protection, and built essential fish and other invertebrate habitats, which are sources of animal protein for over a billion of the world's poorest that depend on healthy and well-functioning marine ecosystems such as coral reefs (Burge et al. 2014; IPCC 2014). Certainly we cannot expect welfare regulations for all Cnidarians or marine invertebrates immediately, but we can start protecting the foundation and keystone species and do so before the total crash and destruction of the important natural communities they help to construct and sustain.

Education is probably the most important tool that needs to be expanded globally to attract the much-needed public attention (Schuldt et al. 2015). The frequent news about bleaching and coral reef decline and how human activities and GCC are impacting these and other important communities around the globe is a good start. However, it seems that we need to continue to convince the general populations about the key problem, to reduce human population growth and, hence, the demand for more natural resources and space. This should help increase the support to combine conservation and welfare principles in the near future. Unfortunately, in today's social media and fast-news environment, these types of news and information are downplayed and avoided or, if listened, are quickly forgotten. The process should start at an early age and continue throughout the whole educational curriculum because it seems obvious that the majority of policy makers and today's politicians are more driven by economic gains than the protection of our future. Education and continuous advertising and news over the social media communication networks may make a difference. The more exposure to the current problems and future forecast to the general public, the higher the chance they will understand the problems and change their attitudes. The current and increasing threats to the world's biodiversity, ecosystems, and natural resources, with cnidarians and other important foundation groups at risk in the near future, underscore the need to act fast and develop comprehensive sustainable conservation/protection measures that include both ecological and ethical (welfare) principles.

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Chapter 7
Assessing the Potential for Pain in Crustaceans and Other Invertebrates

Robert W. Elwood

Abstract All animals face hazards that cause tissue damage, and most have nociceptive reflex responses that protect them from such damage. However, some taxa have also evolved the capacity for pain experience, presumably to enhance long-term protection through behaviour modification based on memory of the unpleasant nature of pain. In this review, I consider various criteria that might help to distinguish nociception from pain. Because nociceptors are so taxonomically widespread, simply demonstrating their presence is not sufficient. Further, investigation of the central nervous system provides limited clues about the potential to experience pain. Opioids and other analgesics might indicate a central modulation of responses, but often, peripheral effects could explain the analgesia. Thus, reduction of responses by analgesics and opioids does not allow clear discrimination between nociception and pain. Physiological changes in response to noxious stimuli or the threat of a noxious stimulus might prove useful, but, to date, application to invertebrates is limited. Behaviour of the organism provides the greatest insights. Rapid avoidance learning and prolonged memory indicate central processing rather than mere reflexes and are consistent with the experience of pain. Complex, prolonged grooming or rubbing may demonstrate an awareness of the specific site of stimulus application. Trade-offs with other motivational systems indicate central processing, and an ability to use complex information suggests sufficient cognitive ability for the animal to have a fitness benefit from a pain experience. Recent evidence of fitness enhancing, anxiety-like states is also consistent with the idea of pain. Thus, available data go beyond the idea of just nociception, but the impossibility of total proof of pain means they are not definitive. Nevertheless, more humane care for invertebrates is suggested.

R. W. Elwood (✉)
School of Biological Sciences, Queen's University, Belfast, UK
e-mail: r.elwood@qub.ac.uk

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7.1 Pain

7.1.1 Introduction to Nociception and Reflexes

The soft tissues of animals are fragile and easily damaged, which could result in disease or death. If not so extreme, the tissue will usually be replaced, and resources are diverted from maintenance, growth and reproduction. Thus, minimising damage is important for fitness (Bateson 1991; Elwood 2011). This can be achieved by several means. One common method is by production of hard coverings that protect soft tissues, but the animal still needs to interact with the environment to obtain food and reproduce and exposes soft tissue to achieve those aims. Further, the hard tissues are costly to develop and may impede movement or at least increase the cost of movement. Another, more common, method is to develop sensory systems that detect tissue damage so that the animal can escape from whatever is causing the damage. This can be the whole animal moving away or just the part that is being damaged can be moved.

The specific receptors that are sensitive to injury are called nociceptors, and they mediate protective reflexes (Sherrington 1906). These receptors are simple in that there are no specialised or complex sensory organs. Rather the nociceptors have bare endings that respond to chemical, mechanical or thermal stimuli or sometimes a combination of these. They are found in virtually all phyla of multicellular animals, including nematodes, annelids, molluscs, chordates and arthropods. This wide occurrence of nociceptors indicates that they evolved very early and certainly before the Cambrian explosion that gave rise to major existing taxonomic groups. Conserved cellular processes thus control the responses of a wide range of animals to noxious stimuli (Burrell 2017).

Nociception is the process of encoding and processing noxious stimuli (Loeser and Treede 2008) or the detection and reaction to stimuli that may compromise their integrity (Besson and Chaouch 1987). Thus, nociception is the perceptual mechanism coupled with the organisation of reflex responses that typically take the animal away from the stimulus or, at least, are effective in terminating the perception. Nociception, however, does not eliminate tissue damage. Nociceptors only respond to the onset of damage and do not enable detection of distant stimuli that have the potential for tissue damage should contact occur. Further, there is no suggestion that nociception necessarily involves higher-level neural processing and the animal may be unaware of the nociceptive responses. Nevertheless, nociception confers major benefits in that tissue damage is clearly reduced. Indeed, even those animals with hard, protective coverings still possess nociceptors in those body regions that are exposed when gathering resources, moving or mating.

7.1.2 Concept and Function of Pain

In humans, and possibly other taxa, there is a second system that we call pain. This is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP 1979, p. 250). Pain typically follows nociception, but with pain, there are inputs to the central nervous system, resulting in awareness of the tissue damage and the stimuli that caused it. In humans, for example, unexpected damage to the hand may result in a nociceptive reflex that moves the hand away from the cause of the damage and a negative sensation follows shortly after. Because the experience is unpleasant, there is a high motivation to terminate the stimuli that caused the pain by escape (Bateson 1991). These attempts to escape might go beyond the reflexive response seen with nociception and might be more successful than is possible with a mere reflex. Further, because the event has a high salience, it is likely to be remembered, and the situation that gave rise to it is avoided in the future. Stimuli associated with pain may be perceived from a distance by various sensory systems so that there is no requirement for close contact to trigger a further nociceptive response. Thus, there is no repeat of tissue damage, which might occur should only nociception be available. Pain might also result in anxiety so that the animal becomes wary of various stimuli, possibly even stimuli that have not been encountered previously, and avoids further damage. Thus, pain offers protection from future tissue damage beyond that available with just nociception (Bateson 1991). Pain might also inhibit specific activities or, indeed, inhibit all activities that might otherwise delay recovering, and might result in guarding behaviour to protect the wound (Wall 1979; Bateson 1991).

7.1.3 Pain in Animals

The generally accepted definition of human pain noted above is not applicable to animals because animals cannot tell us what they experience. Instead, Broom (2001) defines pain as "an aversive sensation and feeling associated with actual or potential tissue damage". It uses the term "aversive" rather than "unpleasant" (IASP 1979, p. 250) because the former is more easily identified by the actions of the animal (Broom 2001). There is no doubt that this definition grasps the essence of the subject and provides a focus on what the implications are for the welfare of the animal (Broom 2014). However, it does not enable easy identification of pain as opposed to a non-pain response in animals for two key reasons. First, we cannot access animal feelings, and thus focusing on feelings might inhibit the identification of pain (Stamp Dawkins 2012). Second, if an animal moves away from a stimulus, we may describe that stimulus as being aversive, yet the movement might be due to a nociceptive reflex rather than pain. An alternative definition that provides some guidance on how to identify pain is "an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and may modify species specific behaviours, including social behaviour" (Zimmerman 1986). Sneddon (2009) adds to this definition in suggesting that an animal in pain should "quickly learn to avoid the noxious stimulus and demonstrate sustained changes in behaviour that have a protective function to reduce further injury and pain, prevent the injury from reoccurring, and promote healing and recovery". However, as the definitions include more potential indicators of pain, they assume a rather unwieldly, list-like quality. Nevertheless, the list approach turns the focus on how to detect when pain is likely to be occurring in an individual and to detect which species are likely to experience pain (Sneddon et al. 2014).

The list approach provides a set of criteria that would be expected to be fulfilled should an animal experience an aversive sensation and unpleasant feeling. But we need to be clear that although fulfilling criteria of pain is consistent with the idea of pain, it is not the same as proof of pain (Elwood and Adams 2015; Magee and Elwood 2016a). There may be alternative explanations for the behaviour following noxious stimulation (Rose et al. 2014; Key 2016), and alternative explanations are often more firmly promoted for invertebrates than for vertebrates (Sherwin 2001). Nevertheless, if many of the expected criteria are fulfilled for a species, we can say that it is possible or even highly probable that pain occurs in that species. Further, because of the close similarity between related species, if pain is considered highly probable in one species, we may view it as highly probable in closely related taxa. Alternatively, if the criteria are not fulfilled, or very few are fulfilled, then the possibility of a pain experience must be considered low. This is a somewhat unsatisfactory outcome of scientific investigations, which normally aim at more definite conclusions, but it is in keeping with other investigations of private mental attributes such as consciousness (Stamp Dawkins 2012).

Finally, attempts to define animal pain face a major hurdle because we relate to those definitions via our own individual experience of pain. We are so familiar with our own experience that despite attempts to avoid introspection, we naturally think of pain as something like our own feelings. To avoid thinking of animal pain in that way, phrases are used about the subjective experience as not necessarily being the same as human experiences (Molony 1992) or that an animal's pain might be totally different from a human's, reflecting its different way of life and differences in body function (Bateson 1991). In many ways, this is unsatisfactory because if the pain felt by an animal species is not necessarily the same, or indeed very different to humans, then what is it? Further, if pain in one species is very different from that in humans, it is unlikely that it would be similar to species from different broad taxa. Thus, as noted above, we have no way of determining what a particular species "feels" when subject to noxious stimuli. We can, however, judge if the behavioural and physiological responses to injury serve the same protective function across phyla (Rutherford 2002). We refer to those responses in humans as those indicating pain, and it seems reasonable to use the same term for animals, be they vertebrates or invertebrates (Sherwin 2001).

7.1.4 Lists of Criteria for Pain

Bateson (1991) proposed eight criteria for animals to be considered as having a subjective experience of pain. The rationale for these eight derived to some extent from the difficulty of judging pain in humans, and the criteria tend to be oriented primarily, although not exclusively, towards vertebrates. The first three refer to morphology and propose that for pain, an animal should possess nociceptors, brain structures analogous to the human cerebral cortex and nervous pathways that connect the two. Additionally, there should be receptors for opioid substances, and analgesics should modify the response to noxious stimuli. Also included is a suggestion that animals should select analgesics to self-administer when they are exposed to noxious stimuli. The last three criteria are entirely behavioural and suggest that the animal should avoid noxious stimuli and minimise damage to the body, that the avoidance should be relatively inelastic, that the responses should be persistent and that the animal should learn to associate neutral events with noxious stimuli (Bateson 1991). These criteria have been modified, particularly when attempting to consider if invertebrates might experience pain (Sherwin 2001; Broom 2007; Elwood 2011). The review by Elwood (2011) did not consider connections between nociceptors and the brain. It added responsiveness to anaesthetics and physiological changes in response to noxious stimulation. It also suggested that trade-offs should occur between stimulus avoidance and other motivational requirements. This was rather different to Bateson's (1991) view of a relatively inelastic response, with Elwood taking the view that pain could be thought of as a motivation and that all motivational requirements tend to trade-off. There was the inclusion of rubbing and attending to the site of a wound. Finally, high cognitive ability and sentience were considered. The most recent and extensive list that might be applied to all taxa is that of Sneddon et al. (2014), which suggests 15 criteria (Table 7.1).

Broom (2001) notes that identification of pain by fulfilling criteria might face problems with some species. First, overt responses to noxious stimuli might serve a function of warning close kin of a danger. If that occurs, then it makes it easy for us to assess that the noxious stimulus has at least been perceived by that animal. If the species is not social, then the selection promoting overt responses may be reduced. Indeed, there are situations where showing overt behavioural responses to tissue damage might be disadvantageous. It might pay the animal to avoid providing information about injury because doing so might make the animal more vulnerable to predation (Broom 2001) or possible defeat in aggressivee interactions. Hiding pain might thus be important in some situations for some animals, and that makes it more difficult for us to assess pain in those species, as animals hiding pain would be judged incorrectly as not feeling pain.

Another problem arises from difference between individuals in personality types. For example, in horses, certain personality types, e.g. extroverted, show increased overt responses to injury, and thus pain is relatively easy to identify (Ijichi et al. 2014). However, pain may be missed in less extroverted individuals. While this

Table 7.1 The 15 key criteria for pain in animals as suggested by Sneddon et al. (2014)

1. Evidence of central processing of nociception involving brain areas that regulate motivated behaviour (including learning and fear)
2. Nociceptive processing sensitive to endogenous modulators (e.g. opioids in vertebrates)
3. Nociception activates physiological responses (one or a combination of the following: change in respiration, heart rate or hormonal levels (e.g. cortisol in some vertebrates)
4. Evidence that responses are not just a nociceptive reflex (i.e. not simply moving away)
5. Alterations in behaviour over longer term that reduce encounters with the stimulus
6. Protective behaviour such as wound guarding, limping, rubbing or licking
7. All of the above reduced by analgesia or local anaesthetics
8. Self-administration of analgesia
9. Pay a cost to access analgesia
10. Selective attention whereby the response to the noxious stimulus has high priority over other stimuli; the animal does not respond appropriately to concurrent events (e.g. presentation of predator; reduced performance in learning and memory tasks)
11. Altered behaviour after noxious stimulation where changes can be observed in conditioned place avoidance and avoidance learning paradigms
12. Relief learning
13. Long-lasting change in a suite of responses especially those relating to avoidance of repeat noxious stimulation
14. Avoidance of the noxious stimulus modified by other motivational requirements as in trade-offs
15. Evidence of paying a cost to avoid the noxious stimulus

phenomenon has not been noted in invertebrates, given that they too show personality types (Briffa et al. 2015), there is the scope for identifiers of pain to be missed in some individuals. This variation in responses makes identification in some species more difficult.

It is clear from the above examples that testing criteria is not without problems. Nevertheless, that approach offers the best chance of identifying taxonomic groups that might experience pain. The aim of the following sections is to give an overview of investigations that test criteria in invertebrates. To improve clarity, some of the following sections comprise more than one criterion.

7.2 Experimental Evidence

7.2.1 Avoidance Learning

Invertebrates have received considerable attention with respect to their learning abilities, and there are many studies on avoidance learning (Sherwin 2001 for a review). Recent examples have specifically tested the criteria of pain experience that avoidance learning should be rapid. Speed of learning is important as swift learning brings about a marked reduction in the number of direct encounters with the noxious

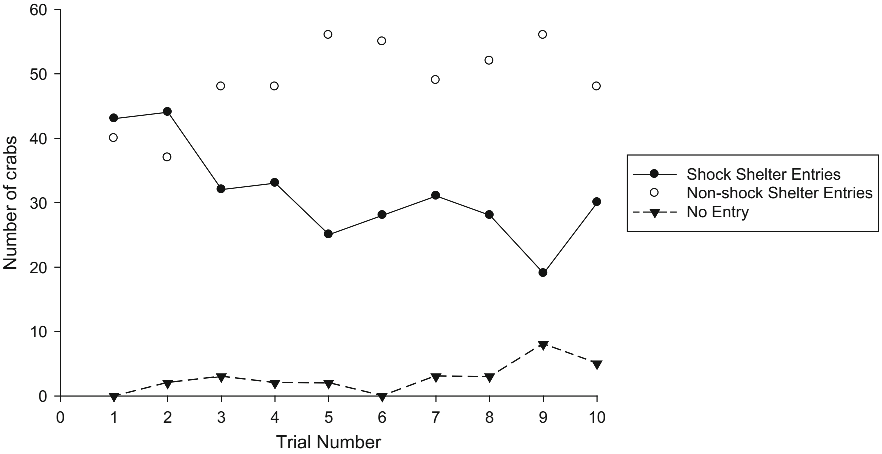


Fig. 7.1 The number of crabs that entered the shock and non-shock shelters in each of the ten trials, as well as the number of crabs that did not enter either shelter (from Magee and Elwood 2013)

stimulus and hence reduction in tissue damage. Magee and Elwood (2013), for example, repeatedly placed shore crabs, *Carcinus maenas*, in a brightly lit rectangular enclosure, at each end of which was a dark shelter, and the crabs typically used one to escape from the light (Barr and Elwood 2011). On the first such choice, all crabs swiftly entered one of the shelters, indicating a high motivation of shore crabs to avoid bright light. Some crabs had previously been selected to receive small electric shocks at 5 s intervals within their first chosen shelter, whereas the remaining crabs only received shocks in subsequent trials if they went to the alternative shelter. After each 2 min trial, the crabs were briefly removed from the enclosure and then returned to the centre so that they could again make a choice of the two shelters. Ten such trials were completed. On the first trial, the crabs had no information about potential shock. The first opportunity for the crabs to use information in their choice was in trial 2, but being shocked had no effect on the choice in trial 2, and most crabs simply went to the same shelter they used in trial 1. However, on trial 3, significantly more crabs that were shocked in the previous trial switched their choice of shelter compared to those not shocked. That is just two trials in which information might be gained resulted in significant avoidance of the shock shelter, and over the ten trials, there was a decrease in selecting the shock shelter (Fig. 7.1). The crabs were free to exit their chosen shelter, and about 55% of those being shocked did so in the first of the ten trials ($n = 41$). By contrast, no crab moved out of a safe shelter in any of the ten trials. In later trials, crabs entering the shock shelter became increasingly likely to move out of that shelter during the trial. Thus, crabs appeared to use a second method of avoiding shock.

Magee and Elwood (2013) placed striped cards at either end of the rectangular enclosure; one card had vertical stripes, while the other had horizontal, and the same stripe positions were used for the ten training trials. Further, the crab was always placed at the start of a trial facing in the same direction, perpendicular to the axis between the two shelters. Thus, throughout the ten training trials, they would always have to walk in one direction and to one stripe orientation (either left or right and either horizontal or vertical) to get to the safe shelter. The 11th trial was designed to test what the crab had learned. In that test, 50% of the crabs were tested with the same stripes used in training, but the others had the cards switched. Further, 50% the crabs started in the same position, but the other half had the start position turned 180°. There was no effect of visual stimuli on the choice made in the 11th trial indicating that visual information was not associated with the safety (or shock). By contrast, crabs turned 180° were more likely to go to the shock shelter than were those not turned. Thus, crabs used response learning and not place learning.

Although the crabs did not use the visual cues that might have been due to the cards being insufficiently different, both had the same number of stripes of identical width. Other studies, however, have shown associations between visual cues and avoidance. For example, spiders discriminate between black and white cues to avoid shock in a place learning paradigm (Peckmezian and Taylor 2015), and honey bees associate colours with shock (Agarwal et al. 2011). Further, non-visual cues can be used as with crayfish that learn to avoid shock associated with either a hard or soft substrate in an otherwise similar paradigm (Bhimani and Huber 2016). Thus, allocentric cues can be utilised by invertebrates as well as the egocentric cues noted by Magee and Elwood (2013). Nevertheless, the speed of learning in that experiment is impressive and consistent with the idea of pain. Further, spiders showed avoidance of the shock side of a petri dish within the first 5 min trial (Peckmezian and Taylor 2015), and crayfish avoided the shock areas within the first 10 min of training, during which time they received about nine shocks (Bhimani and Huber 2016).

In these place avoidance experiments, the choice was simultaneously presented on each trial. An alternative approach is to train animals with just one place at each trial, but that is a more cognitively challenging task (Dyer and Neumeyer 2005). This was attempted with shore crabs (Magee and Elwood 2016b) using the same enclosure as in Magee and Elwood (2013) but with an opaque partition separating the two dark shelters. For each 2 min trial, the crab was placed on one side of the partition and allowed to sample one shelter. As before, the crab was in the same orientation for each of ten training trials, and the horizontal and vertical stripes were placed above each dark shelter. All crabs were shocked on the first trial and in the second trial were placed on the other side of the partition, and the shelter on that side was always safe. The sides in which the crabs were placed alternated between tests. On an 11th trial, each crab was tested without the partition, thus having the first simultaneous choice of shelters but without shock in either shelter. On a 12th trial, the crabs were again tested without the partition, but half the crabs had their orientation changed by 180°, and half had the striped cards switched. With this paradigm, however, the crabs showed no preference for the safe shelter on the 11th trial. In the 12th trial, most crabs went to the same shelter chosen on the 11th trial. However, they were more likely to go in the same direction, i.e. to the crab's left or right, irrespective of their orientation but did not show a preference for stripe orientation. Nevertheless, the crabs showed other methods of reducing the number of shocks. Over the five training trials involving shock, there was an increase in the number of crabs exiting that shelter after receiving shock (as happened in Magee and Elwood 2013). Further, they exited the shelter after fewer shocks in later trials. This is consistent with the idea of avoidance learning and pain, but increased sensitivity might play a role (sensu Crook et al. 2011, 2013; Alupay et al. 2014). However, the main conclusion from Magee and Elwood (2016b) is that no associations were formed between the location of shock and either egocentric or allocentric cues. In this respect, there is no support for the idea of pain. This, however, seems to be because the paradigm requires a cognitive ability that is beyond the ability of these animals rather than their ability to experience pain. With more simple paradigms, discrimination avoidance learning is evident (Magee and Elwood 2013; Peckmezian and Taylor 2015; Bhimani and Huber 2016).

A different paradigm to investigate avoidance learning involves crabs raising one leg out of shallow water to avoid an electric shock to the leg when in the water (Hoyle 1976; Dunn and Barnes 1981; Punzo 1983). This results in a decrease in lowering of the leg compared with non-shocked controls or yolked animals that receive shock whenever a "master" crab is shocked. The technique has been employed in investigations of brain regions involved in this avoidance leaning (Punzo 1983). Remarkably, however, crabs that have the brain destroyed also show the capacity for learning, thus casting doubt on the idea that the learning is driven by a pain-like state (Dunn and Barnes 1981). However, there is reason to believe that the type of learning in these preparations is very different from the place avoidance learning noted above. Intact mud crabs, *Eurypanopeus depressus*, received a mean of 680 shocks during the first minute of training, which reduced to 140 per minute after 10 min of training (Punzo 1983). Decerebrate shore crabs, *C. maenas*, received over 1500 shocks in the first minute, which declined to 400 after 10 min of training (Dunn and Barnes 1981). That is the number of shocks required to train both intact and decerebrate crabs vastly exceed the numbers required for avoidance of locations (Magee and Elwood 2013). Even the intact crabs do not fulfil the criterion of swift avoidance learning, and decerebrate crabs presumably could not experience pain even if intact crab might. This appears to be a very different type of association, possibly involving ventral ganglia and less so with the brain. It tells us little about the possibility of pain in decapods.

There have been many studies on avoidance learning and the CNS mechanisms involved in short and long-term memory in molluscs, particularly *Aplysia* and *Octopus*. In one study, *Aplysia* were exposed to an odour (shrimp extract) either just before electric shocks to the head (paired) or 90 min after the shock (unpaired) (Walters et al. 1981). During training, the shock caused several reactions, including withdrawal of the head and ink release. They were then tested 1 or 2 days later with just the odour. The paired animals showed more head withdrawal than did the unpaired animals, but the two groups did not differ for the other measures when tested with just the odour. However, when the odour was paired with a small shock to the tail, other responses were much greater in paired animals. The siphon was withdrawn for longer in the paired than unpaired groups. The paired group showed inking at a lower intensity of shock than did the unpaired subjects, and there were more escape responses in the paired group and less feeding. That is, a range of responses was now associated with a combination of the odour and small shock, and defensive responses were enhanced and appetitive responses depressed. The authors concluded that the training stimuli had conditioned a fear response that included the anticipation of danger, similar to the fear responses noted in mammals.

One example for *Octopus vulgaris* is a study in which subjects were trained to attack a white ball and received food if they did so (Shomrat et al. 2008). Once trained, the subjects were shown a red ball, which was also attacked. However, some animals received a small electric shock when they attacked, and on subsequent trials, the number of animals attacking declined. Those not shocked continued to attack. By the fourth trial, after just three experiences of shock being associated with the red ball, about 50% stopped attacking.

As noted above, the key to understanding these experiments, with respect to the possibility of pain, is to see if learning is swift. For some experiments, the data comprise the time spent in areas in which shock and no shock occurs, but this does not give information on the number of choices. The data required are those showing the number of entries to the shock area (or area that will produce another noxious stimulus) prior to avoidance. That is, we can judge how quickly the animal changes its choice of action in a way that might protect it from tissue damage. An alternative is to determine if the animal is slower to move to an area in which a required resource is located if that is also associated with a noxious stimulus. Unfortunately, in many studies on avoidance conditioning, these data are not available.

However, experiments designed to investigate avoidance learning have used differing paradigms, and these may be a major source of variation in conclusions. For example, in Magee and Elwood (2013), the animal had to choose between two shelters that were identical except one was associated with shock. The safe shelter was available on each trial, and thus, not taking the shock shelter should cost little to the animal. However, the data show that animals typically return in trial 2 to their originally selected shelter so any change of use must overcome this preference. In an experiment with cuttlefish preying on their preferred food item (prawn or crab) tainted with quinine, there was a take it or leave it choice (Darmaillacq et al. 2004). Leaving it involved not feeding on the preferred prey type so any learning must involve an unlearning of food preference and giving up a valuable food resource. Thus, one might predict slower avoidance in the latter situation because there is a substantial cost in avoiding the noxious stimulus. Nevertheless, the squid showed markedly longer attack latencies in the second trial and reached learning criterion of not attacking in successive trials in eight trials. This avoidance was retained after 3 days, and the normal preference of individual cuttlefish was switched to the originally less preferred prey. In comparing experiments, we need to be aware of such cost incurred when avoiding the noxious stimulus.

Another factor that appears to determine speed of learning is the nature of the response that will enable the animal to avoid the noxious stimulus. This was shown with crayfish placed in a shuttle box when a light onset signalled that a shock would be delivered (Kawai et al. 2004). The animals could avoid the shock if they moved to the other side of the shuttle box within 10 s. Some animals were facing the direction that would lead to safety whereas others faced away. Two reactions were noted. At first, all animals showed a tail flick escape response to the shock. This resulted in those facing towards the safe area to dart backwards and further into the shock compartment. Those facing away from the safe compartment rapidly darted backwards into the safe area. Learning to avoid the shock by responding to the light was very different between the two groups. Those that faced towards the safe area slowly learned to use the signal and walk to safety. By contrast, those that were facing away when the light signal occurred and could have tail flicked to move to safety failed to do so. They only tail flicked when shocked and appeared to show no learning. However, when this group had their position reversed, they showed rapid learning and now walked to safety on the light signal (Kawai et al. 2004). The study shows that invertebrates, as well as mammals (Bolles 1970), are markedly influenced by the nature of the response.

The salience of cues might also influence the speed of avoidance learning. If the animals are being trained to avoid one half of an area and the cues are visual, then the cues for each half might be present all the time. Thus, when in the safe half the cues for the noxious half may be present so there is little salience for the cue, and this might result in slow learning. By contrast, if the onset of a light or odour signals the onset of the noxious stimulus, then the cue will likely have greater salience, and the association should be quicker. Further, if the arena is small, a shock might induce rapid walking, and the animal might move rapidly between the shock and no shock area and back to the shock area. Again, this might result in apparent slow learning. We need to consider what cues are easy for the animal to discriminate because what is obvious to us could be indistinct to the animal.

Various invertebrates can learn to predict shock if that is reliably preceded by a cue. For example, *Drosophila melanogaster* that are trained with a novel odour just prior to a shock will avoid that odour when given the opportunity (Yarali et al. 2008). That is, the odour predicts something that is “bad”, but when paired with the arrival of something “good” such as food, the odour will come to be preferred (Tempel et al. 1983). More surprisingly is the finding that an odour that arrives at the end of an electric shock comes to be preferred when tested against an alternative odour. That is, the odour signals safety or relief, and this learning has been termed “pain relief learning” (Gerber et al. 2014). However, it has different properties than when the odour precedes the shock. Relief learning typically takes more trials for the effect to be shown with 1, 2 or 4 trials not being effective but 6 and 8 trials resulting in odour preference (Yarali et al. 2008). With punishment learning, it can be as low as one trial for avoidance (Tully and Quinn 1985). Further, when the odour precedes shock, a strong avoidance is noted, but when it follows the shock, the preference is weak, with the size of the effect being about a fifth of that shown for avoidance (Yarali et al. 2008). The intensity of the shock used in training also has an effect with increasing learning seen with increasing intensity up to 100v but a marked decline thereafter. This latter finding was ascribed to the very high shocks inducing amnesia and/or damage to the fly. The relief learning in flies has many similarities to that found in rats and humans, but it remains relatively little understood, and studies are required in other species to aid in understanding how it might add to our understanding of pain (Gerber et al. 2014).

7.2.2 *Giving Up a Valuable Resource and Motivational Trade-Offs*

A nociceptive reflex might briefly interrupt an ongoing behaviour, but it unlikely to cause an animal to give up a valuable resource, e.g. food or shelter, for a prolonged period. The rationale for this is that once the reflex is completed, there is no need to presume an awareness and no need to presume a marked shift in motivational state. Thus, the animal should return swiftly to its original activity. However, there are cases when an animal responds to a noxious stimulus by giving up a valuable resource to remove itself from the scene of stimulation. The example noted above of shore crabs moving out of a dark shelter into a brightly lit area is one such situation (Magee and Elwood 2013). The dark shelter is important to shore crabs, and moving from a shelter to be exposed to light in natural situations will normally increase the risk of predation (Fathala and Maldonado 2011). Another example occurs with hermit crabs that are shocked within their shell, which causes crabs to evacuate from the shell and thus abandon the important protection the shell provides (Appel and Elwood 2009a, b). Often, a hermit crab will remain near the shell, and some investigate within the shell by probing it with their chelipeds (claws). Some crabs then move back into the shell, but a large number remain out of the shell for a prolonged period. Indeed, some move away from the shell and may scramble against the wall of the test arena, apparently attempting to escape from the location. This is not predicted by a nociceptive reflex; rather, it indicates a marked change in the motivational state of the animal. Hermit crabs are dependent upon shells, and abandoning the shell is an extreme reaction and clearly demonstrates the aversive nature of the electric shock.

A compelling argument for non-reflex responses can be made when motivational trade-offs occur. The rationale for this is that during normal decision-making processes, various motivational requirements affect the decision and patterning of behaviour. Thus, if we see the response to a noxious stimulus being affected by other motivational requirements, it must be due to a decision-making process, as opposed to a reflex (Elwood and Appel 2009). One example is seen when hermit crabs are induced to occupy either *Gibbula cineraria* or *Littorina obtusata* shells, the latter being considerably preferred to the former (Elwood 1995). Crabs receiving a small electric shock (10v) within their shell were more likely to get out of the less preferred species of shell, showing that the quality of the shell was traded-off against shock avoidance. A second example involves hermit crabs in *L. obtusata* shells that were subject to shocks of increasing intensity (up to 25v) and exposed to different odours (Magee and Elwood 2016a). When no odour was present, 95% of the crabs emerged from their shells, which was not significantly different to 80% emerging when the odour of a non-predator (mussels) was present, but these were both different from the group exposed to the odour of a predator in which only 41% emerged. That is, crabs were trading-off risk of predation with shock avoidance. Two other experimental groups received predator or non-predator odours that were 100× more concentrated. For the concentrated predator odour group, 47% got out of the shell, but this was not significantly different from 57% for the concentrated non-predator odour group. The response to extreme concentration of mussel odours was not statistically different from those to the predator odours, indicating the importance of using concentrations that might realistically be found in natural conditions. The conclusions are that crabs tend to remain in their shells when odours of either unnaturally high concentration or of potential predators are present. Thus, although evacuating from a shell might seem a relatively simple behaviour that might be reflexive, it is clearly influenced by other motivational requirements and thus a product of central decision-making. The data are consistent with the idea of pain.

7.2.3 *Protective Behaviour: Prolonged Rubbing and Grooming/Wound Guarding*

Wounded mammals may show activities that indicate some awareness of the site of the wound and some attempt to reduce further damage. Typical examples are rubbing, guarding of wounds and limping, and these activities are interpreted as being consistent with pain (Weary et al. 2006). Various examples of similar activities have been reported for invertebrates. For example, application of either 10% sodium hydroxide or 10% acetic acid to a single antenna of glass prawns, *Palaemon elegans*, resulted in prolonged grooming and rubbing of that specific antenna (Barr et al. 2008). The grooming involved repeatedly pulling that specific antenna through the small chelipeds (claws) or through the mouth parts, whereas rubbing was pressing and moving that antenna against the side of the tank. Pinching one antenna with forceps did not affect grooming rate but did increase rubbing. The responses were directed at the treated antenna significantly more than the untreated antenna, indicating an awareness of the specific location of the noxious stimulus. Further, application of sodium hydroxide to one eye of a glass prawn caused high levels of grooming of that specific eye with either one or both first walking legs. This behaviour was not seen if just sea water was applied (Barr 2009). Also, shore crabs scratch at their mouth parts if the latter is treated with acetic acid (Elwood et al. 2017), and hermit crabs will groom their abdomen if they had received abdominal shock, an activity not seen without the noxious stimulus (Appel and Elwood 2009a, b).

In some crab fisheries, the claws are twisted and pulled off, and the live animal then returned to the sea. McCambridge et al. (2016) compared the competitive ability of male crabs that had the claw forcibly removed with those induced to autotomise a claw, which does not cause a large wound. Apart from demonstrating a lower ability of the former to gain access to females, several observations suggested an awareness of the wound. These included holding the existing claw over the wound during the competition in a manner akin to guarding. Manually declawed crabs also touched their wound and picked at the broken exoskeleton with their remaining claw and sometimes then showed a "shuddering response". These manually declawed crabs showed a lower motivation to compete for the female and seemed to be more engaged in self-defence than were those induced to autotomise (McCambridge et al. 2016).

Similar behaviour directed at wounds is seen in the octopus *Abdopus aculeatus* that have had an arm crushed by forceps (Alupay et al. 2014). The wounded area was held in the beak for at least 20 min in some individuals. Some 6 h later, this behaviour was not observed after experimentally touching the wound area, but the animals held the wound close to the body and adjacent arms curled around. However, no such behaviour was noted when part of an arm was removed in the squid *Loligo pealeii* (Crook et al. 2011).

7.2.4 Autotomy

Autotomy is another protective motor response in arthropods, such as brown crabs *C. pagurus* (Patterson et al. 2007), and cephalopods, such as the octopus *A. aculeatus* (Alupay et al. 2014). It enables the animal to cast off an appendage that is damaged. For example, cutting a leg-joint membrane at a joint distal to the main body, causes immediate haemolymph loss and autotomy occurs within a few seconds, preventing further loss of fluid (Patterson et al. 2007). This autotomy leaves a clean break at the joint with the main body, which immediately seals to prevent loss of haemolymph. Crabs also autotomise limbs in situations that do not involve haemolymph loss, for example, if the whole animal is placed on a hot plate (Fiorito 1986), injected with formalin (Dyuizen et al. 2012) or injected with acetic acid (Barr 2009) or if the leg is subject to electric shock (Magee and Elwood 2013). The acetic acid treatment rapidly induces autotomy in a dose-dependent manner, and the results are consistent with the idea that pain mediates the autotomy response. In the octopus, *A. aculeatus*, autotomy is induced by crushing an arm with forceps.

Legs may also be autotomised in spiders, e.g. *Argiope aurantia* (Eisner and Camazine 1983). This occurred when these spiders attempted to capture ambush bugs (*Phymata fasciata*), usually when the bug grasped a spider leg and probed a joint with its proboscis (the venomous saliva is painful to humans). Eisner and Camazine (1983) examined the role of chemicals that induce pain in humans. Injected bee and wasp venom both induced autotomy, whereas penetration of the joint with a sterile pin did not. They found that when individual components of the bee venom were injected, some, but not all, produced autotomy. Effective components were histamine, serotonin, phospholipase and melittin, all of which induce pain in humans, whereas ineffective components were acetylcholine, bradykinin, hyaluronidase, adrenaline and dopamine. Acetylcholine and bradykinin induce pain in humans but not autotomy in spiders, and hyaluronidase, adrenaline and dopamine do not induce pain in humans. Thus, there is a concordance between pain effects in humans and autotomy in the spider.

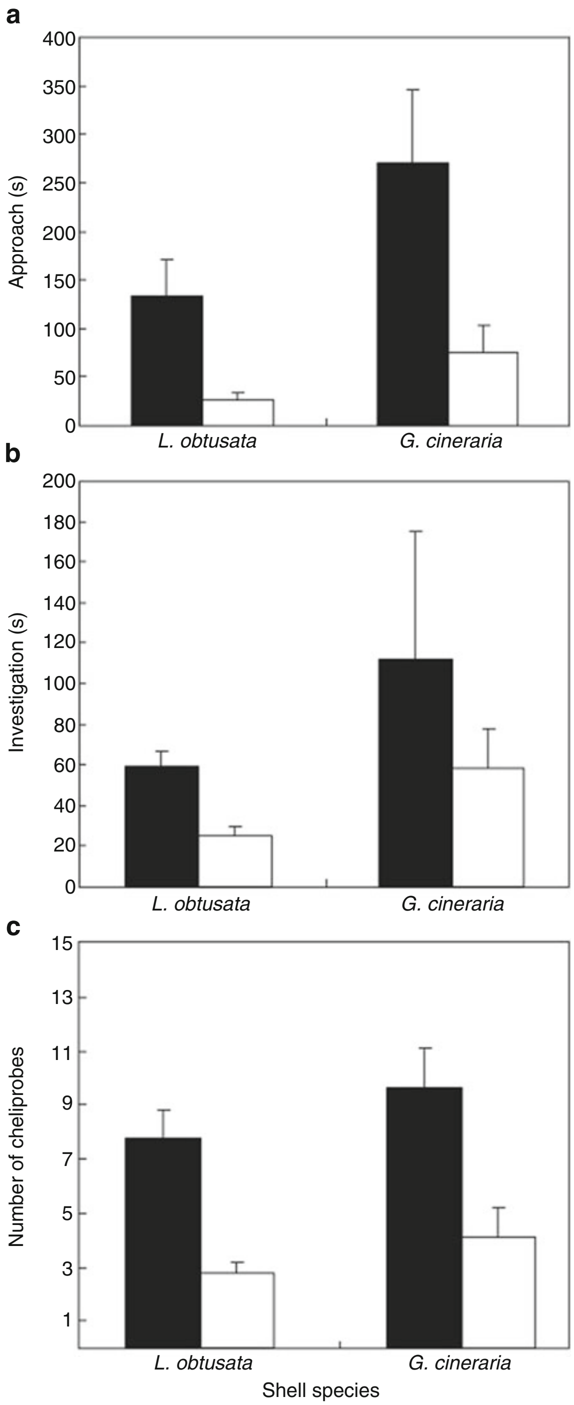
It is possible that at least some incidences of autotomy are mediated by a pain-like experience, but we need experiments to test the effects of anaesthetics and analgesics to determine if they reduce autotomy. Until those experiments are conducted, it is difficult to assess what autotomy tells us about possible pain.

7.2.5 Long-Term Motivational Change

As stated above, the advantage of pain over nociceptive reflex is that it may more easily result in a long-term change in behaviour, including avoidance learning discussed previously. There are, however, instances of long-term change in motivation that may not easily be shown as due to learning. One such example comes from studies in which hermit crabs were given small electric shocks within their shells. Those that did not evacuate from their shells were subsequently offered an empty shell 20 s after the last shock and the responses compared to crabs that were not shocked prior to offering a shell (Elwood and Appel 2009). Many crabs, in both treatment groups, moved toward the new shell, investigated and most then moved into the shell. However, crabs that were shocked were significantly more likely to approach and take the offered shell than were those that were not shocked. Of those that moved into the new shell, most did so after a single approach and investigation; those in the shock group approached more quickly (Fig. 7.2a), spent less time investigating the new shell prior to moving in Fig. 7.2b and used fewer insertions of their chelipeds during the investigation (“cheliprobes”) (Fig. 7.2c) compared to those not shocked. Shocked crabs thus showed a higher motivation to obtain a new shell and acted as if the shell they occupied was of very poor quality. They acted similarly to crabs housed in shells that are far too small and hence had a high motivation to change shells (Elwood and Stewart 1985; Elwood 1995).

To determine how long the motivation to obtain a new shell lasted, a subsequent experiment varied the time from the last shock (or control treatment) to the offering of a new shell (5 min, 30 min, 2 h and 24 h) (Appel and Elwood 2009b). Shocked crabs in the 30 min and 1 day groups were more likely to approach the new shell than were the non-shocked ones. Further, significantly more shocked than non-shocked crabs of the 30 min group moved into the new shell. Of those that contacted the new shell, shocked crabs from the 2 h group approached significantly quicker than the non-shocked group. Of the crabs that entered the new shell after first approach, shocked crabs used fewer cheliped probes in the 5 min, 2 h and 24 h groups. Further, after moving into shells, hermit crabs may thrust their abdomen in and out of the new shell, presumably in a continuation of the shell evaluation. Shocked crabs in the in the 2 and 24 h groups were less likely to show this behaviour than were the non-shocked crabs. That is, 24 h after the shock treatment, crabs still showed a higher motivation to obtain a new shell as evidenced by various activities. Clearly,

Fig. 7.2 Mean ± SE of (**a**) time taken to approach (s), (**b**) duration of investigation (s) and (**c**) number of cheliprobes by crabs in either *L. obtusata* or *G. cineraria* shells that had been shocked (open bar) or not shocked (black bar) (from Elwood and Appel 2009)



behavioural differences shown 24 h after the noxious treatment cannot be described as a reflex. Further, if a key function of pain is to change behaviour in the long term to avoid repeated tissue damage, the ability to remember is a prerequisite. The studies noted above on hermit crabs and shore crabs show such a memory. There are many other studies that demonstrate short- and long-term memory in a wide range of invertebrates (Sherwin 2001).

A key function of pain is to increase subsequent survival, and we expect animals subject to noxious, potentially painful, stimuli to be wary of stimuli that are associated with elevated predation risk. An example of this increased wariness comes from work on crayfish (Fossat et al. 2014). Crayfish were tested in cross mazes in which two arms of the maze were brightly lit and the other two were dark. Crayfish wandered throughout the maze but spent more time in the dark than the light. In natural conditions, crayfish stay in hiding during the day and are mostly active at night, presumably when predation risk is lower. In the main experiment, some crayfish were exposed to repeated short-duration electric fields that induced tail-flicking escape responses, indicating that this treatment provided a noxious stimulus. Animals thus treated spent far less time in the light arms of the maze than did those placed in the treatment area but without the shocks (Fig. 7.3). That is, the normal preference for the dark was considerably enhanced, and the shocked animals were described as showing “anxiety”.

A further example of increased wariness involves squid that had a small section of one arm removed with surgical scissors showing marked changes in response to approaching visual stimuli (Crook et al. 2011). The squid were touched at intervals with a bending filament attached to a long, thin handle to assess changes in sensitivity to tactile stimuli. However, the approaching filament caused the squid to respond by movement or colour change prior to contact. For control squid, there were no temporal changes in the distance between the filament and the animal before the first response. For injured squid, however, temporal changes in responsiveness were noted. Ten minutes after wounding, the squid appeared to be less responsive to the approaching stimulus, but thereafter, it was considerably more responsive than were controls. This increased wariness was apparent for up to 48 h after injury. Further, there was considerably more anticipatory jetting and protective ink plumes by injured squid, and these responses typically increased from the first trials 10 min after injury to the last trials 24 or 48 h later. Comparison of blindfolded squid and those that could see confirmed that the reactions were to visual stimuli rather than vibration, because the blindfolded squid did not respond during the approach of the filament (Crook et al. 2011).

While the increased wariness towards approaching visual stimuli by injured squid is consistent with a mechanism to reduce predation, it does not specifically demonstrate fitness enhancement. Another study on squid, however, clearly demonstrates fitness enhancement. Further, the enhanced fitness is dependent upon the nociceptive input from injury rather than just the injury itself (Crook et al. 2014). The approach was to give some squid a small wound whereas others were unharmed. Half of each of these treatments were also given a local anaesthetic that would block the nociceptive input of the wound. Giving the local anaesthetic to squid that were not

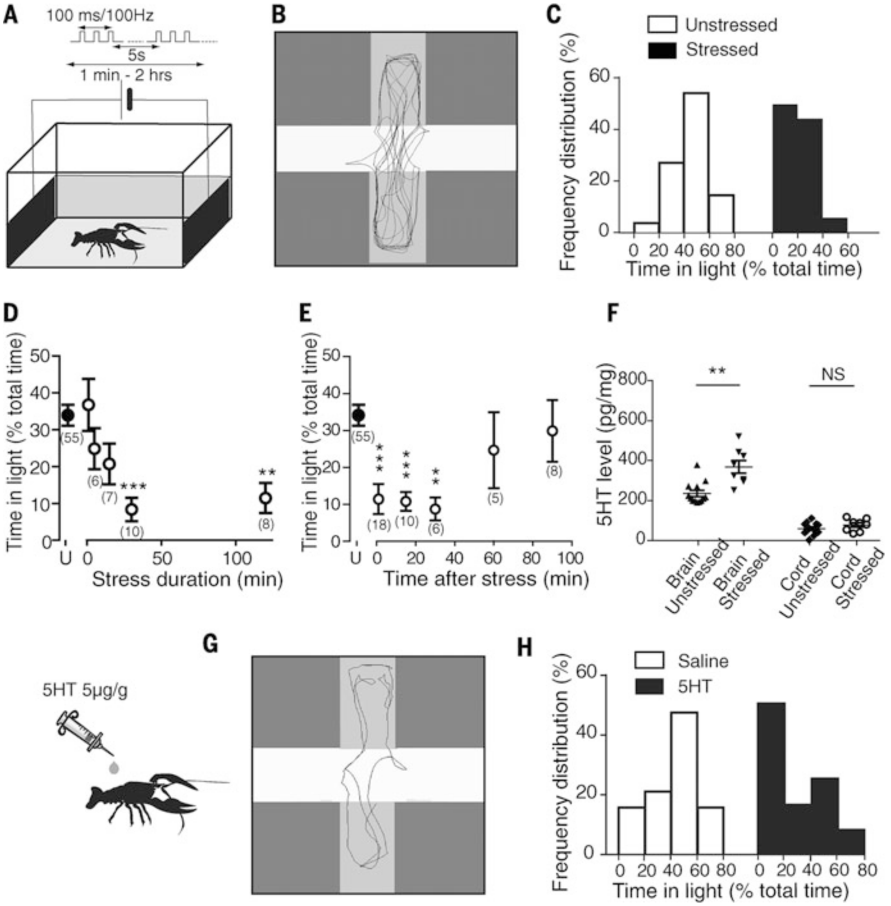


Fig. 7.3 Crayfish develop 5HT-dependent light avoidance after exposure to stress. (**a**) Experimental procedure for stress induction in crayfish (supplementary materials). (**b**) An example crayfish route after a 30 min exposure to an electric field. Walking occurred nearly exclusively in the dark arms. (**c**) Frequency distribution histograms of the percent time spent in light arms by stressed and unstressed crayfish. (**d**) Effect of stress duration on time spent in light arms (U unstressed; P vs. unstressed <0.001 after 30 min and P vs. unstressed <0.01, after 2 h of stress, Dunn's test). (**e**) Time course of behavioural changes (as measured by time spent in light arms) after exposure to a 30-min stressful experience. Crayfish recovered "normal" behaviour after 90 min (U = unstressed; P vs. unstressed >0.05, Dunn's test). The number of animals (n) is in parentheses in (**d**) and (**e**). (**f**) Serotonin concentrations (in picograms per milligrams of fresh weight) measured by means of HPLC in the brain and ventral cord of unstressed and stressed crayfish. Brain concentrations of 5HT were significantly higher in stressed than in unstressed animals. (**g**) After injection of 5 μg/g 5HT into the haemolymph, the crayfish route was similar to that of stressed crayfish. (**h**) The frequency distribution histograms of percent time in light arms for saline- and 5HT-injected crayfish were similar to those for (**c**) unstressed and stressed crayfish, respectively (from Fossat et al. 2014)

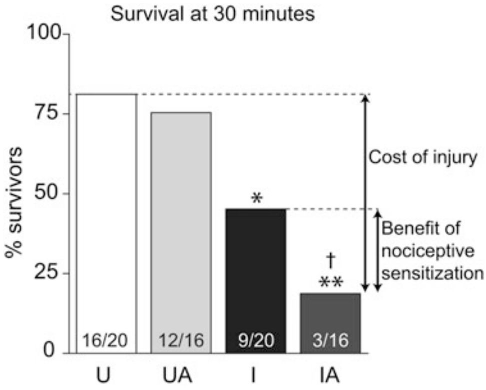


Fig. 7.4 Injured squid lacking nociceptive sensitisation had the lowest odds of survival. At the conclusion of a 30 min trial with free interaction of squid and fish, squid in the I and IA groups had lower overall survival than in the U group, and IA group squid were most likely to be killed. The difference in survival between the U and the IA group can be considered the cost of being injured, while the difference in survival percentage between the IA and I groups ($p = 0.05$) reveals the benefit that nociceptive sensitisation provides to injured animals. Odds ratios, $^{*}p \leq 0.05$, $^{**}p < 0.01$. *U* uninjured, *UA* uninjured with anaesthetic treatment, *I* injured, *IA* injured with anaesthetic treatment (from Crook et al. 2014)

wounded enabled the effect of the anaesthetic per se to be evaluated. The squid were then exposed in groups of four to predatory fish for 30 min and interactions between the squid and fish recorded. Fish could discriminate between injured and uninjured squid and oriented to the injured squid and started to pursue the injured squid at a greater distance than they did to the uninjured squid, irrespective of the application of local anaesthetic on the squid. The local anaesthetic had no effect on the behaviour of uninjured squid but did on the injured squid. Injured squid without local anaesthetic became alert and began to flee at a greater distance from the fish compared to all other groups. The squid that had the greatest chance of being captured by the fish were those with the injury and the local anaesthetic, and these were more likely to be predated than those that were injured but had no local anaesthetic (Fig. 7.4). The nociceptive input from the injury gives some protection, presumably because they were more reactive to the fish. This is consistent with the idea of pain resulting from the nociceptive input inducing a heightened awareness in the squid (Crook et al. 2014). The resulting fitness gain is clearly demonstrated in this study, and it is consistent with a key expectation of pain (Bateson 1991; Sneddon et al. 2014).

7.2.6 Sensitisation of Touch Nociceptors

The increased wariness after injury in squid is not restricted to visual stimuli. Similar changes in general sensitivity have been shown for tactile stimuli applied to areas distant from the site of experimental wounding as well as near to the wound (Crook et al. 2011, 2013). Squid that had a small portion of an arm cut off showed stronger responses to touch, in terms of increased latency to return to crypsis or settled behaviour, compared to unharmed controls (Crook et al. 2011). These responses and changes were similar for touching at different body locations, and responsiveness seemed to peak at 24 h after wounding. By contrast, unharmed squid showed only minor shifts over time, generally becoming slightly less responsive. In keeping with this increased responsiveness after injury was a decreased threshold required to elicit defensive behaviour, which again was not site-specific. Indeed, a wound on one fin increased sensitivity to touch not only on that fin but also on the contralateral fin (Crook et al. 2013). This is different from the site-specific increase in sensitivity following wounding in mammals (Treede et al. 1992) and other molluscs (Walters 1987). In the squid, the changes following wounding appear to be a generalised shift in sensitivity and increased wariness to a range of stimuli (Crook et al. 2011).

In the study of sensitisation of fins noted above, the sensitisation occurred even in fins that had been excised from the main body before crushing of the fin occurred (Crook et al. 2013). Further, if fin crushing was done to intact animals, the sensitisation was seen if those fins were excised 30 min and 24 h later. This increased sensitisation was also observed in the uncrushed contralateral fin after that was excised. Apart from the sensitisation, spontaneous firing of neurons in the fins was noted that did not depend on further tactile stimulation (Crook et al. 2013). When intact animals had a fin crushed, spontaneous firing was noted after the fins were excised not just in the crushed fin but also in the unharmed contralateral fin. However, this spontaneous firing in the contralateral fin did not occur when the other fin was crushed after being excised from the main body. This shows that the spontaneous firing is dependent upon intact neural or humoral connections at the time of crushing and thus suggests a role of the CNS in enabling the spontaneous firing.

The findings noted above are important as they cast severe doubt on the idea that nociceptors simply feed information about tissue damage to the CNS; rather, there is feedback to the nociceptor that is likely to influence their continued functioning (Crook et al. 2013). Such feedback occurs in mammals and occurs at numerous levels from the nociceptor to the brain and involves multiple excitatory and inhibitory process (Burrell 2017). It is thus beyond that expected of a simple reflex mechanism. Remarkably, this overall complexity and the specific modulatory mechanisms are found in at least four invertebrate phyla, e.g. arthropods, nematodes, molluscs and annelids (Burrell 2017). For example, in vertebrates, substance P and bradykinin sensitise nociceptive inputs, whereas opioid reduces the nociceptive signalling. Invertebrates have similar neuropeptides with tachykinins and opioid-like substances. Both vertebrates and invertebrates have glial cells that contribute to nociceptive function (Walters 2014) and have other mechanisms that relate to both nociception and learning/memory. Endocannabinoids are found widely in different phyla and are associated with nociception modulation (Elphik 2012). GABA and glycine inhibit nociceptive signals to the brain in vertebrates, and these substances are also found in invertebrates and inhibit nociceptive signalling (Burrell 2017). The complexity of these systems goes beyond the scope of the present chapter but is reviewed by Burrell (2017). Importantly, these studies demonstrate that the nociceptive mechanisms appear to be bound up with adaptive behavioural changes beyond a reflex. For example, both localised and widespread sensitisation appear to be important in reducing further tissue damage. Other systems regulate nociceptive input to the CNS and thus regulate pain experience, at least in vertebrates, and hence influence behaviour for a prolonged period.

7.2.7 Further Physiological Changes

The increased "anxiety" noted in crayfish exposed to repeated electric shocks has a physiological basis (Fossat et al. 2014, 2015). Stressed animals had higher levels of serotonin (5HT) in the brain, and unstressed animals injected with 5HT showed similar levels of anxiety to those that were stressed (Fig. 7.3). Crayfish, pretreated with a 5HT agonist, did not show the anxiogenic effect of 5HT (Fossat et al. 2015). There were also close correlations between 5HT levels and behavioural indicators of anxiety thus providing further evidence for a role of 5HT in anxiety. Dopamine also increased in stressed animals, but there was no correlation between this biogenic amine and behavioural indicators of anxiety (Fossat et al. 2015).

Fossat et al. (2015) also investigated the effects of chlordiazepoxide (CDZ), which is a drug used to reduce anxiety in humans. There was a marked effect of this anxiolytic treatment. Previously stressed animals, treated with CDZ, spent far more time in the light arms of the cross-maze than did the saline-treated controls. Indeed, they spent about as much time in the light arms of the maze as did animals that had had not been stressed. However, CDZ did not alter 5HT levels, suggesting that the effect of the anxiolytic is independent of the biogenic amine.

In crayfish and other decapods, serotonin also functions to release the crustacean hyperglycaemic hormone (CHH), which elevates haemolymph glucose concentrations (Webster 1996; Bergmann et al. 2001; Toullec et al. 2002). This occurs by mobilisation of intracellular glycogen, with liberated glucose either moving to extracellular fractions or being converted intracellularly to lactate via glycolysis (Stentiford et al. 2001; Verri et al. 2001), which is analogous to the stress responses of vertebrates. Removing one claw of edible crabs by twisting the claw had rapid physiological effects (Patterson et al. 2007). When compared to control animals that were handled, there was a significant increase in lactate and glucose but no decline in glycogen. However, the shift in the glucose to glycogen ratio indicated a marked mobilisation of glycogen to glucose. These physiological changes were not evident in crabs induced to autotomise (Patterson et al. 2007), suggesting that the effects were predominantly due to the tissue damage caused by manual declawing. Physiological changes were even more marked if the crabs were housed with an intact crab immediately after treatment, suggesting that being with a potential competitor was stressful for those animals without a claw. When male crabs were observed competing for a female, those with a claw removed by twisting showed marked deficits in their competitive ability compared to intact crabs and to crabs induced to autotomise a claw (McCambridge et al. 2016). Those with the tissue damage did not compete effectively and seemed to act in a defensive and submissive manner. Thus, it is not the lack of the claw that seems to have wide-ranging effects but those that had tissue damage showed marked physiological stress responses coupled with behavioural changes.

One problem that arises in interpreting studies on physiological change after noxious stimulation is that animals subject to the stimulation often engage in more vigorous behaviour than controls. Thus, there is a possibility that the effect is due to the activity rather than the stimulation (Elwood and Adams 2015). For example, in the studies of Fossat et al. (2014, 2015), crayfish were subject to electric charges repeated at 5 s intervals over a period of 30 min, which caused repeated vigorous tail-flipping escape responses. When the power of the electric charges was reduced so that flipping was not observed, there was no physiological change. At higher shock intensities, the tail flipping decreased over time, and this was suggested to be due to habituation (Fossat et al. 2015). It could, however, have declined due to exhaustion. This problem was examined in shore crabs by shocking at 10 s intervals for 2 min, i.e. less frequent, and for a much shorter time than for the crayfish (Elwood and Adams 2015). Shore crabs do not engage in the vigorous tail-flip response, but some show escape responses by attempting to climb the walls of the tank or showing a threat response. However, this was not seen in all shocked crabs, and some did not engage in behaviour more vigorous than walking. Many control animals also walked, but some remained still during the equivalent 2 min in the test tank. The key comparison in this experiment involved those shocked crabs and those control animals that walked. Lactate was significantly higher in shocked than non-shocked crabs, thus showing that the physiological stress response was caused by the noxious stimulus rather than the behaviour that it elicited (Fig. 7.5).

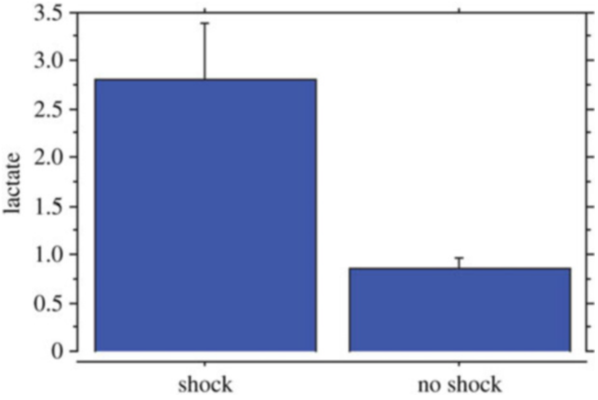


Fig. 7.5 Means and standard errors of lactate (mmol l^{-1}) for shock and control crabs that showed walking as most active response (from Elwood and Adams 2015)

7.2.8 *Opioids and Local Anaesthetics*

Opioids moderate responses to noxious stimuli among vertebrates such as fish (Sneddon 2003), amphibians (Machin 1999), birds (Gentle and Corr 1995) and mammals (Brownstein 1993). Opioid peptides and receptors also occur in various invertebrates and seem to be involved with pain or stress-induced analgesia (Harrison et al. 1994) but also affect feeding, aggression and protective behaviour (Dyakonova 2001). In mantis shrimps, *Squilla mantis*, and crabs, *Chasmagnathus granulatus*, morphine reduces the response to electric shock in a dose-related manner (Maldonado and Miralto 1982; Lozada et al. 1988). In the crab, this was reversed by the opioid antagonist, naloxone. In general, these effects required high doses of morphine, and they declined much more rapidly than in vertebrates. However, morphine also has other non-analgesic effects. For example, the escape response to a moving shadow in the crab, *C. granulatus*, is reduced by morphine (Tomsic and Maldonado 1990). This opens the possibility that the apparent analgesic effects of morphine simply reflect a reduction of responsiveness to all stimuli (Tomsic and Maldonado 1990). This possibility was tested by Barr and Elwood (2011) using the shore crab *Carcinus maenas*. Crabs were either given morphine or water injections and placed into a light area that had a single dark shelter into which the crabs moved. Some crabs received an electric shock within the shelter, but others did not. Each crab was tested for 20 trials; whether they entered the shelters and the latency to enter was recorded. The rationale was that if morphine had an analgesic effect then more crabs should move into the shelter when paired with a shock compared to those without morphine. However, this was not found. Irrespective of shock or not, crabs given morphine showed low numbers of shelter entries during the first ten trials and appeared to be unresponsive and limp. They soon recovered, and in the second ten trials, there was no difference between those given morphine and those given water injections. This supports the idea that the suggested analgesic effects noted in other studies were simply due to a general lack of response rather than analgesia (Barr and Elwood 2011). Local anaesthetics such as benzocaine, however, appear to be effective at eliminating nervous transmission and, hence, nociception. For example, the effects of noxious chemicals on grooming and rubbing of antennae in prawns were significantly reduced when the animal was pre-treated with benzocaine (Barr et al. 2008).

The use of cephalopods in science is now regulated by the EU Directive 86/609/EEC, and the use of analgesics and anaesthetics is demanded for procedures that have the potential to cause pain. However, there is little systematic study on the effectiveness and manner by which different chemicals act on the nervous system of these animals (Andrews et al. 2013). For example, magnesium chloride solution appears to block nociceptive transmission (Crook et al. 2014), but there is a suspicion that it acts as a muscle relaxant (Graindorge et al. 2008) and its use has been queried on welfare grounds (Andrews et al. 2013).

7.2.9 Self-Administration of Analgesics

In this approach, an animal is offered two types of food or fluid that are clearly distinguishable by colour of container, location or taste. With one, an analgesic is included, but the other lacks the drug. The aim is to determine if animals facing long-term pain learn to shift the intake to favour the container with the analgesic (Colpaert et al. 1980). This was achieved in a highly influential study on chickens in which lame chickens consumed more of the analgesic than did control chickens (Colpaert et al. 1980). However, not all such studies on vertebrates have shown an ability to associate the distinguishing features of the container and the effects of the analgesic. Indeed, this is a difficult association to achieve, especially if both containers are sampled within a short time and the analgesic is slow to work. In such a situation, there would be few clues as to which container produced a beneficial effect. To date, there appears to be one such study on invertebrates. Honey bees, *Apis mellifera*, had a single leg amputated compared to unharmed controls (Groening et al. 2017). They were then allowed access to two differently coloured feeders, one of which had sucrose solution and the other sucrose plus morphine. Amputated bees consumed more sucrose overall and thus more morphine than did control bees; however, there was no significant change in the ratio of sucrose/morphine to pure sucrose consumed. Thus, there was no evidence of an association between the colour of the container and effects of morphine, and, therefore, the data do not support the idea of pain in these animals. Nevertheless, this is an interesting approach to the study of potential pain, and further work is warranted. Paradigms should be used that enable temporal separation of the sampling of the containers, coupled with a swift-acting analgesic. Further work might also use different flavoured fluids or food as the vehicle for the drug and the control.

7.3 Specific Brain Structures

7.3.1 A Suitable Central Nervous System Analogous to the Human Cerebral Cortex?

We know much about human pain, and the complex pathways involved in human pain are well established (Key 2016). These pathways and neural structures differ in many other vertebrates and are absent from invertebrates. For this reason, it has been argued that fish and invertebrates are unable to experience pain (Rose et al. 2014; Key 2016). It is suggested that only animals having a system that closely matches that found in humans may reasonably be expected to have the potential for pain, e.g. primates. The argument rests on the idea of functional homologies being mapped onto structural homologies. Key (2016), for example, uses the example of the vertebrate visual system comprising a laminated optic tectum, and it is this structure that specifically enables vision.

However, it has been noted repeatedly that animals may show similar function with completely different neuronal structures (Elwood et al. 2009; Elwood 2012). The complex brains and eyes of humans, octopus and honey bees have separate evolutionary histories, each developing independently for many hundreds of millions of years. The result is that the eyes are very different, with the honey bee having a convex arrangement of numerous ommatidia and humans and the octopus having concave arrangements of light-sensitive pigments with a lens to focus the incoming light. These latter two have evolved independently, and the superficial similarities between the octopus and human eye are examples of convergent evolution. The structures are not homologous. Further, the brain structures of the honey bee and octopus are very different from each other, and both lack the optic tectum of vertebrates. Nevertheless, both have an ability to use light from distant sources to gather information about the world. That is, they have the same function as that in humans despite having different evolutionary histories and different structures.

Key (2016) notes that for pain to have survival value it must be mapped to specific body locations. Specific structures in the human cortex are presumed to enable such mapping. However, we have seen above that crabs, prawns and octopuses will attend to specific locations on the body that have been subject to noxious stimuli but they do this without the human cortex. Further, damage to the cortex in humans can lead to a loss of pain sensitivity (Key 2016), and thus there is no motivation to escape from the stimulus. We have demonstrated above that many invertebrates show high motivation to escape from or avoid noxious stimuli yet they have no human cortex. This demonstrates that at least some of the components of pain identified as requiring the cortex in humans nevertheless are found in animals that lack it. The conclusion is clear; those invertebrates are showing the same functions with different anatomy. Thus, we may conclude they must have brains that are in part analogous to those of mammals.

Of course, noting that different taxa have similar sensory abilities with different structures does not mean that they must experience pain. The point of the argument is to indicate that the possibility cannot be dismissed because of morphological differences. If pain confers such evolutionary advantages that it has developed in at least some vertebrates, then other taxa might well have developed a similar solution to long-term avoidance and protection from noxious stimuli.

Another argument that invertebrates do not experience pain is that their brains are too small. Indeed, the brain of a honey bee only has approximately 1 million neurons compared to about 68 million for a mouse (Klein and Barron 2016). Size and weight are particularly important to a flying animal, and there has clearly been considerable selection pressure to reduce unnecessary weight whenever possible. However, Klein and Barron (2016) query if neuron number is the key to understanding the capabilities of a brain. They maintain that functional organisation is the key and argue that bees and mammals are similar in that respect. Bees have surprising cognitive abilities, and the processing capacities of vertebrates and insects are not as different as the neuron numbers might suggest (Chittka and Niven 2009). It should be noted, however, that the brain of the octopus is large, complex and distinctly divided into specialised lobes (Young 1963; Crook and Walters 2011). The CNS comprises some

500 million cells and enables the highly complex behaviour of this animal, but many of these are in ganglia in the arms (Mather 2011). However, there seems to be no compelling argument to link brain size and or neuron number to the ability to experience pain (Broom 2007).

A key aspect of mobile animals is that they monitor internal states and external environment and prioritise actions in what has been termed the final common path (sensu McFarland and Sibly 1975). Most can distinguish between changes to their perceptual input that is caused by their own movement and those not affected by such movement. That is, they must have some basic awareness and sentience. In this respect, Klein and Barron (2016) argue that some fundamental aspects of brain structure of vertebrates, arthropods and molluscs, but not nematodes, are conserved. That is, the structures and abilities that enable decision-making likely predate the divergence of these major taxonomic groups. It is suggested that these groups share the ability of subjective experience, which is presumably a prerequisite for pain experience (Klein and Barron 2016).

7.4 Conclusions and Thoughts on Humane Treatment

Invertebrates have traditionally been considered to respond to noxious stimuli purely by nociceptive reflex, and some still argue that that remains true (Rose et al. 2014). It is clear from the evidence, however, that in many cases, the responses go far beyond reflex. They show rapid avoidance learning involving both egocentric and allocentric cues (Magee and Elwood 2013). They make behavioural decisions about responses to noxious stimuli, and the requirement to avoid the noxious stimuli is traded off against other requirements (Magee and Elwood 2016a). They show long-term motivational changes that reflect memory of their prior experiences (Elwood and Appel 2009; Appel and Elwood 2009b) and show similarities to the anxiety seen in vertebrates (Crook et al. 2011; Fossat et al. 2014). These changes are mediated by complex physiological processes that are analogous and, in many cases, homologous to those of vertebrates (Fossat et al. 2015; Elwood and Adams 2015). They have complex, compartmentalised brains that in some cases have more neurons than some vertebrates. They have complex cognitive abilities and show an awareness of the noxious stimulus and about the part of the body that was affected, and this improves survival (Crook et al. 2014).

This mass of evidence, in terms of criteria that are fulfilled, is consistent with the idea of pain and shows similarities to the available evidence for many classes of vertebrates. As pointed out by Sherwin (2001), however, the acceptance of experimental evidence is often guided more by our views of specific taxa than by the data. There is a public dislike of invertebrates because they are strange and alien and thus a marked lack of empathy (Kellert 1993). As Sherwin (2001) argues, however, the degree of empathy should not affect our acceptance of data. If data are accepted for vertebrates, they should also suffice for invertebrates.

This is not a trivial point because little thought or protection is provided to invertebrates, particularly with respect to their treatment in the human food chain (Elwood 2012). With respect to crustaceans captured or farmed for human consumption, the numbers are vast. The number of tiger prawns (*Penaeus monodon*) used in 2008 is estimated at 214 billion (i.e. 214 million, million). Further, this species comprises only about 12% of the number of crustaceans used per year, making the total number over 1600 billion animals. This greatly exceeds the combined numbers of chickens, pigs, sheep and cattle killed (Elwood 2012). Even if we conclude that the probability of these species being able to suffer is low, or that the degree of suffering is likely to be low, we should weigh that potential suffering by the vast numbers involved.

It is important to reiterate that there is no absolute certainty that any animal experiences pain. Nevertheless, our treatment of vertebrates is frequently influenced by the possibility that they feel pain and hence might be able to suffer. However, the current lack of control measures enables food processors to treat lobsters in the most extreme ways. In processing factories, live lobsters may have the appendages bearing the claws twisted off. The still living animal may be impaled on a spike to remove the abdominal (tail) muscle and the living head and thorax is discarded (PETA 2013). Further, in several crab fisheries, the claws are twisted off and retained, but the animal is returned alive to the sea. Here, it may be unable to feed (Patterson et al. 2009) and may show marked stress response and low survival (Patterson et al. 2007). In the light of evidence being consistent with the idea of pain, more humane methods to kill the animal before dismembering should be encouraged.

Other species in the food industry also need attention. Billions of live individuals are boiled, and we need to consider for which species death may be swift and suffering minimised and which species may die slowly. Some processes currently employed for crustaceans and cephalopods might result in slow death with a high possibility of pain, and these should be modified. Arguments that because we cannot prove pain in animals then those animals should not be protected must be rejected. We now have established criteria that we expect to be fulfilled should pain exist in these animals, and we now have numerous tests of those criteria. In many cases, the criteria have been fulfilled. It is possible that not all criteria are equal in indicating pain and we need to establish which of those are the more persuasive and how many of those should be fulfilled. It has been suggested that protection should be given if just one criterion is fulfilled (Birch 2017), but it is likely that the food industry will demand more. It is important to accept that closely related groups of species are likely to have the same capabilities and thus avoid delays in giving protection that could arise from demands that each species be tested (Birch 2017). The evidence is now extensive, and we should be in a position to use it to enhance the welfare of a considerable number of animals.

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Chapter 8
Care and Enrichment for Captive Cephalopods

Gavan M. Cooke, Belinda M. Tonkins, and Jennifer A. Mather

Abstract Cephalopods have become an archetype for invertebrate cognition, sentience and welfare studies. Their convergence with so-called 'higher' vertebrates (birds, mammals) in memory, learning, problem-solving, tool use and likely sentience has made biologists completely rethink the nature and commonality of cognition in the animal kingdom. Cephalopods are a model in many areas of biological sciences, often key attractions in public aquaria and kept in private collections, as well as being important for the future of aquaculture. Modern animal welfare practice should demand that, in addition to maintaining good environmental parameters (e.g. water quality), sufficient environmental, cognitive and social stimulation are provided in a design that fully engages an organism's cognitive, sensory and motor abilities. Cephalopods' abilities are far-ranging and must be considered when providing captive care, to not only provide adequate welfare and well-being but to also ensure normal development, allowing confidence in results obtained from their use in experimental settings or conservation programmes. Their sensory capability, inter- and intraspecific communication, personalities and life histories require thoughtful and specific environmental design. Here, we outline their cognitive abilities and likely captive conditions and suggest how their abilities can be appropriately stimulated.

8.1 Introduction

This book acknowledges that some invertebrates require welfare considerations. It has not been written to provide suggestions for encouraging growth rates, increasing fecundity or making them more visible or interesting, but for the sake of the animals

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G. M. Cooke (✉)
Department of Life Sciences, Anglia Ruskin University, Cambridge, UK
e-mail: gavan.cooke@anglia.ac.uk

B. M. Tonkins
The College of Animal Welfare, Godmanchester, UK

J. A. Mather
Department of Psychology, University of Lethbridge, Lethbridge, AB, Canada

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themselves. The cephalopods are the most obvious of all the invertebrates that might deserve such attention. There are approximately 800 extant cephalopods (Young et al. 1998; Roper et al. 1984; Jereb et al. 2005), ranging in size from a few millimetres long (the pygmy squid, *Idiosepius notoides*) to more than 14 m (the colossal squid, *Mesonychoteuthis hamiltoni*), but most are not larger than a mantle length of 200 mm. Of the species identified to date, all but one, *Vampyroteuthis infernalis* which eats marine snow and is not a squid at all (Hoving and Robison 2012), are obligate predators. They are limited to marine habitats (Norman 2003) but utilise many niches within them.

Approximately 10% of the known cephalopod species were kept in captivity in 1981 (Boyle 1991). Cephalopods have been used extensively in science over the centuries, in a wide variety of fields including anatomy, physiology/neurology, animal behaviour/behavioural ecology/ethology, ecology/evolution and conservation/fisheries (Vidal et al. 2014; Fiorito et al. 2014). As a result, they are found in laboratories across the globe (Smith et al. 2013). A large proportion of cephalopod species, such as cuttlefish, bobtail squid and octopuses, spend a good proportion of their lives just above or in the substrate (Hanlon and Messenger 2018).

Many cephalopods (bar cuttlefish) have a paralarval stage, which has become a bottleneck in captive breeding as it can be difficult to provide the appropriate environment, including food (Sykes et al. 2014; Iglesias and Fuentes 2014). Other cephalopods show direct development to immature mini-adults; rapidly progressing through a variety of transitional stages, including changes in diet, behaviour and preferred habit choice—see Robin et al. (2014) for a detailed review of cephalopod development.

This chapter first aims to explore their sensory/cognitive abilities and how these attributes can be utilised in care and enrichment programmes. Then, the nature of cephalopod captivity is described, before we make suggestions for applied enrichment ideas and avenues of future research. Some of the enrichment ideas presented in this chapter are clearly difficult to perform in aquaculture, research and even public aquarium settings, but they can be implemented, if only in part. No two captive environments are ever the same, and aquarists are excellent at making use of what they have to hand.

8.2 Cephalopod Sensory and Cognitive Abilities

The sensory capabilities of cephalopods have been studied for over a century, but as new species are discovered all the time, especially in the deep oceans, more work remains before we have a thorough understanding of cephalopod sensory abilities. The studies on cognition, pain and suffering are limited to a few model species, e.g. *Octopus vulgaris* and *Sepia officinalis* (Hochner et al. 2006; Ponte et al. 2013), and while evidence for their high levels of cognition, sentience and the ability to ‘feel’ and therefore suffer is compelling (Mather 2008), much work is needed to understand the extent of these abilities across the Class Cephalopoda.

8.2.1 *Visual Abilities*

Cephalopods possess a lens eye, comparable in complexity (Serb 2008), but different anatomically from the vertebrate eye (Gleadall and Shashar 2004; Zylinski et al. 2011). They focus through movement of the aperture (Yamamoto 1985) like camera lenses, unlike vertebrate eyes in which the lens changes shape. Many cephalopods, such as the octopuses, squids and cuttlefish, can discriminate the plane of polarised light (Shashar et al. 1996). This is due to the orthogonal nature of the photoreceptor arrangement, which differs from that of the rods and cones found in vertebrate eyes (Mäthger et al. 2009). The precise use of polarised light is not yet fully understood, but it may be used in predation and intraspecific communication (Shashar et al. 1996; Mäthger et al. 2009) and to increase visual acuity in turbid waters or low-light conditions (Cartron et al. 2013). One species perceives wavelength of light, i.e. colour (*Watasenia scintillans*—Matsui et al. 1988). Interestingly, visual learning may determine preferences for prey and substrate before hatching in *S. officinalis* and imprinting (Darmaillacq et al. 2006) which has significant implications for captive rearing.

8.2.2 *Auditory/Mechanoreceptive Abilities*

Cephalopods are not 'deaf', but they have no organs analogous to ears (Hanlon and Budelmann 1987), and there are differences in their abilities to receive mechanical stimulation from those of marine vertebrates. Cephalopods may only be able to receive low frequencies (<10 Hz) (Kaifu et al. 2008; LaRoe 2011). They use a statocyst system for balance and orientation, in a similar arrangement to that of vertebrates (Williamson and Chrachri 2007). This organ possesses hair cells which detect motion and so may have some auditory processing features. To detect water motion, some cephalopods have an analogue to the lateral line seen in fish (Budelmann and Bleckmann 1988), lines of enclosed ciliated cells on the heads and arms of *S. officinalis* cuttlefish and *Lolliguncula* sp. squid.

8.2.3 *Olfactory and Chemosensory Abilities*

Cephalopods possess a structure which appears to serve olfactory detection abilities, an olfactory pit with nerve fibres leading to a defined olfactory nerve, which in turn enters an 'olfactory bulb' (Polese et al. 2016). A few studies (e.g. Boyle 1983, 1986; Alves et al. 2007) suggest cephalopods can detect waterborne chemicals. Behavioural changes in octopuses and cuttlefish were observed when water from a tank containing predatory fish was added (Wells 1963; Messenger 1977), and these animals sometimes inked after the liquids were added (Di Cosmo et al. 2006; Derby 2014). Conversely, adding crustacean 'juices' to tanks increased the ventilation rate in octopuses, a sign that chemical cues might be used in search for prey (Boyle 1983). Mate choice appears to be determined by olfactory cues rather than vision in cuttlefish (Boal 1996). Chemical cues probably play a role during mate searching in cephalopods that are dispersed (Budelmann 1996) and may also trigger agonistic interactions in the southern blue-ringed octopus *Hapalochlaena maculosa* (Morse et al. 2017).

Coleoid cephalopods produce and detect a viscose-, melanin- and dopamine-based fluid or ink (Derby et al. 2007), which acts as visual concealment, confusing predators (Hanlon and Messenger 2018), and is toxic to some of them (Russo et al. 2003; Derby et al. 2007). Cephalopod ink may act as an alarm (Nair et al. 2011), alerting conspecifics to the threat of predation, as has been observed by Wood et al. (2008). This would be useful in species such as squid that gather in groups. However, Lucero et al. (1994) found a previously unidentified antioxidant molecule that may prevent rapid oxidation, therefore reducing dilution, in seawater, suggesting the alarming properties may also serve more dispersed species (see Wood et al. 2008).

One of the common ways in which cephalopods can sense the world is by contact chemoreception, ‘tasting’ using suckers on their arms. The suckers possess numerous chemoreceptors. An *O. vulgaris* sucker ~3 mm in diameter will contain tens of thousands of receptors, and there are similar and more dispersed receptors found all over the skin (Graziadei and Gagne 1973). Each arm can control 40 million tactile and chemical receptors (Nesher et al. 2014), and octopuses search for invisible prey using their eight arms and this sensory system (Mather et al. 2014). It is likely an adaptation for the foraging strategy of many octopuses, which involves daily trips to crevices, large rock works, reefs and other aggregations where they use chemotactile investigations to seek out prey (e.g. Leite et al. 2009). After a visual survey initiates movement to an appropriate foraging site, non-visual chemo-tactile senses dominate the hunting process. In a laboratory analogue of this, octopuses given a crab in an enclosed jar did not learn to open the lid to acquire the prey inside unless there were chemical cues on the outside of the jar (Anderson and Mather 2010).

Tactile/kinaesthetic receptors in the muscles, for all eight arms, may number into the hundreds of thousands (Graziadei 1971). Octopuses may exhibit much localised motor autonomy (Sumbre et al. 2001; Grasso 2014) in the chains of brachial ganglia and a large degree of decentralisation of neuronal motor control compared to vertebrates (Nesher et al. 2014; Zullo and Hochner 2011; Hochner 2013; Mather and Dickel 2017). This allows them to explore their environment, to coordinate the actions of the eight arms in crawling, which have no known gait (Levy et al. 2015) and combine camouflaging postures with arm propulsion (Huffard 2006).

8.2.4 Cognition and Sentience/Consciousness

Cephalopods possess brains comparable in brain body ratio to those of mammals and birds (Sykes et al. 2012) and are thought to be highly intelligent (Mather and Dickel 2017). Octopus species possess ~500 million nerve cells in total (Young 1963, 1971), a number comparable with that of domestic cats (Solnick et al. 1984). They are capable of many types of learning (Young 1991; Dickel et al. 2000; Karson et al. 2003; Alves et al. 2007; Jozet-Alves et al. 2013), from habituation through operant conditioning. Although most octopuses are solitary (though see Scheel et al. 2016), most squids are obligate members of groups and the reef squid, *Sepioteuthis sepioidea*, is considered social (La Roe 1971; Moynihan and Rodaniche 1982; Mather 2016). Some cuttlefish aggregate, particularly during reproduction (Hall and Hanlon 2002), and others have been observed to school (Yasumuro et al. 2015).

Octopuses use learning extensively (Wells 1978). *Sepia officinalis* memories can be episodic-like, i.e. they integrate 'what', 'where' and 'when' components (Jozet-Alves et al. 2013). This ability may enable 'mental time travel' (e.g. Clayton et al. 2003); the individual can 'look' into the past to recall events and plan for the future. This is an ability demonstrated via coconut carrying in an octopus (*Amphioctopus marginatus*: Finn et al. 2009), but also in foraging over time (see Mather 1994). As a result of these findings, cephalopods are considered aware and conscious (Mather 2008; Mather and Anderson 2007; Edelman and Seth 2009). They also exhibit play, exploration and problem-solving behaviour (Kuba et al. 2006; Mather and Anderson 1999).

Cephalopod species have shown individually different behavioural responses across a variety of contexts, as demonstrated in octopus (Mather and Anderson 1993), bobtail squid (Sinn et al. 2006, 2008; Sinn and Moltschaniwskyj 2005) and cuttlefish (Carere et al. 2015), which is considered a definition of animal personalities (Sinn et al. 2008).

The public do not generally accept that consciousness or sentience is present in invertebrates (Carruthers 2007; Horvath et al. 2013). Major global legislators (e.g. the European Union) state that *all* animals should be treated as sentient creatures and have especially noted the cephalopods for consideration. They have special protection in some countries when used in science, e.g. UK/EU, Canada, Norway and some states of Australia.

8.2.5 Ability to Feel Pain and Suffer

Cephalopods have cutaneous free nerve endings, and recent research has found direct evidence for mechano-nociceptors in squid and octopus (Crook et al. 2013; Alupay et al. 2014). Smith et al. (2013) suggest that evidence of 'feeling' should include the presence of nociceptive receptors, possession of 'higher' integrative brain centres, connection of the nociceptive pathways to these centres, opioid receptors in the CNS, analgesics modifying responses to stimuli that would be painful to humans, learnt associations of stimuli to events and CNS actions based on these stimuli. As the evidence is scattered, Birch (2017) argues for a precautionary principle in the face of our ignorance.

Cephalopods have learnt avoidance of shocks from sea anemone stings (Boycott 1954) and distasteful prey (Darmaillacq et al. 2004). Following an injury, there is sensitisation of the area and general hyperresponsiveness in squid (Crook et al. 2013). In addition to these responses, there is evidence of wound-directed behaviour in octopuses (Alupay et al. 2014). Although it has not been directly studied, phylogenetically, the endogenous opioid system is highly conserved (Zhu and Stefano 2009), i.e. the opioid system originated earlier than cephalopods evolved. For it to not be present in cephalopods, it would need to have been selected against early in cephalopod diversification or repeatedly removed via natural selection, which is unlikely. There is some evidence for the presence of enkephalin-like peptides and opioid receptors in the peripheral tissue (Andrews et al. 2013). At present, there are no published data on whether cephalopods have cannabinoid or steroid receptors in the brain, which could modulate pain, or whether analgesics modify the response to a painful stimulus (Andrews et al. 2013).

Reviews of these properties leading to pain/suffering in cephalopods have been extensively published elsewhere (Moltschaniwskyj et al. 2007; Fiorito et al. 2014), so we can evaluate whether many of the key criteria are met and apply the 'precautionary principle' (e.g. Croney and Millman 2007; Birch 2017). An experiment that may cause pain or suffering requires central authorisation, which would seldom be granted.

Part of the care of cephalopods in laboratory settings may be controlled by legally enforced welfare guidance. One aspect of this for research is known collectively as the 3Rs: refinement, replacement and reduction (Russell and Burch 1959). These are general directives but may also be sometimes legally binding. They specify that in experimentation, proper care must be used in procedures, models or 'lower' animals should be used and as few experimental subjects as possible should be tested. They may specifically state that enrichment is required during housing, and even the type of enrichment for specific taxa.

Senescence

Nearly all cephalopods are semelparous (Mangold 1987), that is, they go through one breeding period and then die. Senescence (Mather 2006) occurs after animals have mated with multiple partners, the females have laid all their eggs and the octopuses have tended them (Anderson et al. 2002). Senescence physiologically begins at full sexual maturity and involves secretions from the optic gland (Tait 1987; Wodinsky 1977) that rapidly mature the reproductive organs. This appears to inactivate the salivary/digestive glands, reduces or stops appetite and might be the cause of the physical deterioration observed (Tait 1987 but see Anderson et al. 2002 for a thorough description of octopus senescence). Females are less active, and males, possibly more active, may lose their daily activity cycle (Meisel et al. 2003), wandering freely; public aquariums have reported male octopuses becoming more diurnally active (Anderson 1987; Anderson and Martin 2002). They may stop changing their skin displays at disturbance and often show signs of infection, including cataracts or rapidly decreasing muscle tone (Anderson et al. 2002). Those species that offer no egg care tend to die quickly. Typically, in the wild, this is because of predation (Anderson et al. 2002), as listless and seemingly careless cephalopods are presumably easy targets for their many predators. Prevention of breeding may extend some species' lifespan (in cuttlefish, Panetta et al. 2017; in octopuses, Wodinsky 1977). Senescence is first noticed in captivity when the normally eager animals begin to slow down their feeding, eventually ignoring food (Anderson et al. 2002). Displaying animals in this condition may be a problem for public aquaria.

8.3 Captive Conditions

The conditions of captivity for cephalopods will vary between settings. However, all situations can be fitted into one of four categories: *research* (including educational contexts), *aquaculture* (i.e. aquatic meat production), *public display* (i.e. public aquaria and zoos that possess aquaria) and *companion animals* (i.e. as pets in a home or similar environment).

Except for the shelled nautiloids, coleoid cephalopods are physically fragile, lacking an internal or external skeleton to protect them, and do not always heal well from injuries (Sherrill et al. 2000; Oestmann et al. 1997). When threatened, cuttlefish, squid and octopuses jet unpredictably (Forsythe et al. 1991) or in a zig zag, heading backwards away from perceived threats. This may cause damage in confined areas as they crash into objects or walls (Cooke pers. obs.). Infection may then result in posterior mantle tip dermatitis (Hanley et al. 1999), a bacterial infection in their posterior epidermis (Sherrill et al. 2000; Oestmann et al. 1997). The injuries may retard growth and even kill the individual if a cuttlefish cuttlebone breaks (Hanley et al. 1999) and are common in captive coleoid cephalopods (Cooke pers. obs.). This needs to be considered in tank design.

8.3.1 Research

Cephalopods degrade the quality of the water they live in by excretion and degradation of food remains. They remove the flesh from their molluscan or crustacean prey but leave discarded remains, especially of their crustacean diet, near their shelter. In the wild, scavengers will eat anything edible in these discards (Mather 1992), and the seas are so vast in volume that water chemistry is unaffected by rotting remains. In captivity, remains need to be removed, but this may not happen immediately, which puts additional pressure on filtration systems or creates potentially time-consuming husbandry. As coleoid cephalopods release ink when under threat (Lucero et al. 1994; Derby 2014; Wood et al. 2008), a supply of new water can be vital should a major inking episode take place. Inking may also black out tanks, leading to damage if the cephalopods flee erratically (Cooke and Tonkins 2015). Degradation of water quality by ammonia release can be countered by biological, chemical and mechanical filtration, especially important in a closed circulation system. Although no data exist, many captive cephalopods that are used in research are probably near a natural source of sea water due to the requirements for constant and large water replenishment.

Natural sea water, although likely settled to reduce turbidity before being supplied to the aquarium, filtered and possibly sterilised (using UVc sterilising bulbs, ozone is not recommended for cephalopods—K. Perkins, SeaLife pers.com), may itself be enriching. Coastal research aquarium facilities are less environmentally sterile than artificial saltwater systems due to the replenishment of natural water. Sterility, both in terms of water quality and in environmental objects, is an overriding feature of animal experimentation (Dawkins 2003), despite arguments suggesting it is bad for science as well as welfare (e.g. Baumans 2005).

Provision of physical enrichment such as rocks, shells, sand or barriers may limit the ability to see every individual every day for health inspections, as many cephalopods, especially octopuses and cuttlefish, will use these items to hide themselves (Mather 1986, 1994). Research protocols may demand a controlled, therefore possibly sterile experiment, a pressure that counters the requirement for enrichment. Educational institutions may display animals for simple observational or decorative reasons, being more akin to a public aquarium environment, in which case, viewer demands may dictate a more ‘cluttered’ environment.

8.3.2 Aquaculture

There is a wide range of studies on growth and survivorship in cultured cephalopods (Boyle 1991; Sykes et al. 2011, 2014; Correia et al. 2005; Iglesias and Fuentes 2014) but few dealing with welfare per se in captive environments (see Tonkins et al. 2015). Cephalopods have many characteristics ideal for aquaculture, including high protein content, high fecundity, high food conversion rate and rapid growth (Sykes et al. 2014), and have been cultured commercially since the 1960s (Schroder 1966). The European cuttlefish (*S. officinalis*) has some other useful traits, such as high survival rate compared to other cephalopods and large eggs. Many of their behaviours are understood (Hanlon and Messenger 2018), and they are resistant to crowding, disease and handling, which enables easy shipping (Sykes et al. 2014). The average world production of *S. officinalis* is approximately 18,000 tonnes per year (2000–2010 approximately 9 million individuals per year based on a 500 g adult). They are produced on a medium scale in aquaculture in Italy, Portugal and France (Sykes et al. 2014). Large-scale aquaculture of cephalopods is a nascent industry, and their use is increasing (Correia et al. 2005).

Squids are difficult to keep in confinement because of their escape jetting. Tanks of various shapes such as circular tubes may be suitable for normally pelagic squid (Lee et al. 1994) as they reduce damage when squid jet defensively (Hanley et al. 1999). Squid have also been successfully kept in floating sea pens, but these pens are prone to catastrophic losses during very bad weather (Saso 1979). Raising most octopus species is difficult because the paralarvae are tiny and planktonic (Vidal et al. 2014). In addition, solitary and cannibalistic octopuses may eat each other in confinement. Often, normally solitary species are housed in high densities (Sykes et al. 2011; Correia et al. 2005) which, even if all survive, can result in poor welfare. Boal et al. (1999) found that increasing density of *S. officinalis* adult males and females caused more agonistic behaviours, reduced resting time, reduced feeding and caused more damage to females. These impoverished or crowded environments may also be detrimental to yield (Sykes et al. 2014). Some aquaculture researchers have investigated the use of ‘seminatural’ conditions, and the aquaculture industry is beginning to take the idea of environmental enrichment seriously (Ashley 2007; Martins et al. 2012; Näslund and Johnsson 2016).

8.3.3 Public Aquaria

There are approximately 350 public aquariums worldwide (Penning et al. 2009), and cephalopods often make exciting attractions, especially as the public aquarium sector continues to mature and visitors expect new and exciting animals to view. Indeed, the Monterey Bay Aquarium showed two *Sepia* species and eight octopus species, as well as one squid and nautiluses, in its special exhibit, ‘Tentacles’. Some species adapt to this confinement and others do not. Commonly seen octopus species (e.g. *Enteroctopus dofleini* and *O. vulgaris*) are wild caught due to the difficulty in captive breeding, while other species produce viable offspring for five to seven generations, e.g. *S. officinalis* (Sykes et al. 2014) and *Metasepia pfefferi* (Read et al. 2005).

The giant Pacific octopus (*Enteroctopus dofleini*) (GPO) is likely the most commonly seen cephalopod in public aquaria (Cooke and Mather pers. obs.), although it has a relatively short lifespan compared to comparatively sized birds and mammals. As a result, there is a whole handbook on its care (AZA 2014), including sections on feeding regimens, how to keep it confined and enrichment to prevent its boredom. The GPO is one of the species we deem ‘charismatic megafauna’—large, easily viewed and exotic, easily caught in the wild, transports well—and is relatively active in captive conditions (Anderson and Wood 2001). The true squids (order Teuthida) are rarely seen in public collections, due to their mostly pelagic life style. *Loligo vulgaris* has been kept in Spain (Aquarium Finisterrae) successfully, and Monterey Bay Aquarium has famously exhibited the vampire squid (*Vampyroteuthis infernalis*—although not technically a squid, its demands are significant) and the gelatinous open-ocean octopus (*Japetella* sp.) in 2014. As with all the coleoid cephalopods, they are very fragile and presumably damaged easily when caught, which makes transporting them to aquariums very difficult.

The non-coleoid cephalopods, the nautiluses (family Nautilidae), are relatively common in public aquaria. This is likely due to their hardiness, suitability to be kept in groups, unlike most other cephalopods, which are prone to cannibalism (Iglesias and Fuentes 2014), and relative ease of feeding. They are at least partially scavengers and accept dead food readily. However, although they live a comparatively long time (~20 years), breeding success has been very poor (Fields 2006), eggs can take up to 12 months to hatch (Carlson et al. 1992) and they have not lived longer than just over 12 months (Fields 2006) in captivity. They are now listed as threatened under the US Endangered Species Act, due to harvesting for their shells, and CITES protected, requiring a licence to collect, so acquisition should be discouraged (Table 8.1).

8.4 Enrichment

Enrichment is the term used for attempting to improve captive animal welfare by providing or giving access to stimulation thought to be important or by promoting activity and behavioural variety (Wells 2009). The concept is broad, but enrichment is any technique that helps the biological functioning of a captive animal by

Table 8.1 Examples of cephalopod species in captivity, types of captivity with other salient information

	Species	Common name	Type/s of captivity	Notes	References
Sepiida	*Sepia officinalis*	European cuttlefish	Aquaculture, public aquarium, home	Short lived, breeds well up until G6–7	Hanlon and Messenger (2018) and Sykes et al. (2014)
	Sepia bandensis	Dwarf cuttlefish	Home (very common)	Short lived, breeds well up until G6–7	
	Metasepia pfefferi	Flamboyant cuttlefish	Home (rare), public aquarium, lab	Short lived, breeds well up until G6–7	Read et al. (2005)
	Sepia pharaonis	Pharaoh cuttlefish	Aquaculture	5 generations cultured	Minton et al. (2001)
Sepiolida	*Euprymna* spp.	Bobtail squid/dumpling squid	Laboratory, home (rare)	Model for symbiotic bacteria research	Nabhitabhata et al. (2005)
Octopoda	*Abdopus aculeatus*	Algae octopus	Home	Most kept pet species	
	Octopus vulgaris	Common octopus	Home, laboratory, aquaculture, public aquarium	Very common lab model	Fiorito et al. (2014)
	Octopus bimaculoides	Two spot/ Californian octopus	Home, laboratory	Young can be raised successfully	
	Hapalochlaena spp.	Blue ringed	Home (rare)	Dangerous and endangered	
	Enteroctopus dofleini	Giant Pacific Octopus (GPO)	Public aquarium, home (rare)	Can get very large (3 m)	Slater and Buttling (2011) and AZA (2014)
	Eledone cirrhosa	Curled or Northern octopus	Laboratory		Boyle (1981)
Squid	*Sepioteuthis lessoniana*	Pacific long-finned squid	Aquaculture	Most common of rarely seen captive squids	La Roe (1971) and Walsh et al. (2002)

changing its environment, including the encouragement of natural behaviours (Newberry 1995). To ascertain whether enrichment has succeeded in its purpose requires an understanding of the species' physiological needs, its behavioural repertoire and sensory capacities (Young 2003), also the measurement of changes due to the enrichment (Alligood et al. 2017). These might be biological processes such as activity levels, respiration rates, skin displays and also time spent using enriching objects. Without objectively assessing enrichment results, we will never know if the intervention has achieved what was intended. AZA now asks for formal evaluation of enrichment programmes (Alligood et al. 2017), but many staff lack the time and/or understanding to conduct publishable studies (Hoy et al. 2010), so that in many instances of aquaculture and research, the bare minimum of environmental enrichment is provided. Often there is no substrate to burrow in or camouflage, or even a physical refuge. There may be no behavioural enrichment, e.g. live/dead food is dropped right in front of the cephalopod (e.g. Fiorito and Gherardi 1999, see also Anderson et al.'s 2009 statement that live food is enriching for octopuses). Environmental limitation can influence development in some cuttlefish species, causing delayed growth and impaired memory, slower maturation rate (Dickel et al. 2000), lesser burying ability and longer latency to burrow in sand (Poirier et al. 2004). Environment changes can affect food preference, before hatching via olfactory cues (Guibé et al. 2010), or by visual cues (Darmaillacq et al. 2006). It may also affect preferences in background matching (Poirier et al. 2005; Yasumuro and Ikeda 2016) (Fig. 8.1).

Enrichment must be tailored to individuals; knowledge of personalities of octopuses (Mather and Anderson 1993) and cuttlefish (Carere et al. 2015) may allow us to predict the individual's repertoire and possible influences. This individual variation adds a layer of complexity when we consider the type or measure the result of enrichment. For example, a cephalopod may not use enrichment items placed in the centre of its tank if it is 'shy' and stays in close physical proximity to safe objects (Mather 1980). If animals come from impoverished captive environments, they may be less prone to adopt enrichment (Dallaire et al. 2012). Further, if an aquarium has only one individual, keepers may believe that it represents its species in response to this specific form of enrichment. Frequency of some activities may also vary between species, e.g. some cephalopods have fixed and often nocturnal activity and some, such as *Octopus vulgaris* (Meisel et al. 2006), are much more flexible. Latency, or the speed at which a cephalopod engages with objects, may significantly differ within and among species, although it has been suggested as 'the' measure to assess the welfare of cephalopods (e.g. Amodio et al. 2014; Ponte et al. 2017; Fiorito and Gherardi 1999). For all these reasons, individual responses and baselines need to be established to accurately determine enrichment effects (Ponte et al. 2017).

Behavioural Enrichment

Before providing enrichment to animals, aquarists and researchers make at least two assumptions. The first one is that the animal that the enrichment is designed for requires it. Cephalopods are by no means all the same behaviourally, so may need different enrichment quantity and type. Enrichment is required to be species-specific, which can be challenging, as species-specific behavioural repertoires are absent or incomplete for all cephalopods. The only available ethogram is for the family Octopodidae (Mather and Alupay 2016), although there are more fragmentary ones for *Octopus vulgaris* (Packard and Sanders 1971) *Octopus insularis* (Mather and Mather 1994), previously thought to be *vulgaris*, and *Abdopus aculeatus*

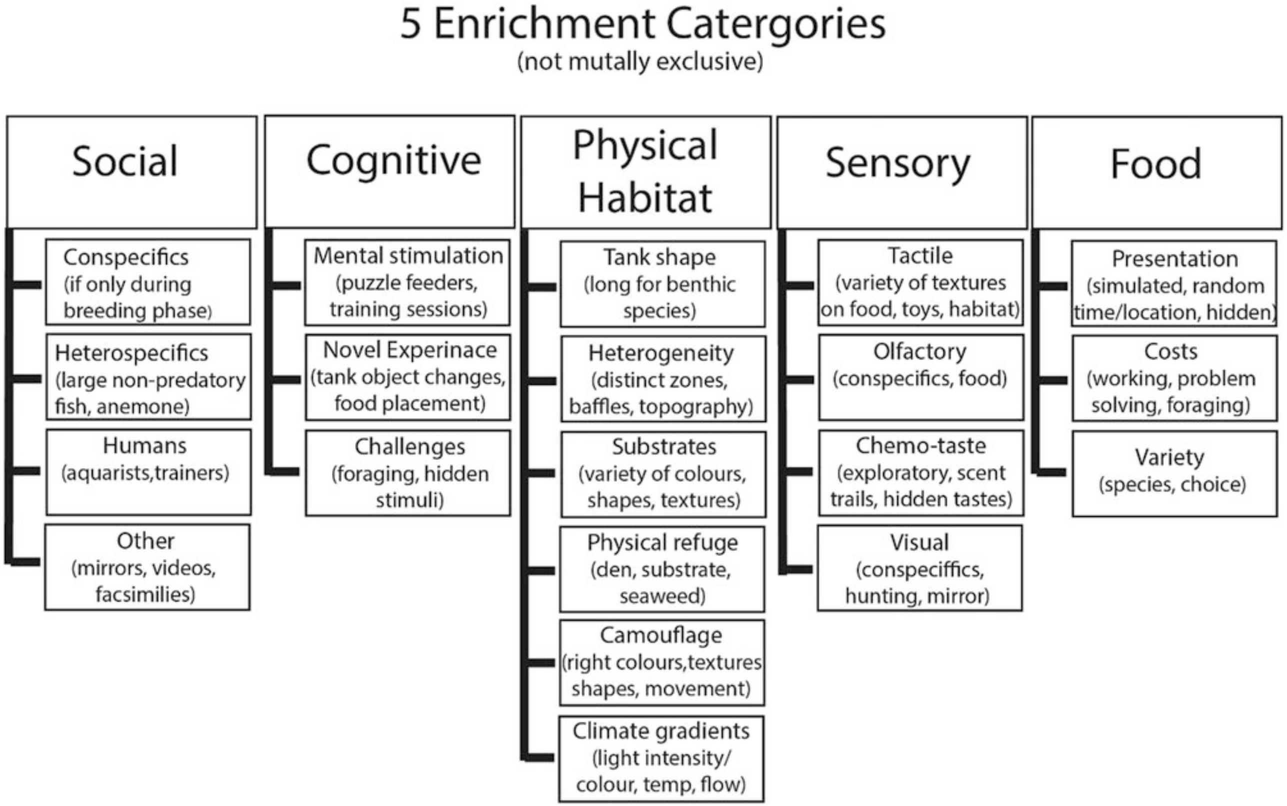

Fig. 8.1 Modified with permission from The SHAPE of Enrichment for use with cephalopods. The five categories use knowledge of sensory, cognitive and physical abilities. For cephalopods except squid, ‘social’ is limited to short periods for breeding

(Huffard 2007). Conversely, what is enriching for one octopus species may also be enriching for another, so the lack of species-specific knowledge should not prevent suggestions for enrichment for closely related taxa.

The second assumption is that the enrichment is useful or desired by the specific individual. Although experience and scientific knowledge about their behaviour and ecology helps us to suggest enrichment based on a species' life history, sensory capabilities and behavioural repertoire, the motivation to use one type of enrichment over others is rarely tested and has been investigated only once in cephalopods (Tonkins et al. 2015), for cuttlefish environmental preferences. Other aspects of life history, such as developmental stage, sex, reproductive motivations and personality, need to be considered. Males and females may be differentially responsive to the same stimuli at various times of their lives, especially as adults. For instance, male adult cuttlefish use different navigation cues than juveniles and females (Jozet-Alves et al. 2013).

8.4.1 Captive Environment (Table 8.2)

Table 8.2 Cephalopod environment considerations, modified from Cooke and Tonkins (2015)

Consideration	Group	Potential advantages	Potential disadvantages	Notes	References (context)
Substrate	Cuttlefish Octopus	NB, RA, RF NB, RA, RF	HC, PE, IH HC, PE, IH	Facsimile of substrates may remove issues	Tonkins et al. (2015) Boal (2011) and Mather and Anderson (1999) (Research)
Pipes/caves	Octopus Cuttlefish	NB RA	HC, IT HC, IT	In the wild, an octopus may spend 88% of time in dens	Mather and O'Dor (1991) (Natural observation) Tonkins et al. (2015) (Research)
Fake/real plants	Cuttlefish	NB, RA	If real PE, EC, IH	Fake plants can have many uses	Boal (2011) and Tonkins et al. (2015)
Fast water flow	Squid	NB, RM	EC	Essential for squid	Sykes et al. (2012, 2014) (Aquaculture)
General environmental heterogeneity	Octopus Cuttlefish	RA, RF, NB RM	HC, IT, II	Not appropriate for squid or nautilus	Tonkins et al. (2015) (Research)

(continued)

Table 8.2 (continued)

Consideration	Group	Potential advantages	Potential disadvantages	Notes	References (context)
Learning/ novel objects/ problem-solving	Octopus Cuttlefish	ST, IG ST, IG	HS	Used in studies investigating a variety of topics	Rehling (2000) and Anderson and Wood (2001) (Zoo) Boal (2011) (Research)
Conspecifics	Octopus Cuttlefish	NB, ES, RA	IT, BB, II	Required for breeding but at least one squid species shoals	Boal et al. (1999) (Research)
Live food/varied diet	All	NB, IG, RM	EC, EI, PE, IH	Bar reproductive opportunities might be the best enrichment	Octopus: Anderson and Wood (2001) Mather and O'Dor (1991) and Wood and Wood (1999) (Zoo) Cuttlefish: Sykes et al. (2012, 2014) (Aquaculture)
Tank design	*Nautilus*—tall tank Cuttlefish—rounded and soft-sided tank Squid—raceway	NB RI NB	EC EC EC	Nearly all cephalopods require large tanks in large recirculating systems	Needs evidence for value in Nautilus For all groups, Sykes et al. (2012, 2014) (Aquaculture), Hanley et al. (1999) (research) Lee et al. (1994)

Potential advantages and disadvantages are given. Abbreviations: *ST* stimulation, *ES* eustress, *RA* reduced aggression, *RF* reduced fear/threat behaviours, *RI* reduced injury, *IG* increased growth rates, *EB* exploratory behaviour, *HC* reduced ability to health check individuals, *PE* pollutes local captive environment, *IH* increased husbandry, *IT* increased territoriality, *BB* breeding behaviour, *EC* excessive cost/time for husbandry, *II* increased injury, *RM* reduced mortality, *HS* hastened senescence

8.4.2 *Physical Environment*

In the ocean, cephalopods are adapted to specific regimens of illumination and daily cycles, temperature, salinity and, in many cases, substrate and available space. Such features allow expression of natural behaviours but also reduce stress. Captive environments must attempt to mimic the environment in which the species is found, which requires both knowledge of the niche of the species and its flexibility, as captive environments can never exactly match natural ones. Fiorito et al. (2014) discuss some of these variables and adaptations. Cephalopods may benefit from changes mimicking seasonal environmental shifts in variables such as temperature, salinity or turbidity, and changes may be necessary to induce maturity, although these are difficult to implement.

Captive cephalopods appear tolerant of lights bright enough to illuminate a laboratory, which may not mimic natural light in the ocean. In addition, water quickly filters out light selectively, eliminating longer wavelengths such as red at around 640–650 nm. Giant Pacific octopuses and *O. vulgaris* are often given dim red lighting in public aquaria, presumably to attempt to match the 'natural' illumination. If cephalopods are given full-spectrum lighting in captivity, only the ones that live in shallow waters are being given 'normal' illumination. This wider light spectrum appears to have no effect on welfare, but it has not yet been investigated. However, cephalopods can often see beyond their tank, as they can learn to recognise individual people (Anderson et al. 2010), so sufficiently lit environments may be enriching.

Normal daily activity varies within and between cephalopod species, and responsiveness may depend on matching peak activity times or selecting species that are flexible about activity. Meisel et al. (2006) found *O. vulgaris* to have flexible activity cycles, but *Callistoctopus macropus* to be inflexibly nocturnal. As light is usually a rhythmically occurring natural phenomenon (Cobb et al. 1995), the illumination regimen can be switched. A species with a variable activity cycle such as *O. vulgaris* that is normally nocturnal (Brown et al. 2006) can be entrained by feeding regime (Wells et al. 1983). Cuttlefish tend to swim more at night (Denton and Gilpin-Brown 1961: Oliveira et al. 2017), and many sepioids bury in the sand and sleep (Frank et al. 2012) during the daytime. They can also be exhibited with a switched light regimen or tested in research, nocturnally with dim red light. In the wild, *Sepioteuthis sepioidea* hunt individually at night but gather in schools in the daytime and exhibit considerable sexual behaviour during this time as adults (Mather 2016), so activity related to them might depend on the situation and expected outcome.

The physical aspects of confinement should match the microenvironment as much as possible (e.g. AZA 2014). Substrate is important to octopuses so a relatively flat (height = 1–1.5 m) tank is optimal. Various parts of the substrate could be similar to the octopus's natural home. Octopuses are commonly given dens, which likely reduce stress in the individual given it has a safe refuge. In laboratories or aquaculture settings, dens are often ceramic pots or rocks/brickwork (e.g. Vidal et al. 2014), and similar structures are provided in public aquaria (Slater et al. 2013). Such pots can also be used to transport octopuses (AZA 2014). In the field, octopuses may 'build' walls to cover inadequate naturally formed dens (Mather 1994; Katsanevakis and Verriopoulos 2004) or shelter in human trash. It would be more enriching to give such materials that an octopus needs, rather than a fully formed artificial den, as it would 'rearrange' the materials over time. Temporary partitions could be introduced to allow these solitary animals spacing from one another. Conspecifics could be introduced into these mini-biotopes, which allows for slow integration for breeding via 'partial partitions', barriers so chemical cues could be passed with less risk of physical aggression and injury.

Cuttlefish tend to bury into sand a large proportion of the time (Mather 1986; Tonkins et al. 2015), until reaching sexual maturity (Hanlon and Messenger 1988). Aquarists outside of the public sector (i.e. in a research setting or aquaculture facility) may argue that providing substrates reduces water quality or can be harder to maintain, as in removal of prey waste. Adult cuttlefish do not bury so much, suggesting lifetime changes in physical needs. Tonkins et al. (2015) discussed how adult cuttlefish in large groups could be given simple visual 'baffles' (which can be artificial, but large seaweed also works well), but this reduces line-of-sight vision. This provides another area of their environment to match, and at sexual maturity, baffles can reduce male-male agonistic interactions and male-female harassment (used frequently with terrestrial animals, Wells 2009). The more an animal can use its environment, the better it can cope through the opportunity to make choices (Bassett and Buchanan-Smith 2007). Photographs could visually mimic substrates, albeit with a loss of burying opportunities (Tonkins et al. 2015), and fake seaweeds are now easily purchasable.

Nautiluses kept in public aquariums are often given relatively sparse tanks due to their natural behaviour of floating in the water column. In the wild, they go through considerable daily vertical migrations—*Nautilus pompilius* moves ~200 m (Dunstan et al. 2011), so vertical surfaces might be optimal, but whether this is required for well-being is unknown.

As nearly all cephalopods are obligate predators, the use of live food needs to be considered (Ponte et al. 2017; Anderson et al. 2009; Cooke and Tonkins 2015). Live food is always preferred by cephalopods and might be necessary for feeding studies. Then again, octopuses and cuttlefish have many foraging strategies (Hanlon and Messenger 2018), and enrichment suggests that prey should be placed in hiding. There is always an ethical trade-off in that some living animals die for cephalopod food. In some countries, it might be illegal to provide live fish as food, as many protect all vertebrates, unless the cephalopod would otherwise starve. Crustaceans were suggested for ethical regulation in the EU, and while they were not adopted, there is a move in the UK for decapods to be protected. Some species of cephalopods may base their lifetime-preferred choice of decapod crustaceans on experience during a key developmental window via olfactory (Guibé et al. 2010) or visual cues (Darmaillacq et al. 2006) before hatching, and again this must be considered. Coleoid cephalopods inject their prey with a neurotoxin (Cornet et al. 2014) which paralyses and kills the prey. We cannot know for sure whether the prey suffers during this process, although death is swift. Recent research suggests decapod crustaceans may feel pain (Elwood and Adams 2015; see Elwood 2019) or experience suffering.

8.4.3 Cognitive Enrichment

Many octopuses are provided with 'toys' or puzzles which they are supposed to find stimulating, although there is no evidence that this is the case as it was never tested. This enrichment may take the form of a food treat hidden in a glass jar or trapped in Lego™ or similar structures. There are several scientific papers investigating these enrichment ideas (e.g. Anderson and Wood 2001; Rehling 2000; Boal 2006; Wood and Wood 1999), based on the premise that cephalopods possess high cognitive abilities (Mather and Dickel 2017) and require motor and cognitive stimulation. The studies appear to focus almost solely on manual dexterity and cognition by having animal remove something or themselves from a closed object, though they may occasionally focus on chemo-tactile sensory abilities (Rehling 2000).

8.4.4 Social Experience

Many cephalopods are solitary for most of their lives (Hanlon and Messenger 2018). Squid are considered social because they gather in groups, but they show no cooperative behaviour. Cuttlefish gather for mating (Hall and Hanlon 2002), and some may school outside of this time (Yasumuro et al. 2015). Octopuses are generally solitary, but see Huffard et al. (2008) for the case of *Abdopus aculeatus*. If suitable shelter is rare in its environment, the gloomy octopus *Octopus tetricus* (Scheel et al. 2017) may gather and interact. Some octopus species are tolerant of conspecifics, see *Eledone moschata* (Mather 1985) and *E. cirrhosa* (Boyle 1991), and some will form a dominance hierarchy in captivity e.g. *O. joubini* (Mather 1980). However, many cephalopods are cannibalistic (see Ibánez and Keyl 2010 for a review).

8.5 Enrichment Suggestions

8.5.1 The Octopuses

Anderson and Wood (2001) suggest we cannot know if the Giant Pacific octopuses require enrichment, but, given their cognitive abilities, we can err on the side of caution. Water quality/chemistry is always a major priority in aquatic husbandry, and monitored near constantly, food is plentiful, so it is hard to see many other explanations for common problematic behaviours, such as frequent escape attempts. There are a few papers (e.g. Anderson and Wood 2001; Rehling 2000; Rehling 2001; Boal 2006; Wood and Wood 1999) that describe behavioural enrichment and how the commonly kept octopuses may benefit from it. However, none of the papers consider what goal the enrichment should achieve. It is clear the octopuses engage with toys/puzzles and are therefore stimulated. Grasso (pers. comm.) commented that octopuses seemed to perform a manipulation task for stimulation, rather than the food reward provided.

As many cephalopods have complex sensory abilities, these toys may not be maximising an octopus's potential to be appropriately stimulated. Octopuses can bend their arms in any direction at any point (Mather 1998) giving them an unparalleled ability to search for things in otherwise inaccessible places. Their suckers are on stalks (Mather 1998), providing even greater abilities for manipulation of hidden objects. Foraging-based enrichment has been shown to be very effective in intelligent captive aquatic mammals, for example, the Australian fur seal (*Arctocephalus pusillus doriferus*) (Hocking et al. 2015) (Fig. 8.2).

8.5.2 The Cuttlefish

Cuttlefish (like the octopuses) camouflage against a variety of objects and providing habitat choice might be a key aspect of enriching their lives (Wells 2009). Therefore, different options for camouflaging should be considered. A substrate is essential for encouraging normal burying behaviours. As active predators, cuttlefish also seem to benefit from providing live prey—many more behaviours in are exhibited when live crabs are provided compared to dead ones, including a previously unexpected variability in prey capture sequences (Cooke and Tonkins 2015; Zoratto et al.

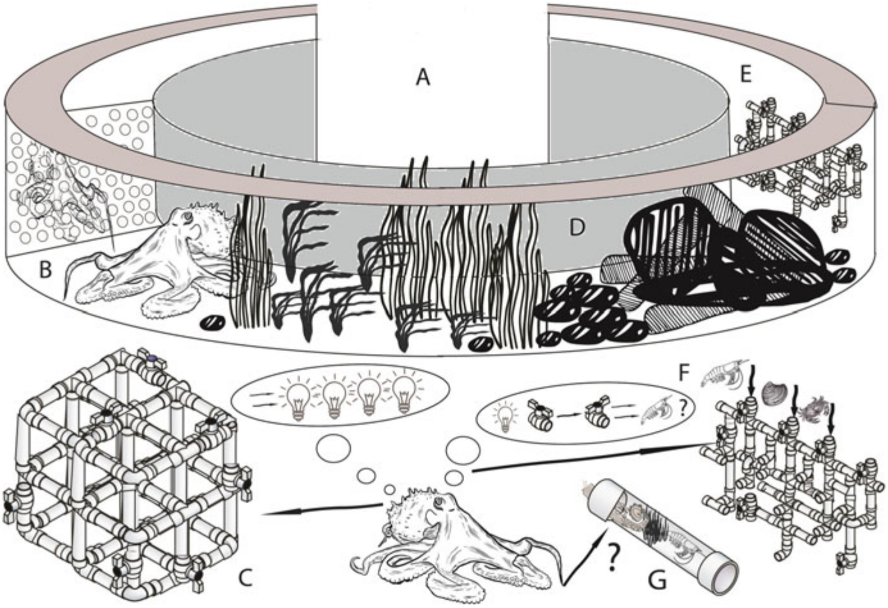

Fig. 8.2 (**a**) Octopus habitat including baffles for safe conspecific interaction (**b**), with an enrichment device (**c**), which encourages more natural feeding where chemical and tactile senses are employed to find the food (**f**). The pipework can be rearranged and is often in abundance in an aquarium. The whole lattice can be masked with fake corals/kelp, etc. to maintain a natural look (**d**) and placed away from the central area to encourage foraging (**e**). The octopus learns to open valves (**f**) to gain further access within the puzzle. Parts of the puzzle may have aversive properties (**g**) (AstroTurf, a known repellent) that require changes in approach. The addition of open/close valves offers a new opportunity for the octopus to use learning/memory, fine motor control and senses. Making the pipe too small for the octopus to get fully in and having it opaque prevents visual guidance of the arms

2018), including dynamic displays such as the 'passing cloud'. If feeding live prey that may suffer, such as decapod crustaceans (e.g. Magee and Elwood 2013; Chap. 7) and fish (e.g. Sneddon 2003) is incompatible with an institution's ethical policy, live prey can be simulated. After tying translucent cotton/fishing line to a euthanised prey appendage/tail, it can be dragged or otherwise moved to trigger a gamut of predation behaviours that might otherwise only be elicited by living prey. It is very convenient but not enriching just to drop food into a cuttlefish's tank and watch them feed.

8.5.3 The Squids

True squids are open water pelagic hunters, but it is very difficult to mimic open water conditions in captivity, although pumps/water jets can create an endless flow of water from one end of a tank to another. Zoos face similar challenges with big cats and have come up 'Cheetah Runs' (e.g. Fota Wildlife Park, Republic of Ireland), where the carcass is tied to a rope connected to a zip line and pulled very quickly by a motor across part of the exhibit; something similar could be done for squid. Dead fish could be attached to a line/s via translucent wire/s in such a way that they are quickly released when the animal tugs on it. Multiple lines are made up like this and attached to a horizontal bar, which is raised above one end of the raceway just outside the edge of a cylindrical tank. This bar is then pulled by staff or a motor, or even gravity if the arrangement is on a slope across the squid's environment (Fig. 8.3). This drags the dead fish, through the water and encourages the squid to chase down their prey.

8.5.4 Nautilus

Nautiluses detect prey by sensing chemical cues carried by currents across reefs using their olfactory organ (O'Dor et al. 1993), and then they use their many tentacles to locate and handle their prey. This natural behaviour could be replicated under captive conditions using a simple brush attached to a stick, which is then generously washed in liquids from a crab and smeared along the tank until it reaches the food. Dropping 'crab juice' into the inflow, where it meets the tank water, could encourage olfactory-based foraging behaviour.

8.6 Suggestions for the Future

We can infer a cephalopod's inability to cope with its present levels of stimulation by measuring normal behaviour as well as physiological and behavioural features before and after an enrichment intervention. Many forms of captive animal

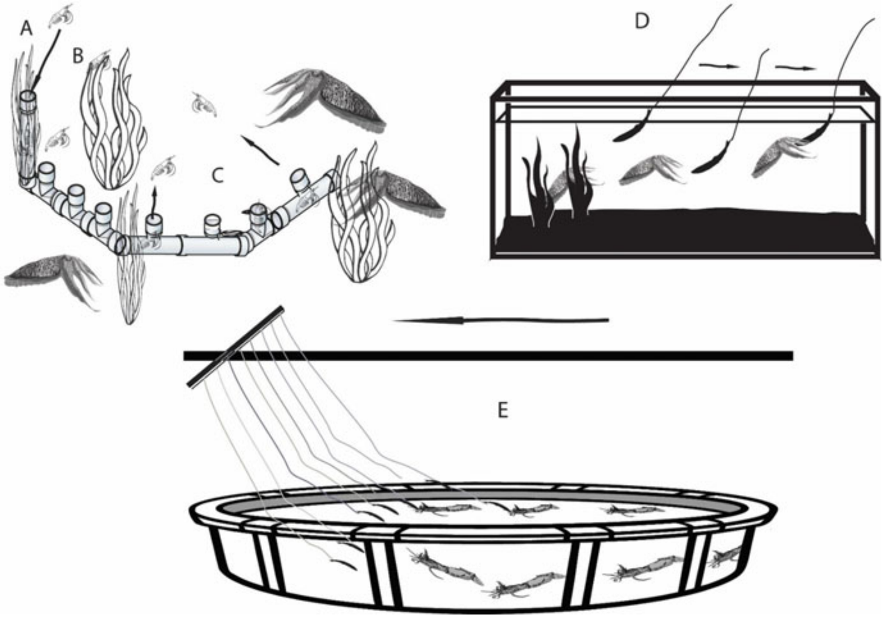

Fig. 8.3 (**a**) This device allows food to be delivered to cuttlefish either by quick release (**b/c**) or by towing, to stimulate predation by action and movement (**d/e**)

enrichment come from what we either intuitively think is good for the species or what provokes a response we benefit from (i.e. entertainment). Exploring enrichment interventions may also stop if the initial action provokes any kind of seemingly favourable response.

Octopuses notice their reflected self in a mirror but do not treat the image as a conspecific (Mather and Carere 2019). Fragmentary evidence suggests other cephalopods may also do so, as cuttlefish retreated from a mirror image (Shashar et al. 1996). Since cephalopods are solitary, mirrors might instead produce agonistic/courtship displays at sexual maturation.

Many cephalopods are nocturnally active. Terrestrial zoo taxa may have more or different behaviours at night, e.g. elephants (Greco et al. 2016). Nocturnal species are not likely to be kept if their daily increase in activity does not coincide with visiting hours if in a public aquarium, and animals should be tested at the peak times of activity in research. Since light is the normal cue for activity, the daily cycle can be switched (Mather pers. obs.), and as cephalopods are relatively insensitive to red light, sufficient illumination can be provided for humans in the ‘dark’ period. There may be other approaches, which still utilise their natural abilities but use artificial objects. Zoos have been experimenting with using technology for improving captive animal welfare (see Clay et al. 2011 for a review). Some of the audiovisual technology, such as that used by Pronk et al. (2010), may be employed to improve the lives of our captive aquatic animals, including cephalopods, simply by covering one aspect of its tank in a modern (e.g. LED) screen; a small screen, tablets rather than televisions, might be of use. Animals appear to cope better when given choices (Wells 2009), perhaps providing a choice of images would be a novel way of providing them with two visual choices (e.g. shrimp or crab) to tell us exactly what they want. This may lead to even more control by giving them access to other features of their environment such as lighting and temperature; see Carlstead and Sheperdson (2000) for examples of animal-based environmental controls in zoos (Table 8.3).

Table 8.3 Suggestions for cephalopod enrichment

Taxon	Specific species	Detail of enrichment	Sensory abilities utilised	Cognitive/ physical	Behaviour
Cuttlefish	All	Network of pipes, with some open ends, food is forced down—see Fig. 8.3	Visual	Hunting, locomotor	Natural hunting behaviour
Octopus/ cuttlefish	Applies to all epibenthic species	Distinct microhabitats within the main tank see Fig. 8.2	Visual, chemo-tactile	Memory, learning, camouflage, locomotor	Choosing where and how to camouflage, seek refuge or forage
Octopus	Applies to all epibenthic species	Tanks with large surface area—Fig. 8.2	Visual, chemo-tactile	Memory, learning	Benthic exploration, boldness, foraging
Octopus	Applies to all benthic foragers	3d lattice of opaque pipework with a variety of textures within—Fig. 8.2	Visual, chemo-tactile	Learning, memory	Natural foraging behaviour
Octopus	Applies to all epibenthic species	Frequent rearrangement of rockwork/refuges away from central den	Visual, chemo-tactile	Learning, memory	Exploration, boldness, natural foraging behaviour
Cuttlefish/ squid	Applies to all	Simulating live prey using translucent/ invisible wire; see Fig. 8.3	Visual	Hunting	Encourages natural hunting behaviour
	All	Tank flow	Visual		
Cuttlefish/ octopus	Possibly all benthic species	Swell/wave creation	Visual	Camouflage	Adds new dimension to regular camouflage behaviour
Nautilus	All	Chemical trail	Olfactory/ chemo-tactile	Hunting, locomotor	Natural hunting behaviour

8.7 Conclusion

Some cephalopod biologists have taken up the challenge long before laws changed to try and improve well-being in captive cephalopods, but interventions need quantifying. Even small quantitative studies can be peer reviewed and published in special sections of *Zoo Biology* or the *Journal for Zoo and Aquarium Research*. A few papers or cephalopod management guides are mentioned repeatedly throughout this chapter (Slater and Buttling 2011; AZA 2014). What has been done has shown that aquarists and aquatic scientists care for their animals.

The debate regarding the sentience or consciousness of cephalopods (Mather 2008) and other invertebrates might not be the point. Providing an enriched environment can have measurable positive effects, irrespective of proof of consciousness or sentience, and Birch's (2017) precautionary principle applies here. The lobby against recognising sentience/consciousness to our animals often comes from those who are set to lose if it is true (Dawkins 2003). Restrictions imposed on what they do affect the economic value of the species they are exploiting, and the conflict is exemplified by the discussion of whether fish feel pain (Braithwaite 2010). By asserting cephalopods cannot think or feel, or refusing to acknowledge their cognition, some people justify treating them inhumanely, but we can do better.

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Chapter 9

Cephalopod Welfare, Biological and Regulatory Aspects: An EU Experience

Giovanna Ponte, Paul Andrews, Viola Galligioni, João Pereira, and Graziano Fiorito

Abstract Recent increased interest in the welfare of cephalopods may be mainly due to their inclusion in Directive 2010/63/EU, which regulates—in Member States of the European Union—the use of animals for scientific research and educational purposes. Here we aim to challenge this view by providing information and considerations that have a broader impact than the use of these animals in an experimental context. We aim to provide a discussion around arguments linked to cephalopods' welfare.

We (1) discuss historical contributions to the knowledge of welfare of this class of molluscs, (2) review the most significant elements required to assess and manage animals' well-being in a research context, (3) review issues related to fisheries and aquaculture and their implications for cephalopod welfare, (4) suggest that the identification of a list of "needs" for these animals is required and review how deviations in their management may affect their welfare, and finally comment (5) that standardization is required to assure the quality of data and also (6) on some ethical and public perceptions of cephalopods as laboratory animals vs their use as food for human consumption.

G. Ponte (✉) · P. Andrews · G. Fiorito
Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Napoli, Italy

Association for Cephalopod Research 'CephRes' – a non-profit research organization, Napoli, Italy
e-mail: giovanna.ponte@szn.it

V. Galligioni
Association for Cephalopod Research 'CephRes' – a non-profit research organization, Napoli, Italy

Comparative Medicine Unit, Trinity College Dublin, Dublin, Ireland

J. Pereira
Direcção-Geral dos Recursos Naturais, Segurança e Serviços Marítimos, DGRM/DRI, Lisbon, Portugal

Departamento do Mar e Recursos Marinhos, IPMA, Lisbon, Portugal

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We believe this chapter will promote discussion around arguments that drive the consideration of welfare issues in all aspects of cephalopod science, from basic to applied, that extend well beyond the boundaries of the European Union.

9.1 Introduction

The Treaty of Lisbon, signed by European Member States on December 13, 2007, is the international agreement amending the two treaties which form the constitutional basis of the European Union (EU). More importantly for us, it is where the recognition that animals are sentient beings was first made in European Union legislation and ultimately how the concept of animal welfare was introduced into the European Union legal framework. Article 13 (Title II, Provisions having General Application) provides a recommendation to implement the EU's "agriculture, fisheries, transport, internal market, research and technological development and space policies" in order to "pay full regard to the welfare requirements of animals."[1] According to the EU policy, animals under human care should be treated in such a way that they do not suffer unnecessarily and should be held in accordance with the "Five Freedoms" (Brambell 1965),[2] i.e., the ideal states of animal welfare (see also Ohl and van der Staay 2012; Mellor 2016). In the context of laboratory animal research, this also contributes to ensuring that the results will have biological relevance and be reproducible and standardized (e.g., Kilkenny et al. 2010; Carbone and Austin 2016; Crabbe 2016; Spangenberg and Keeling 2016; Aske and Waugh 2017). Conditions should therefore be provided to reduce or eliminate the stress induced by captivity.[3] To do so, researchers must identify and reproduce those aspects of species-specific natural environment/lifestyle habits that are essential for the well-being of animals (Lahvis 2017), for example, the need to be free "to explore, problem-solve and overcome challenges" (Lahvis 2017, p. 623). This should be somehow managed for a mouse living in a space (the cage) that is at least 300,000 times smaller than its natural area of "exploration" in the wild (Lahvis 2017). A similar limitation may certainly apply to an octopus that is taken from the wild and introduced to an entirely novel environment (the tank) in an experimental setting. The foraging area of an octopus is estimated to be about 200 m^2 at sea (Mather 1991), but it is constrained to

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[1] http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:12012E/TXT&from=EN

[2] Freedom from hunger and thirst; Freedom from discomfort; Freedom from pain, injury, or disease; Freedom to express normal behavior; and Freedom from fear and distress (see also http://kb.rspca.org.au/Five-freedoms-for-animals_318.html).

[3] Directive 2010/63/EU specifically refers to animals bred for experimental purposes. It also dedicates an article (Article 9) to "animals taken from the wild," providing that their use shall be limited in experimental procedures, and only allowed in cases where scientific justification is provided, and/or considering the limitations of breeding them in purpose (see Article 10). In addition, the Commission recommendation is that the capture of animals from the wild shall be carried out only by competent persons using methods which do not cause the animal's avoidable pain, suffering, distress, or lasting harm.

an aquarium representing an area more than 300 times smaller than the one explored by an animal in the field during sorties or excursions (Borrelli 2007; see also Marini et al. 2017). The same principles should be considered even for animals bred on purpose in captivity and not taken from the wild, where adaptation to the captive condition is not necessary.

In January 2013 the Directive 2010/63/EU came into force in Member States of the European Union, regulating the use of animals for scientific research and educational purposes. All 28 EU Member States have transposed the Directive into national legislation.[4] Among several innovations in this revision of EU legislation on the use of "laboratory animals," the most remarkable change is arguably the inclusion for the first time of a taxon of invertebrates in the list of regulated species. All "live cephalopods" (e.g., nautiloids, cuttlefish, squid, and octopus) were for the first time given equal footing with all vertebrates. From hatching to death, all species belonging to the class Cephalopoda are the sole representatives of the invertebrates that are now included in the Directive.

Despite being limited in number, with about 800 species described to date (Sweeney and Roper 1998; Jereb and Roper 2005, 2010; Jereb et al. 2016) when compared with the very populous phylum Mollusca to which they belong (e.g., Ponder and Lindberg 2008), cephalopods are an astonishing example of diversity of forms and functions. The taxon provides a paramount example of how evolution can drive potential limitations in design, based on the molluscan clade, to extreme complexities (Albertin et al. 2015; Shigeno et al. 2015). Being molluscs, cephalopods belong to the clade of Protostomia (Lophotrochozoa) but evolved behavioral repertoire and morphological complexities unparalleled among invertebrates. But they are still invertebrates!

What does this mean in terms of Directive 2010/63/EU? It means that for the first time an entire class of animals (Cephalopoda) has been specifically regulated and as outcome it is for the first time ever that a class of invertebrates is regulated.

This implies also that in all EU Member States, procedures exceeding the threshold for induction of pain, suffering, distress, or lasting harm carried out on cephalopods are regulated in an identical way to any vertebrate "laboratory" species (Smith et al. 2013; Fiorito et al. 2014, 2015). According to the Directive 2010/637EU, the threshold is indicated as "any procedure which may cause pain, suffering, distress or lasting harm equivalent to, or higher than that caused by the insertion of a hypodermic needle in accordance with good veterinary practice." It should be noted that this covers both invasive and noninvasive procedures, and therefore studies involving behavioral interventions may reach this threshold. Furthermore, the EC Expert Working Group noted that "applying several such (below threshold) techniques in one animal may require the procedure to be classified as mild or higher" (see also Andrews et al. 2013).[5] Finally, considerations about "severity"

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[4] See http://ec.europa.eu/environment/chemicals/lab_animals/transposition_en.htm

[5] EC 2009, Moderator P. Nowlan; see ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf

and humane end points in procedures should be taken into appropriate account when planning experiments with living cephalopods (see also Stokes 2002; Andrews et al. 2013; Fiorito et al. 2015).

This is a paramount change in the consideration of cephalopods *and invertebrates* in any research context.

The application of a regulatory framework to cephalopods corresponds to a significant turning point in policies requiring accountability from those investigating cephalopod biology, including aspects of aquaculture research (reviewed in Di Cristina et al. 2015). Complying with this legislation provides a challenge for EU-based researchers working on these molluscan species. It also impacts on those working outside the EU as, for example, scientific journals and funding agencies are adopting some of the principles of the Directive 2010/63/EU, for example, the application of the "3Rs" (Replacement, Reduction, Refinement) to cephalopod research (Fiorito et al. 2014, 2015). In addition, there are policies that potentially also affect cephalopod research, such as (1) the revision of the current EU Common Fisheries Policies (ICES 2014), (2) the inclusion of several species into the revision of the IUCN Red List (Allcock 2011; Cardoso et al. 2012), and (3) the explicit mention of these animals in the Marine Strategy Framework Directive (Di Cristina et al. 2015; Xavier et al. 2015).

The other challenge is identified by the fact that despite only ~4% of the known cephalopod living species being currently utilized for experimental purposes (Smith et al. 2013), these are representatives of a great diversity in biological, physiological, and ecological adaptations and of marked behavioral variability (e.g., Packard 1972; Kröger et al. 2011; Albertin et al. 2015; Marini et al. 2017; Villanueva et al. 2017).

9.2 Animal Welfare in the Current Context of Cephalopod Research

We counted 206 scientific publications on the "welfare" of "cephalopods" (i.e., terms utilized for the query), indexed in the Web of Science over the last years (time span 2013–2018; source WoS-Clarivate Analytics, last update August 2018). This number is more than double that of the previous 5 years, but it is at least 38 times fewer than the hits counted when the same search query is applied to fish (938 results from Web of Science Core Collection). The term "welfare" appeared in the abstract, and the title of several scientific papers and reviews focused on different aspects of cephalopod biology, including aquaculture, but the pool of "concerned" authors is not yet extensive (e.g., Estefanell et al. 2011, 2012a, b, 2015; Gonçalves et al. 2012; Sykes et al. 2011, 2012, 2013).

Considerations of cephalopod welfare have been discussed in publications by Mather and colleagues (Mather 2001; Mather and Anderson 2007) and by Moltschaniwskyj and colleagues (Moltschaniwskyj et al. 2007). However, the topic may be traced much further back, to the work and recommendations by Grimpe

(1928), and those of Brian B. Boycott and John Z. Young for the use of octopus in learning studies in the search for a model of the brain (reviewed in Marini et al. 2017).

Recent efforts have provided tools and findings to fill the gaps and increase knowledge of cephalopod welfare. These are here summarized by studies on (1) optimal conditions of care and maintenance of animals (Sykes et al. 2012; Amodio et al. 2014; Fiorito et al. 2015); (2) evidence of the capacity for cephalopods to possess nociception and experience pain (e.g., Crook et al. 2011, 2013; Crook and Walters 2011; Alupay et al. 2014; Oshima et al. 2016; Perez et al. 2017)[6]; (3) anesthesia (e.g., Gleadall 2013; MacCormack et al. 2016; Pugliese et al. 2016) including the recent efforts to collate available knowledge to assist the EU regulatory framework (Fiorito et al. 2015); (4) methods for humane killing (Andrews et al. 2013; Fiorito et al. 2015); (5) physiological and behavioral analysis, including brain organization and functioning (e.g., Hochner et al. 2006; Borrelli and Fiorito 2008; Hochner 2012; Huffard 2013; Ponte and Fiorito 2015; Shigeno et al. 2015; Shomrat et al. 2015; Hough et al. 2016; Schnell et al. 2016; Darmaillacq et al. 2017; Marini et al. 2017; O'Brien et al. 2017), and evaluation of the effect of stress and suffering in cephalopods (reviewed in Fiorito et al. 2015) including immune response to challenges (e.g., André et al. 2011; Mooney et al. 2012; Castellanos-Martínez and Gestal 2013; Grimaldi et al. 2013; Le Pabic et al. 2013; Locatello et al. 2013; Solé et al. 2013; Castellanos-Martínez et al. 2014a, b; Gestal and Castellanos-Martínez 2015); and (6) the use of noninvasive or minimally invasive approaches as applied to cephalopods (e.g., Grimaldi et al. 2007; Margheri et al. 2011; Ponte et al. 2017; Sykes et al. 2017).

The increase in the breadth and number of studies over the last 5 years is a sign of how scientific interest in cephalopods has increased, thus boosting the amount of knowledge available on cephalopod biology in different fields. Recent advances in cephalopod genomics and transcriptomics are an important complement to the former (e.g., Sousounis et al. 2013; Albertin et al. 2015; Petrosino 2015; Liscovitch-Brauer et al. 2017), which will further boost future knowledge of the biology and physiology of these animals including nociception and analgesia, cognition, and adaptive capabilities. Thus, in our view, the inclusion of cephalopods in Directive 2010/63/EU has been (and still is) a driver for research on this class of molluscs, encouraging the development of a wider international cooperation between researchers, regulators, veterinarians, aquarists, and all responsible for care and welfare of these animals.

However, our starting point for discussion is that consideration for the welfare of cephalopods is not only a requirement due by law and policy but an emerging research effort on its own, driven by the widespread acknowledgement of the sentience of these animals.

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[6] See also discussion in Andrews et al. (2013).

9.3 Animal Welfare in the Recent Scientific Context

Broadly, animals are recognized to have the ability to have subjective feelings, but this is not always considered relevant to how animals should be treated (Carruthers 2011; Dawkins 2017). On the other hand, the fact that animals may have "conscious feelings" and the capacity to experience pain and *emotions* provide the justification for treating them with moral consideration (for discussion see Mendl and Paul 2004; Dawkins 2017). This view can be traced back to the work of the English philosopher Jeremy Bentham (original edition dated 1789, here referred to as Bentham 1907) who claimed that despite the lack of proof that an animal can "reason," we should ask ourselves and assess if they may "suffer." This provides the basis for investigating a range of unpleasant states, including fear and anxiety, to mention just two.

Many definitions of welfare stem from assessment of "feeling," which raises the closely linked issue of conscious awareness of "feelings," both negative and positive. But animal welfare may also be considered without the need for "conscious feelings" (Dawkins 2017). Authors have, for example, examined in a captive context: longevity, the health status of an individual, reproductive success, and the ability to exhibit natural behavior. On the other hand, physiological measures of "stress" have been promoted as indicators of changes mediated by the autonomic nervous system and hormones. However, we share the view of other authors that the problem with these "ways of assessing welfare is that while they are objectively measurable. . ., they are often difficult to interpret in terms of good or bad welfare" (Dawkins 2017, p. 4). There is emerging evidence that "stress hormones" are released not only when an animal is confronted with a risk (e.g., predator or other immediate danger) but also occurs before feeding or a sexual experience and even in instances where the animal is assessing new environments (Dawkins 2017). Therefore, an accurate evaluation of physiological responses of individuals to any condition/state, and an increased attention to the use and outcomes of the application of some methodological approaches, is required (e.g., Otovic and Hutchinson 2015; Hüske et al. 2016; Mesa-Gresa et al. 2016; Burnard et al. 2017; Huber et al. 2017). In the examples cited, the changes observed may be more "safely" interpreted as arousal or changes/anticipation of activity, and their correlation with welfare status should be better evaluated.

The same situation occurs when the assessment of "natural" behavioral responses is required. Despite the fact that an adequate and expert observation of behavior may provide distinction between captive and wild responses, it may be difficult to show that an individual in a captive situation is in a "safe" status because—for example—not exposed to a predator has a "reduced welfare because it lacks the 'natural' behavior of running away from a predator" (Dawkins 2017, p. 4).

In a recent essay, Marian Stamp Dawkins (2017) proposed two criteria for assessment of "good welfare": "physical health" and "what the animal wants." An overview of the examples provided by the author is outside the aims of this chapter. However, we introduce them for context, in the framework of cephalopod "laboratory animal" science. Fiorito and co-workers (2015) provided indications and guidance on the assessment of health and welfare of cephalopods in a research context (i.e., in captivity). Based on the assumption that Directive 2010/63/EU requires the application of a strategy to "ensure that the state of health of the animals," which also "safeguards animal welfare and meets scientific requirements," Fiorito and co-workers (2015, p. 20) recommend regular health monitoring based on "objective monitoring and recording of the health and welfare of the animals and recognition of the factors likely to cause deviations from optimal status" (p. 20). In the "guidelines" key parameters are suggested to be both animal behavior and appearance, and these are recommended to be supplemented with measurement of a number of physiological "biomarkers" (Table 5 in Fiorito et al. 2015). These authors included generic indicators of health and welfare based on available knowledge for cuttlefish, squid, and octopus and identified possible signs, proposing a gradation (indicated in the table from green to red) as an indication of an escalation from normal to mild to moderate and severe responses/conditions. These include (1) appearance (physical state, 7 indicators) including skin color, texture, and its integrity, abnormal body appearance (e.g., arms unaligned or dangling); (2) behavior (psychological state, 12 indicators) including unprovoked behaviors (e.g., withdrawal, abnormal motor or locomotor coordination, grooming, wound-directed behavior), responses to food, and provoked behaviors (defecation, inking, etc.); and (3) clinical (physiological/biochemical state, 8 indicators) such as reduction in body weight and changes in the rates of ventilation or heart frequency (Fiorito et al. 2015).

This chapter is also an occasion for us to consider the second criterion suggested by Dawkins, i.e., "what the animal wants" (2017). This provides the grounds for proposing a critical evaluation of previous (whenever data are available) or future behavioral experiments with cephalopods.

To provide an evaluation of what the animal wants, Dawkins (2017) suggested the use of a couple of simple behavioral paradigms such as the choice test (e.g., providing alternatives in the ways food is presented, environment containing both shelters and open spaces, repeated exposure) and the cognitive bias "which has the great advantage that it establishes not just short-term preferences but long-term effects on an animal's long-term mood" (Dawkins 2017, p. 5).

Cognitive bias may provide indication and distinction of positive and negative "emotions" and be applied to paradigms enabling individual animals to indicate their emotional state through operant responses (Mendl and Paul 2004). In cognitive bias tests, animals must solve discriminative tasks (positive and negative responses associated to either stimuli), and once learning occurs, individuals are presented with an "intermediate" stimulus. The response to the test will provide evidence whether the animal is able to "classify" the intermediate stimulus as a positive or a negative experience.

We agree with Dawkins that the "importance of this approach is that it measures long-term effects of living in a particular environment" and will provide a measurement of an "animal's mood even when it has been removed from that environment. As such, it has the potential to provide the animal's point of view of living in that environment over a long period of time" (Dawkins 2017, p. 5; see also Mendl and

Paul 2004). Considering the remarkable learning capabilities of cephalopod species (reviewed in, e.g., Hanlon and Messenger 1996; Huffard 2013; Marini et al. 2017), it is relatively easy to apply the aforementioned notions to these animals and provide support to measures on welfare.

However, this behavioral approach may require an integration of other "indicators" (e.g., "stress hormones") and how these are associated with positive or negative status of the individual animal. The analysis of physiological changes/measures taken in combination with animals' response/behavior will allow us to link the "physiology" and the biology of individuals to positive or negative welfare. These two criteria proposed by Dawkins are complementary (Dawkins 2017) and should be "objectively measurable." Nevertheless, we "need a science of animal welfare that is firmly rooted in the observable" as well as "what can be scientifically tested. If future research brings us closer to understanding how brains of any sort or size give rise to conscious experiences that can only" improve animal welfare science and result in direct benefits to animals. "But given the very real problems that attempting to study conscious experiences still pose, such knowledge should be seen as a welcome addition, not a current requirement for a science of animal welfare" (Dawkins 2017, p. 7).

9.4 Cephalopod Welfare, a Short Historical Framework

We mentioned above that a starting point for considering welfare of cephalopods as laboratory animals is represented by Georg Grimpe's *Pflege, Bahndlung und Zucht der Cephalopoden für zoologische und physiologische Zwecke* (Grimpe 1928). This is the first comprehensive treatise of care for cephalopods as experimental animals. Grimpe's attempt at systematizing the methods of care and handling already covers a good deal of the major problems issuing from capture, care, and maintenance of cephalopods. Similar to later authors (e.g., Boletzky and Hanlon 1983; Boyle 1991; Boal 2011), Grimpe focuses mostly on the littoral species of cephalopods and, among them, on those most adapted to survival in zoological stations and especially inland aquaria. His advice is not to waste these precious and costly resources ("guinea pigs of the sea," as he calls cuttlefish, squid, and octopus) and, where possible, "reuse" them for different experiments (whole animal or otherwise). The issue, however, of providing optimal conditions for ensuring the best possible "material" is always central, and Grimpe closely scrutinizes the biological characteristics of the different species with this end in sight. Thus, his guidelines can be considered a state-of-the art collection of the early twentieth century of information on life expectancy and requirements for reproduction, food, sediment and hiding places, water, and water temperature and salinity, diseases, autophagy, autotomy, and their ability to regenerate. Large parts of Grimpe's text justify the impression that standardization is required concerning at least some aspects, such as the minimum requirements for maintenance and care, including water quality, light, housing, and feeding. The bases for these requirements are (1) the biological characteristics of specific cephalopods including external protection, mobility, response to stress, food, life span, reproductive biology, respiration, social behavior, life history, and early-life behavior (also in Moltschaniwskyj et al. 2007) and (2) conditions met in the natural habitat of the respective species which should be mimicked as much as possible in the aquaria (Grimpe 1928). With nature as a source of constraint, the conditions that must be met in aquaria can be formulated in standardized guidelines. However, enough information about the natural habitat and lifestyle of any species must have been gathered as a prerequisite, as there is a need for species-specific requirements to be taken into account. On the differences between maintenance, rearing, and culture, see also Boletzky and Hanlon (1983); efforts to provide an accurate description of behavioral catalogues of various cephalopod species produced knowledge (e.g., Moynihan and Rodaniche 1982; Hanlon 1988; Hanlon et al. 1999; Jantzen and Havenhand 2003; Huffard 2007; Trueblood et al. 2015; Mather and Alupay 2016; Lin et al. 2017; Nakajima and Ikeda 2017) that we hope will continue to be expanded also by studies in the wild.

Any species that could presently (and economically) be cultured in aquaria would become a very strong candidate for becoming the new “model cephalopod,” and this would arguably have a decisive impact on the research landscape (Moltschaniwskyj et al. 2007).

The flexibility and opportunism of coleoid cephalopod behavior are inspiring. These animals are known to be able to solve different problems and select different solutions to similar “problems” in different circumstances (Borrelli and Fiorito 2008; Huffard 2013; Marini et al. 2017). In the words of Martin Moynihan: “I cannot believe that they are not deliberate, and in some sense, conscious. [. . .] The frequency with which coleoids have to make choices among complicated inputs and outputs may help to explain an (other) interesting aspect of their biology. They are supposed to have evolved relatively large as well as more complex brains than teleost fishes, their principal competitors [. . .]. It could be, perhaps in part, because they must make difficult or delicate decisions more frequently than their rivals. [. . .] Perhaps ‘awareness should be assumed to exist whenever its existence is the simplest possible assumption’ [original quotes, NdA]. The same point can be made less flippantly. All individuals, at least among vertebrates, arthropods and coleoid cephalopods, distinguish between themselves and ‘others’: other individuals, other sexes, other species. On logical grounds, it is difficult to understand how one could distinguish others without having an idea or impression, conscious or not, of what one is. Biologists, sociologists and historians make different emphases. [. . .] The alternative approaches may be tweedledee and tweedledum, blanc bonnet or bonnet blanc. Yet a conclusion is obvious and plausible. There is some sort of personal identity” (Moynihan 1997, p. 217).

This brings us back to the different types of animal welfare conceptions, i.e., function- or feeling-based, and those focusing on natural living. These “conceptions” both overlap and conflict. A comprehensive approach is the one we consider here in agreement with other authors (Nordgren 2010; Dawkins 2017). This requires coordination and bringing together people of different expertise (scientists, veterinarians, regulatory authorities) allowing a harmonized assessment but still requiring the generation of valid data (and approaches) on how to measure welfare in all laboratory animal species.

Initiatives like the consensus document we provided for the care and welfare of cephalopods (Fiorito et al. 2015) "are necessary to fill this gap" (Bert et al. 2016, p. 791). This is seen as the only way into which "scientists maintain their option to participate in the practical application of a law that fundamentally affects their work" (Bert et al. 2016, p. 791).

In the words of Moltschaniwskyj et al. (2007), "When using cephalopods as experimental animals, a number of factors, including morality, quality of information derived from experiments, and public perception, drives the motivation to consider welfare issues. Refinement of methods and techniques is a major step in ensuring protection of cephalopod welfare in both laboratory and field studies. To this end, existing literature that provides details of methods used in the collection, handling, maintenance, and culture of a range of cephalopods is a useful starting point when refining and justifying decisions about animal welfare" (Moltschaniwskyj et al. 2007, p. 455).

In the review the authors provide a short overview of the knowledge available on the care of a diversity of cephalopods. This information is regarded as guiding scientists in better applying their approaches when using cephalopods for scientific research. The "refinement" of experimental techniques (i.e., experimental design and procedures, housing conditions, and handling) is claimed to reduce the stress of the investigation on the animals, and a recommendation for the assessment and management of the impact of the experiments is also provided (Moltschaniwskyj et al. 2007). There is no reason to doubt that "it will be necessary to use existing studies and knowledge base as the starting point in justifying how biologists address issues of welfare and ethics (animal and environmental) when using cephalopods as experimental animals" (Moltschaniwskyj et al. 2007, p. 467).

9.5 Cephalopod Welfare, Elements to Consider

Fiorito et al. (2015) provide a list of indicators and suggest a gradation of responses that may be useful to assess the impact of captive holding and procedures on cephalopod species in a research context (see also above). The considerations taken by Fiorito and colleagues are also included in Moltschaniwskyj et al. (2007) and are based on the solid knowledge of the biological characteristics of the different species. Among the biological features that characterize cephalopods, some are summarized below for the reader to consider:

1. Marine animals. They are all marine-living organisms and with limited capacity to tolerate salinity changes.
2. The skin. Animals lack external protection, except for *nautilus*; the delicate skin is potentially damaged by physical contact, as occurs—for example—during handling and contact with the walls of the tanks.

3. Respiration. Oxygen is taken up via the gills and skin, and limitations may affect the animals (e.g., Finke et al. 1996; Seibel 2016; Capaz et al. 2017).
4. They are all mobile, active animals and some need to swim constantly (e.g., squid), such that they may possibly repeatedly hit the sides of tanks. On the other hand, some species need dens or places in the tank to hide (reviewed in, e.g., Fiorito et al. 2015).
5. Responses to stressors and negative experiences (e.g., nociception) have been reported in various instances, including effects due to toxic substances, disease, aversive conditions (e.g., Darmaillacq et al. 2004; Crook et al. 2011, 2013; Alupay et al. 2014; Oshima et al. 2016; Perez et al. 2017; Zepeda et al. 2017; see also discussion above).
6. The species are active predators (Villanueva and Norman 2008; Villanueva et al. 2017) and in captive settings are reported to require live prey, especially during early life stages; alternative diets have been developed in some instances (reviewed in Iglesias et al. 2014; Fiorito et al. 2015). In addition, prey size is limited by body size rather than mouth size (Packard 1972). Prey items up to 150% larger than the juvenile can be captured and eaten (reviewed in Moltschaniwskyj et al. 2007; see also Nande et al. 2017).
7. The life span is relatively short, and animals are subjected to natural senescence (e.g., Anderson et al. 2002).
8. A range of reproductive traits is known (Rocha et al. 2001), and some species take care of their eggs. Egg size varies with species; large-egg species are easier to culture (Iglesias et al. 2014).
9. Some species are solitary living and others naturally school; thus adjustments are required to facilitate animal needs (Borrelli 2007; Iglesias et al. 2014; Fiorito et al. 2015; Hofmeister and Voss 2017).
10. The behavior is very complex and rich (reviewed in Hanlon and Messenger 1996; Borrelli et al. 2006; Borrelli and Fiorito 2008; Huffard 2013; Marini et al. 2017; Villanueva et al. 2017).
11. Marked inter-individual differences in behavioral repertoire and responses are reported that require attention in providing assessment of individual welfare (e.g., Sinn et al. 2001; Sinn and Moltschaniwskyj 2005; Borrelli 2007; Borrelli and Fiorito 2008; Carere et al. 2015; Marini et al. 2017).
12. Cephalopods show complex behavior, problem solving, “play,” and planning. This supports arguments that they possess cognition (Edelman and Seth 2009; Edelman 2011; see also Mather 2011) and probably evolved this ability in competition with other animal groups and in response to demanding environments. They are reported to form cognitive maps, and use a win/switch hunting strategy, going to different places every day to forage (e.g., Mather 1991; Mather et al. 2014). Neural centers (i.e., the vertical lobe) are reported to be involved in this motor control and memory recall. It seems that cephalopods “construct” internal schemata of important aspects of their environment and store them for later use (reviewed in Huffard 2013; Mather and Kuba 2013; Marini et al. 2017).

The above is a very short list of the most significant elements to be considered to assess and manage animal well-being in a research context (see also Moltschaniwskyj et al. 2007; Fiorito et al. 2015) and represent the basis upon which studies of welfare for these species should be promoted.

Welfare is not only linked to research settings. It is also related to fishery and aquaculture. While cephalopods are still not a volume culture (Vidal et al. 2014), they are an important fishery resource (Xavier et al. 2015), commanding a significant effort, particularly in areas where they are a local traditional marine product (either for local consumption or shipment to other markets). This means that a disproportionately large number of animals are killed in fisheries, in comparison to the number of animals held in research or in aquaculture facilities. Traditional killing methods such as the destruction of the individual's brain are scientifically held to be relatively benign to the animals, provided they are performed by skilled individuals (Andrews et al. 2013), but higher volume enterprises tend to deal with groups rather than individual animals, with a potentially deleterious side effect for animal welfare. However, higher volume enterprises are also higher stakes investments, where welfare implications are regarded less favorably than in research settings, and peer pressure is less present (for discussion on various aspects, see Antunes 2011; Merkin et al. 2014; Bovenkerk and Braithwaite 2016; Grimsbø et al. 2016; Vardanis et al. 2017). That is why a current initiative is underway under the auspices of FELASA (Federation for Laboratory Animal Science Associations) to educate fishers from small-scale fisheries, where animals are still dealt with individually, aiming to gradually increase awareness and peer pressure. The aim is to improve quality of food for human consumption by decreasing animal stress and therefore grow a culture of care from the economical, rather than the emotional, perspective. Ideally, cephalopods will be given a swift death, and overall stress can be reduced to a minimum. This can be achieved by application of the knowledge gained in research settings, to validate the effort of treating cephalopods in the most humane and ethical manner, while having an impact that bears the highest numerical weight.

9.6 Closing Remarks

In this chapter we overviewed issues related to welfare, quality of data, and concept-based approach that need to be promoted for extending the welfare of these animals from laboratory settings to food sources for human consumption. In the words of J. Mather and R. Anderson, cephalopod utilization also commands "Contractarian/Kantian, Utilitarian, and Rights-based" ethical considerations, "and what these lead us to conclude about how we use and care for these animals. [. . .] physiological responses to stress [and nociceptive situations] are widely similar across the animal kingdom and most animals show behavioral responses to potentially painful stimuli. Since cephalopods are often used as a test group for consideration of pain, distress and proper conditions for captivity and handling, we evaluate their behavioral and cognitive capacities" (Mather and Anderson 2007, p. 119). According to Mather and

Anderson, practical issues, such as “minimization of their pain and suffering during harvesting for food; ensuring that captive cephalopods are properly cared for, stimulated and allowed to live as full a life as possible; and, lastly, working for their conservation” (Mather and Anderson 2007, p. 119), should be taken into account to increase our ethical concern toward them.

However, from the Directive perspective, it does not matter if cephalopods “feel” pain or not as they are included in the legislation. The outcome (and our perspective of use) will not change if it turns out that evidence arises that there is no conscious perception of pain but only reflex responses from nociceptors.

The inclusion of cephalopods as the sole invertebrate taxon in Directive 2010/63/EU has been seen as a challenge and may be a limitation to the continued growth of the cephalopod scientific community (Nosengo 2011). This is not our view! The Directive provided an extraordinary opportunity, and the community responded in a way to promote a complete revision of the discussion on the ethical issues, including animals’ welfare, in relation to the use of live cephalopods. This impacted not only on European countries but workers anywhere, and we believe that this will promote further discussion. Cephalopods have been an “experiment,” maybe the first of a series, and we are convinced that in the future, other invertebrate taxa will face the same challenges.

It is without doubt that cephalopods show behavioral responses to both positive and negative experiences and that these reactions are exhibited by a wide range of different levels in the behavioral hierarchy, from movement “reflex” actions to body patterns and a plethora of other responses that include physiological effects. Their inclusion in Directive 2010/63/EU has been based on a precautionary principle, and more and more recent scientific evidence supports the fact that octopuses and their allies are somehow sentient animals (see discussion in, e.g., Adamo 2017; Birch 2017; Mather 2017). Nevertheless, we here stress the point that the revolution we recently assisted in for the consideration of welfare of these molluscs is a paramount change in the consideration of cephalopods (as invertebrates) as research subjects.

Despite the impressive amount of knowledge on these species that has been gained through several decades of work (reviewed, e.g., in Hanlon and Messenger 1996; Huffard 2013; Mather and Kuba 2013; Marini et al. 2017) and particularly more recently, we are still in the infancy of science to be able to prove something as so apparently “simple” as the conscious perception of the sensation of pain in a cephalopod. There is no doubt that cephalopods possess nociceptors, but what sensation (if any) results from their activation, and if so how does the sensation compare to the effects pain has on the psyche of vertebrates, particularly human beings?

Future efforts to address these questions, which we aim to encourage with this chapter, will facilitate understanding of cephalopod welfare, which in turn will impact not only cephalopod science but understanding of invertebrates, their ‘feelings’, and animal welfare in general.

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Chapter 10
Consider the Individual: Personality and Welfare in Invertebrates

Jennifer A. Mather and Claudio Carere

Abstract Personality, defined as consistent between-individual variation in clusters of behavioral traits independent of factors such as age or sex, emerges in most animal species tested so far. The number of invertebrate species discovered to have clear personality profiles is rapidly increasing. This previously neglected variation harbors many unsolved questions about its evolutionary maintenance and consequences, as well as about underlying proximate mechanisms, and it relates to the way individuals cope with stress behaviorally and physiologically. Importantly, it poses new challenges about welfare consequences, since the individuals emerge as the primary target of assessment and adjustment, and not the species. In fact, the effect of individual personalities on suitability for captivity and the efforts necessary to accommodate individuals of any given invertebrate species have not been considered, despite some anecdotal evidence from keepers, e.g., in octopuses, indicating its potential relevance. After an overview on what personality is and why this concept may be relevant to welfare, we enlist challenges and opportunities offered by invertebrates by presenting a series of case studies: cnidarian aggression, spider sexual cannibalism, cephalopod enrichment and escape, and colony personality in social insects. We conclude that because animals of many invertebrate phyla have distinct personalities, fine-tuning welfare provisions to what suits the individual best is recommended.

J. A. Mather
Department of Psychology, University of Lethbridge, Lethbridge, Canada

C. Carere (✉)
Department of Ecological and Biological Sciences, Ichthyogenic Experimental Marine Centre (CISMAR), University of Tuscia, Tarquinia, Viterbo, Italy

Laboratoire d'Ethologie Expérimentale et Comparée EA4443, Université Paris 13, Sorbonne Paris Cité, Paris, France
e-mail: claudiocarere@unitus.it

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10.1 Introduction

Hundreds of species of all taxa, but mostly vertebrates, have been shown to possess individual personalities (Carere and Eens 2005; Carere and Maestripieri 2013). This previously neglected variation is one of the most important recent foundations in behavioral biology, with the realization that it harbors unsolved questions about its evolutionary maintenance and consequences, as well as about underlying proximate mechanisms (Reale et al. 2007; Caramaschi et al. 2013; Koski 2014; Roche et al. 2016). Importantly, it poses new challenges about applied welfare consequences, since the individual emerges as the primary target of welfare assessment and adjustment, not the species. Since individual personality profoundly affects behavior and physiology, it thereby influences individual welfare, while welfare conditions could directly influence behavior, physiology, and personality (Fig. 4 in Finkemeier et al. 2018). Notably, individual needs (e.g., for compatible housing in social species and environmental enrichment) and individual lifetime experience are repeatedly recommended to be taken into account in the Directive 2010/63/EU.

First, this is an intersection of two poorly studied and often neglected topics. We do not know much about the personality of invertebrates (see Mather and Logue 2013; Jandt et al. 2014; Kralj-Fišer and Schuett 2014, for reviews). Second, information about what welfare of invertebrates might consist of is thin. In fact, the effect of individual personality on suitability for captivity and the efforts necessary to accommodate individuals of any given invertebrate species have not been considered.

Until quite recently the variation of behavior among individuals of any animal species was overlooked. Consistent behavioral differences (behavioral syndromes or personalities) were denied, especially for animals other than mammals and birds. Research has begun to change that, as testified by Carere and Maestripieri's (2013) book and Gosling's (2001) explicit linkage of animal personality studies to human research. However, invertebrates are often ignored (Horvath et al. 2013). Kralj-Fišer and Schuett (2014) report finding nearly 4000 references to vertebrate personalities in December of 2013. In contrast, Mather and Logue (2013), with data extracted several years previously, found studies in only 19 invertebrate genera, 15 within the Arthropoda. Kralj-Fišer and Schuett (2014) report an increase in studies of personalities of invertebrates, with 243 publications, only 47 of which were empirical studies. Again, most were in the Arthropoda, though this is the most numerous phylum in the animal kingdom, yet the contrast in focus between the invertebrates with 34 phyla and the single subphylum Vertebrata is clear. Very recently the tendency to study personality in invertebrates has further increased (e.g., Cronin 2015; Carere et al. 2015a; Planas-Sitjà et al. 2015; Blight et al. 2016; Santostefano et al. 2016; Udino et al. 2017), in parallel with studies on their cognitive abilities and sentience (especially in social insects) that are significantly boosting the general attention to their welfare (e.g., Barron and Klein 2016; Perry et al. 2016; d'Ettorre et al. 2017; Baracchi et al. 2017).

It is therefore clear that the welfare of invertebrates has received little and only recent attention. One reason for this is that we tend to care about animals similar to us, and invertebrates simply appear very different (see Boppré and Vane-Wright 2019); empathy is easier if one can consider behavioral similarities (Kellert 1993). Invertebrates were considered "things," not animals, and the discussion as to whether "lower" animals can even experience pain and suffering has not receded; note Key (2016) and the recent debate of whether fish feel pain as well as the recent similar debate on crustaceans (Elwood and Adams 2015; Elwood 2016; Stevens et al. 2016). Even with the 3R (refine, reduce, replace) animal welfare approach, one of the tenets is "replace" (Horvath et al. 2013), and this was sometimes considered to be replacement of "higher" vertebrates with "lower" invertebrates. Another factor that led to lack of consideration of the welfare of invertebrates was linked to the underestimation of their behavioral complexity and flexibility. Putting an animal in a simple learning situation in controlled conditions gave simple responses. Animals such as *Aplysia* gastropods have been used as a simple model for learning, but when they are given variety and choices, we are finding that invertebrates' behavior is not at all simple. This was suggested long ago by Leonard and Lukowiak (1986) for this species but has been emphasized much more widely by Brembs (2013) for neurophysiological testing, in evaluation of natural behavior, and when animals are given situations with choices. Research on cognition is spreading from the vertebrates to social insects, cephalopods, crustaceans, and spiders (e.g., Bateson et al. 2011; Loukola et al. 2016; Perry et al. 2016; Alem et al. 2016; Desmedt et al. 2017; Udino et al. 2017; Marini et al. 2017; Wolff et al. 2017). De Waal (2016) has commented that scientists' finding that one animal or group has some cognitive ability stimulates others to find it in quite different species.

A pressure, which has brought gradually to increase consideration of individuality in invertebrates, is the ethical changes that have spread over the last 50 years. Gluck (2016) describes how 50 years ago animals, even vertebrates, were units or numbers, and the psychological testing did not admit them to be actual living beings. Early in her career, Jane Goodall was discouraged from giving her chimpanzees names because that was "too subjective." The Cartesian value, that animals were no better than machines, was the dominant ethos (Mather and Anderson 2007). This was gradually replaced by a utilitarian ethic, one that is often still prevalent, of relative value and gains and losses. This is evident as many advocates of invertebrate research suggest it would advance our knowledge for human well-being or benefit us by providing food or behavioral models (Horvath et al. 2013; Kralj-Fišer and Schuett 2014; Vitale and Pollo 2019). It is mainly the Animal Rights approach (Regan 2001) that focused on the individual and its welfare. Regan believed that we should look at what is good for the individual animals and asked to ensure them a full and complete life. That is what this book and particularly this chapter will ask: how could we use that viewpoint?

Cooke et al. (2019) point out that there are several settings in which we control the lives of invertebrates, and that the issues and possibilities are different for each. Animals used for research are tightly controlled so that only experimental variables can be manipulated, and this may lead to unrealistic restrictions (see van Akker et al. 1994, for the use of chimpanzees in AIDS research). Animals that are raised in culture for food or other human ends may be similarly restricted—for instance, cephalopods would not be allowed to reproduce as this results in the end of their life. But the utilitarian approach also puts pressure on animal keepers for better welfare, because animals that are better kept for human consumption are often thought to taste better or in general to be of higher quality. There is now a huge industry in animal display, in zoos, aquariums, and zoological parks. Here there is public pressure toward animal welfare, though also pressure to manipulate animals to groups and situations that "seem right." The Disney film 'Finding Nemo" featured a family-like setting and ignored the fact that anemone fish are protandrous hermaphrodites (Cooney 2013). Hobbyist keepers of individual pets, of course, have a huge investment in good welfare for their animals, although they are often ignorant about their needs and benefit from education, as well as being very anthropocentric about animals' lives.

Given these pressures, what kind of decisions can keepers make about individual invertebrates in their care? One is surely to select particular individuals in the first place. Anderson (1984) wrote about the selection of giant Pacific octopuses for the Seattle Aquarium based on personality, and one particularly shy individual nicknamed Emily Dickinson was hopeless as a display animal. A similar constraint would be on when to keep individual animals—are they allowed their full lifespan, including reproduction or other key life events so that animals can express what Regan (2001) calls a full, rich life (Anderson 2000)? A second kind of decision, possibly the most important, is to decide how to construct or alter their environment when they are in captivity and how to find the individual's behavioral capacity and make sure it can exercise it (and see Cooke et al. 2019 for a discussion of this). Finally, of course education of all individuals concerned builds a better future.

Personalities are clusters of behaviors that are repeatable across time and/or contexts, at the level of the individual (Reale and Dingemanse 2012). Over the past two decades, there has been an explosion of research on this topic in animals belonging to diverse taxa ranging from invertebrates (especially cephalopods, social insects, crustaceans, and spiders; for the former see Table 10.1), fish, birds, and mammals (Carere and Maestripieri 2013). This body of work shows within-species interindividual repeatability in typically studied personality traits: aggression, boldness, activity, exploration, and sociability (Reale et al. 2007; Bell et al. 2009; Carere et al. 2010; Roche et al. 2016). Research on invertebrates so far has highlighted similar personality dimensions, but, as highlighted by Kralj-Fišer and Schuett (2014), such an approach is needed because of the life history aspects that are often rare or absent in vertebrates, thereby offering new research perspectives. One could think, for example, about eusociality, complete metamorphosis, or asexual reproduction and the possibility to tackle, respectively, questions like individuality versus collectivity, (in)consistency across metamorphosis, or gene-environment interactions in genetically identical individuals. A common sense notion is that invertebrates are somehow more "rigid" than vertebrates in their behavior, with little interindividual differences. However, no significant differences were found in repeatability of behavior of invertebrates and vertebrates in the meta-analysis conducted by Bell et al. (2009), while the "stereotyped" hunting sequence of the

Table 10.1 Cephalopod studies assessing consistent behavioral differences over time, situations, and/or contexts

Species	Common name	Behavioral trait(s)	Time consistency tested	Evidence time/situation consistency	Context consistency/BS tested	Reference
Octopus rubescens	Red octopus	Threat response, feeding	Yes, short term	Partly	Yes (among tests)	Mather and Anderson (1993)
Octopus vulgaris	Common octopus	Threat response, feeding	Yes, short term	Partly	Yes (among tests)	Pacchiarotti (2018)
Euprymna tasmanica	Dumpling squid	Threat response, feeding	Yes	Yes	Yes (among tests)	Sinn and Moltschaniwskyji (2005)
Euprymna tasmanica	Dumpling squid	Threat response, feeding	Yes	Yes	Yes (among tests)	Sinn et al. (2008)
Octopus tetricus	Gloomy octopus	Response to conspecific video	Yes	Episodic	Yes (among tests)	Pronk et al. (2010)
Sepia officinalis	Cuttlefish	Threat response, feeding	Yes, short term	Partly	Yes (among tests)	Carere et al. (2015a)
Sepia officinalis	Cuttlefish	Threat response, hunting sequence, hunting ability	Yes, short term	Yes	Yes (among tests)	Zoratto et al. (2018)

Among tests: correlations conducted between variables measured in different tests; BS tested: correlations between behaviors tested (behavioral syndrome). Modified from Kralj-Fišer and Schuett (2014) cuttlefish harbors an interesting and meaningful variation across adult individuals, which relates to personality differences (Zoratto et al. 2018).

Another avenue of research suggests that personality should be taken into consideration when evaluating the welfare of animals, since personality types are differently linked to affective states. For instance, some personality types are likely to score more pessimistic than others in cognitive bias tests (honey bees, Bateson et al. 2011; carpenter ants, d'Ettorre et al. 2017). Invertebrates are widely used in animal experimentation as well as in zoos and aquaria, and there is a growing interest and concern about their welfare upon realizing that many species possess advanced cognitive abilities, consciousness, individuality, pain suffering ability, etc. (Carere et al. 2011; Horvath et al. 2013). An important tenet of research in animal personality (including humans) is that different personalities typically have a differential vulnerability to stress and thereby also a different susceptibility to disease (coping styles, Koolhaas et al. 2001; Carere et al. 2010). Such aspects directly relate to welfare, and although invertebrate studies in this respect are indeed scanty, their stress response is similar to vertebrates in many respects (Stefano et al. 2002; Adamo 2012, see also Elwood 2019). So it can be argued that similar personality-related differences in coping with stress as in vertebrates could be found in invertebrates.

Finally personality could have significant welfare-related implications in captive breeding and restocking/reintroduction projects, either because of methodological issues (sampling bias of bold individuals in capture-recapture studies) or because of the (in)adequate behavioral profiles assessed when animals have to be released in the wild (Gherardi et al. 2012; Carere et al. 2015b).

By presenting four cases where—we believe—personality matters for welfare, we would like to extrapolate relevant issues, questions, and challenges that need to be pursued with the perspective offered by invertebrates.

10.2 Case I: Cnidarian Sea Anemones and Aggression

Sea anemones are in the phylum Cnidaria, and they look so much like marine flowers that even people who know they are animals have difficulty remembering that they are not. Although they have a diffuse nervous system with no central control, they are predators. They are equipped with stinging nematocysts that are deadly to some animals and uncomfortable to humans. Depending on the species, they are somewhat mobile, as they can detach their pedal disc from the substrate and move to a new location. They have feeding tentacles, which initiate contact with potential prey and other items. Some anemones such as *Actinia* also have specialized tentacles called acrorhagi containing nematocysts, which damage conspecifics and potential prey, and anemones have conflicts (Rudin and Briffa 2011). Anemones that come in contact with one another begin with assessment by the feeding tentacles, and often the smaller individual moves away, and the contest simply resolves as withdrawal. But in some cases, they advance their acrorhagi and begin to sting one another, and the one that receives the most damage from the stings then withdraws. When related individuals fight, the damage is greater (Foster and Briffa 2014).

What does this have to do with personality? Individuals vary in response to a puff of water aimed as a startle test, and a shorter latency to re-extend the feeding tentacles after startle indicates boldness, a highly repeatable characteristic (Briffa and Greenaway 2011). Winners of fights show less startle, and the difference is greater after fights (Rudin and Briffa 2012), so they have both simple personality and plasticity. *Condylactis* anemones, sometimes called purple-tipped, are more mobile and common in shallow water, often living in crevices or moving slowly by detaching and attaching the pedal disc (Zahra 2017). They also have difference along the shy-bold continuum when given the startle test, and they vary in habitat choice and number of nearby conspecifics, depending on personality (Hensley et al. 2012).

There are obvious welfare concerns when keeping more than one anemone, and even the most liberal animal welfare regulations do not cover Cnidarians. If animals fight and inflict damage on one another, it would be ethical to prevent this, although anemones "make a good showing" on display in professional aquariums when they are presented in groups for a greater visual effect. Equally, if we could predict how to select non-aggressive anemones for display, we should do so. Given that we do not know how to select against aggressive anemones, we could alter the environment with a couple of physical design features that would obviously assist the anemones in avoiding damaging fights. First, if they are dispersed in a tank or given the opportunity to disperse, fights would be reduced. Second, if anemones choose different habitats partly dependent on their personality, then a tank should contain a variety of habitat that anemones could select. There are likely species or strains, which have fewer damaging fights, but the personality of the individuals also has to be taken into account.

There is a different problem for hobbyists keeping pets in home aquariums. Anemones are colorful animals and attractive components of a home aquarium. A general guide to keeping them (Barrington 2018) comments that anemones are difficult to keep and that they need good water quality, high levels of dissolved oxygen, a stable salinity, and some water flow, as well as lighting appropriate for keeping their algae healthy. Fortunately, hobbyists probably do not think in terms of multiple individuals, as she talks of "your anemone." But there is nowhere a suggestion that anemones might be harmful to each other and that their welfare might have social as well as physical concerns. This aspect of keeping anemones obviously needs a clear education effort to assure the animals' welfare.

10.3 Case II: Spiders and Sexual Cannibalism

The theoretical basis for the research on female spiders that kill prospective or actual mates is a bit different than that of personality research. It is based on the idea of behavioral syndromes, which are correlations of differences in individuals through time and across situations (Sih et al. 2004; Bell 2007). Syndromes are a property of populations rather than characteristics of individuals. They are firmly based in ecology, in that they expect that natural selection favors different optima in different situations. Thus a tendency like aggression or shyness that is optimal for one context or life event might not be optimal for another and yet would persist. The benefit of this viewpoint is its emphasis on carryover, and its drawback is that it may focus too closely on one dimension when actually a suite of them is inherited together. However, the behavioral syndrome approach offers a logical explanation for situations where a trait does not seem to be adaptive. One of these situations is sexual cannibalism, especially as studied in female spiders to their prospective mates. This is a welfare issue too, as death and its avoidance is central to biology (Maderspacher 2016), but those who keep animals in captivity do not usually intend their death.

The problem of sexual cannibalism was raised first in desert spiders both in terms of their adaptive behavior to different habitats and the foraging tactics of different individuals (Pruitt and Riechert 2012). Several behaviors were studied—prey breadth, attack latency, excessive killing, antipredator boldness and sexual cannibalism—in these spiders, and there were clear correlations. Johnson and Sih (2007) found that fishing spiders that live on the water's edge also have a carryover between boldness in predator threat and as adults, including in mating. The fact that cannibalism occurred especially in spiders that were eager also to attack prey, seen also in comb-footed spiders, led them to suggest that an "overflow" of aggression might lead to sexual cannibalism. Pruitt and Reichert (2009) suggested a trade-off in fitness for these species of spiders and found a correlation between aggression with conspecifics, eagerness in foraging, and precopulatory cannibalism. Foellemer and Khadka (2013) also saw that orb web spiders that were "aggressive" foragers were more likely to attack mates, so this began to look like a general pattern across the solitary spiders.

Of course, this produces a dilemma for scientist who keep spiders to study them and institutions that might want to display them. The researcher with a limited supply of animals that she/he wants to keep must also be sensitive to the supply of the next generation, and if the best way to get more offspring is to sacrifice some of the present one, that is a dilemma. The institution that displays them also faces a dilemma: is it reasonable to display the whole lifespan of a species, remembering the Regan (2001) concept of giving animals a full, rich life, when it results in the death of some of the members? Western society is death-avoiding (Kellehear 1984) and choosing aggressive female spiders that kill their mates is not easy to show. There is always a public outcry at sacrificing any captive animal (see Levin 2015, for Marius the giraffe). But how to tell whether an individual spider should be chosen because she doesn't eat well and won't eat her mate?

More detailed work should make sure this is really the case. If sexual cannibalism the inevitable outcome of voracity, do we have to select spiders that feed well or those that won't kill their mates? With detailed research in lab and field, the results are mixed. With the orb web spider, Foellemer and Khadka (2013) found that there is a correlation between aggressive foraging and attack on mates with the insertion of one of two pedipalps but suggest an alternative possibility, that it has evolved to allow a female more mate choice. Lichtenstein et al. (2016), looking for a relationship with hunger, find that web-building spiders only show these differences clearly when food is readily available. On the other hand, Johnson (2001) found that 20% of fishing spiders remained unmated, but this was not due to cannibalism. While cannibalizing females were more likely to have a hatch of eggs, they were also larger, so this could explain the outcome. Andrade (1998) studied male redback spiders in lab and field. Even though males put themselves in front of females in "sacrifice," only around 50% of females actually killed and ate them, and that choice depended on female hunger levels. Kralj-Fišer et al. (2013) suggested instead that there might be assortative mating in that aggressive females accepted aggressive males. They rejected the aggressive spillover hypothesis for sexual cannibalism (Kralj-Fišer et al. 2012), instead suggesting that *Nephilengys* spiders did show boldness in attacking prey and avoiding predators but that their sexual cannibals were not more likely to attack a prospective mate; rather they attacked the less aggressive suitors.

Clearly there are differences depending on the species and the conditions, but the overflow hypothesis is not universally supported, and the trade-off between keeping a spider that will feed well and be ready to produce eggs and keeping one that will attack mates is not absolute. Researchers can alter the environment to generate the conditions that will avoid cannibalism and yet select aggressive females so that they can keep the next generation. Those who want to display the full life cycle of spiders and who want to carry out Regan's (2001) emphasis on animals living a "full, rich life" do not have to choose between their animals' safety and their survival. Bold spiders may be more likely to attack their mates, but provision of sufficient food (Andrade 1998) and males also of sufficient quality (Kralj-Fišer et al. 2012) can avoid the likelihood of demonstrating the death of one of the protagonists, though there is obviously no guarantee.

10.4 Case III: Octopus Enrichment and Escape

Octopuses are arguably the most obvious case for invertebrate welfare. They have big brains and manipulative arms, and they also explore, play, and have the capacity for many forms of learning (Mather 2008). In addition, they have clear personalities (Mather and Anderson 1993), along three dimensions of activity, reactivity, and avoidance, and the conditions that are right for one octopus to thrive are not necessarily the ones that will suit another. Given the opportunity to play with an object, a floating pill bottle, only two of eight individuals did so. While we do not understand the cognitive capacity of animals in other phyla, keepers of octopuses generally believe that they can become bored, and see Anderson and Wood (2001) and Cooke et al. (2019) for techniques to keep them occupied. From a utilitarian approach that might be necessary if one cultured them, people find that octopuses given an enriched environment do better and gain weight (Beigel and Boal 2006). Given a sterile environment, octopuses can also escape. As they are also the subject of animal welfare regulations (see Ponte et al. 2019), octopuses need special consideration, and one of the necessities is to find out what works for the individual octopus, not just the group or even the species.

For professional aquariums which display octopuses, individual differences are a big problem, and Anderson (1984) writes of the criteria for the large *Enteroctopus dofleini* that make an octopus suitable to select for display. A shy animal, one that stays in hiding nearly all the time, is not visible and needs to be released into the wild and replaced. An active animal is best for public viewing, but active animals are also exploratory and may escape or damage their tank. He writes of one octopus that clipped off the wires holding the plastic pan under the gravel substrate, dug it up, and tore it into pieces, leaving them floating on the surface of the water in the tank. Other octopuses may be more reactive and shoot jets of water at their keepers, not damaging but disconcerting. On the other hand, Montgomery (2016) writes of her encounters with an octopus that could only be called “friendly” which gave her and others a rich experience with another animal’s reality. Still, other octopuses have been known to grasp the hand of keepers when they are feeding or cleaning the tank and pull strongly enough to leave suction marks all along their arms, and this might be a concern for human welfare. If you can do so, selection of appropriate octopuses is advisable.

Individual difference in feeding may also be a problem for hobbyists who want to keep their pet octopuses in good condition. Octopuses can be considered to be specializing generalists (Anderson et al. 2008). They are predators, mainly of crustaceans and molluscs, and prefer prey that is alive and moving. They can be taught to take thawed frozen prey such as shrimp or even pieces of squid, but this is not preferred, and an octopus that is really a specialist may not adapt to this kind of food. On the other hand, a generalist animal may accept such items as pieces of chicken or even eggs, adding variety to the diet and pleasure to watchers (Anderson 2008). Since octopuses are manipulative, both professionals and amateurs have provided them with “puzzle boxes” enclosing food so that they have the opportunity to work at attaining the food, also enhancing their display. Cooke et al. (2019) point out that such enrichment will continue to be a problem until we understand the animal better, as what is enriching for one individual may be threatening for another.

By far the biggest problem for anyone keeping an octopus is their tendency to escape. Octopuses have no bones, rather move by jet propulsion or by the muscular hydrostat movement system in their eight arms (Kier and Smith 1985). As the arms are also lined with suckers, they can exert considerable force (Dilly et al. 1964), and octopuses can also compress their body to a very small diameter. An active octopus that perhaps is bored will be able to escape even well-crafted enclosures, and individuals over the years have gained considerable notoriety by doing so—except that they may end up dead on the floor. Wood and Anderson (2004) have evaluated the tendency of different species to escape, and perhaps it is good advice for a hobbyist to choose to select a species as a pet with fewer escaping tendencies. Again an active octopus is more interesting to view on display, but both the loss of an expensive animal and the concern of animal’s welfare committees (see Ponte et al. 2019), who take a dim view of an animal’s loss, counter this advantage. Altering the physical environment is the best response, and until we understand the individual better, overreaction is the only route to confining an octopus (see Wood in Mather et al. 2013). Heavy weights on top of the tank lid (bricks are often used) can decrease the likelihood that an octopus can escape by that route; locking lids are a good solution if they can be crafted. Accessory areas such as filtration chamber provide escape routes, and they should be as blocked off as well as possible, keeping in mind that the octopus' pulling power is formidable as well.

10.5 Case IV: Colony and Individual Personality in Social Invertebrates

While most solitary animals have obvious variation in personality, the situation is more difficult in social animals. Jandt et al. (2014) point out that there are several levels of variation for social insect populations. There is within-colony variation in morphological castes that are specialized for such behavior as foraging, brood attention, and aggression but also non-morphological behavioral variation, as well as variation in task selection across time (temporal polyethism). These can lead to colony-level variation, which can correlate with the success of the whole colony, and one interesting area of investigation is which individuals are influencing the collective most. Influenced by the behavioral syndromes approach (Bell 2007), several authors have looked for trade-offs due to the influence of one tendency in several situations, and of course for culture of social invertebrates, this success is the "bottom line." Authors have looked, too, at social spiders, ants, and bees, and the conclusions for one of these groups might not hold for others.

Why does individual personality of members of a social colony matter to our interest and investment in one? Many authors have looked at productivity of the group, broadly conceived. Muller and Chittka (2008) point out that a mixture of personalities in bee foragers might match them to a varying environment, with slow accurate ones being better at finding food in different situations than fast sloppy individuals. Wray et al. (2011) found that bee colonies' variation was predicted by two factors, and these influenced both productivity of young and weight of the comb and also ultimately survival of the colony. Scharf et al. (2012) did a factor analysis of ant colony personalities and found that one factor, predicted mostly by nest reconstruction, influenced their productivity. Ant colonies were also influenced by a trade-off due to their position on the shy-bold axis. Boldness led to more exploration, aggression, and food supply but also to higher mortality because ants foraged in dangerous situations such as too high temperatures.

Pinter-Wollman (2012) asked what major factors might facilitate the influence of individual personality on colony personality and suggested that it might be the average personality of the group, external environment factors, or some influence of environment and gave strategies for finding answers. For this, Jandt et al. (2014) advanced the trade-off behavioral syndromes approach. Aggression across situations of feeding and reproduction has been stressed in solitary animals; see the section on spiders. Scharf et al. (2012) found that those ant colonies that showed low aggression failed to remove dead members and moved to new locations, although what mattered to productivity in the protected lab was nest reconstruction. Blight et al. (2016) pointed out that ant colonies differ in what might be called boldness and that more aggression does lead to more food for the colony and better defense but also to more mortality from this same tendency. This emphasis on particular measures or factors may depend as much on the approach of the researcher as the tendencies of the animals. Wray et al. (2011) studied collective colony personalities of bees and found that factors included "runniness," defense, foraging behavior, and lack of honey comb repair, but only the second and third factors predicted survival over the winter, critical to bee keeper cultivators. Similarly, Walton and Toth (2016) found that worker bees tended to divide into those doing more or less interactive tasks and that trophallaxis (food exchange) might be an important behavior. They also found that being part of the queen retinue loaded separately from the other supportive behaviors and components of nest maintenance were important but poorly understood. This paper reminds us to use an ethological approach and keep the basic behavior of the species in mind and also that bees, ants, and spiders might be quite different from one another.

Pinter-Wollman's (2012) question of the sources of colony personality has not been conclusively answered. Carere et al. (2018) found that ants grouped by personality emphasized the similarities and generated similar collectives, which suggests the first influence. However, Modlmeier et al. (2014b) emphasize the concept of the "keystone" individual, across many types of social group (see Sih and Watters 2005, for its origin), which would be the second. Such an individual or small group would not be one playing a social role but a distinct personality that was not replaceable. They might either enhance group performance or totally disrupt it. One example is in social spiders (Pruitt et al. 2013), where a few bold individuals may influence a colony to be much bolder than the average would be. Another example is found in honey bees, where 5% of colony individuals influence the whole swarm to lift off and move to a new location (Dornhaus et al. 2008). The external environment of habitat structure does influence the colony-level personality in some social spiders (Modlmeier et al. 2014a), so the third influence also matters. Clearly there will be no simple answers to such a fundamental question across such diverse groups.

Yet the possibility of a few keystone individuals changing the behavior of a whole group raises interesting questions and gives us a chance to think of opportunities for interventions in situations of cultivation. If aggression is the result of a few colony members, perhaps we can use selection by member removal so a social spider group can be made more docile and be more suitable for display. If a few bees cause the whole group to relocate and it's not a favorable time, perhaps their removal can change the timing. If different habitats can cause different spider personalities, alteration of the physical environment can make them more suitable for a zoo or insectarium public display setting. Research in this area is recent, the field is quite dynamic, and the combination of personality variation in groups and individuals is complex, but advances will no doubt help those who want to influence animal personality in these groups.

10.6 Conclusion

Ironically, while this book asks readers to evaluate animal welfare much more widely, this chapter does the opposite. We have made the case for broad evaluation of welfare of all animals, not just the vertebrates commonly considered. Yet conversely, the exact requirement for welfare may differ according to the individual. Some considerations of welfare, such as provision of social experience or housing, will depend on the phylum, class, or species. Because animals of many invertebrate phyla have distinct personalities, we argue here also for fine-tuning welfare provisions to what suits the individual best.

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