Biological Cybernetics (2019) 113:201–225
https://doi.org/10.1007/s00422-018-0787-5

Biological Cybernetics

REVIEW

A review on animal–robot interaction: from bio-hybrid organisms to mixed societies

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Received: 25 October 2017 / Accepted: 19 October 2018 / Published online: 14 November 2018

Abstract

Living organisms are far superior to state-of-the-art robots as they have evolved a wide number of capabilities that far encompass our most advanced technologies. The merging of biological and artificial world, both physically and cognitively, represents a new trend in robotics that provides promising prospects to revolutionize the paradigms of conventional bio-inspired design as well as biological research. In this review, a comprehensive definition of animal–robot interactive technologies is given. They can be at animal level, by augmenting physical or mental capabilities through an integrated technology, or at group level, in which real animals interact with robotic conspecifics. Furthermore, an overview of the current state of the art and the recent trends in this novel context is provided. Bio-hybrid organisms represent a promising research area allowing us to understand how a biological apparatus (e.g. muscular and/or neural) works, thanks to the interaction with the integrated technologies. Furthermore, by using artificial agents, it is possible to shed light on social behaviours characterizing mixed societies. The robots can be used to manipulate groups of living organisms to understand self-organization and the evolution of cooperative behaviour and communication.

Keyword Animal–robot interaction · Ethorobotics · Bio-hybrid organism · Mixed society

1 Introduction

Animal–robot interactive technologies represent a relatively novel research field of bio-robotics and are opening up to new opportunities for multidisciplinary studies, including biological investigations, as well as bio-inspired engineering design. This new field introduces the possibility to have, beyond the traditional bioinspiration, the merging of natural and artificial worlds in synergistic systems (Webb 2000; Krause et al. 2011; Garnier 2011; Halloy et al. 2013).

In this review, the hybrid context connecting the natural and artificial components that takes place at individual level has been defined as "bio-hybrid organism". In bio-hybrid organisms, also called cyborgs, bio-robots or animal–robots (Sato et al. 2008; Peng et al. 2011; Wang et al. 2015), an artificial component or a biological organ is incorporated into an animal or into a robot, respectively, with a direct interaction between the nervous or muscular system and the electronics. Nowadays, bio-hybrid organisms are a hot spot in the research community since they merge animal capacities to adapt to dynamic environments with the possibility to control them as robots (Krause et al. 2011). Indeed, animals have physical and cognitive features that are often source of inspiration for hardware design and control methods (Cham et al. 2002; Pfeifer and Bongard 2006; Long et al. 2006; Kim et al. 2008; Bonsignori et al. 2013; Lin et al. 2011; Zahadat et al. 2015; Bodi et al. 2015; Schmickl et al. 2011). In addition, the integration of biological entities with artificial devices can also involve biological information processing units and computing elements. This research domain named "cyborg intelligence" produces bio-hybrid cognitive functions such as perception and learning (Wu et al. 2016; Brown and Brown 2017). Despite great efforts in developing novel

Communicated by Jean-Marc Fellous.

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biologically inspired artefacts, animals are still outperforming the current bio-inspired robots by orders of magnitude. Therefore, the possibility to directly control their actions could give interesting advantages (Yang et al. 2015). Their potential applications are numerous, involving high multidisciplinary efforts. For instance, they could be used as reliable micro-vehicles for security and rescue tasks. Besides, they represent valid tools for advancement in neuroscience research and bio-electronic interfaces (Wang et al. 2013; Katz 2014).

When living animals and artificial agents interact and modulate each other, following the principles of social behaviour, an animal–robot mixed society is established. These mixed societies are dynamic systems (Halloy et al. 2013), where artificial agents are no longer simple dummies triggering specific reactions in animals. In mixed societies, robots can evoke behavioural responses adjusting the behaviour according to the animal's one (Halloy et al. 2013). Behaviour traits play an important role in biological adaptations and conservation. Robots can be useful to study behavioural adaptation, since they are easier to handle if compared to real animals, and it is possible to control their position in the environment, allowing a highly standardized and reproducible experimental design. This novel paradigm for behavioural ecology investigation, merging robotics with ethology, is also known as ethorobotics (Partan et al. 2011; Kopman et al. 2013; Romano et al. 2017b). Experiments for social behaviour have been revolutionized by introducing physical models providing important methodological advantages. In an animal–robot mixed society, a biomimetic animal replica is located in a definite place and time simultaneously with real animals, and it may be accepted as a conspecific by animals. In order to establish a mixed society, the robot, accepted as a conspecific, can interact and modulate the animal behaviour and/or can adjust its behaviour according to the animal response. However, since this domain involves both roboticists and zoologists, it should be considered that from a biological point of view, the term "population" instead of "society" is more appropriate, since "society" in zoology has a precise and rigorous definition, often confused with gregariousness and other terms, referring to temporary animal aggregations of any nature (Sherman et al. 1995).

The state of the art of the current research on bio-hybrid organisms, as well as animal–robot mixed societies, was surveyed by following a comprehensive and innovative approach. Indeed, one of the novelties of this review, if compared with the previous ones (Garnier 2011; Krause et al. 2011; Mondada et al. 2013; Wang et al. 2013; Katz 2014; Mitri et al. 2013; Frohnwieser et al. 2016; Stojnić 2017), is that here the interactions between living organisms and artificial systems were investigated from individuals to populations, by summarizing in a more detailed and comprehensive way the different animal species used as biological models as well as the biological interfaces involved in each research. Pioneer works on animal–robot interactions (Vaughan et al. 2000; Halloy et al. 2007) provided different interactive concepts mainly based on the physical localization and mobility of the artificial devices with respect to living organisms (Garnier 2011; Krause et al. 2011; Mondada et al. 2013). In particular, Mondada et al. (2013) named "mobile nodes" the autonomous mobile robots interacting and moving with animals, "static nodes" the immobile artificial agents including distributed sensors and actuators that are fixed in the environment and "mounted nodes" consisting in sensors and actuators fixed on living organisms.

In this review, the nature of the interaction has been described not only at level of spatial localization of the artificial device with respect to the animal (e.g. embedded in the organism or mixed in the animal population), but it branches at level of the physiological/behavioural inputs and outcomes produced during the interaction. In particular, how the artificial components of bio-hybrid organisms affect the physiology of the animal to manage their motor outputs, sensory systems, as well as metabolic processes is reported. Furthermore, which behavioural stimuli, communication channels and/or blend of cues are produced by artificial agents to establish different ecological interactions with animals in mixed societies is also documented.

In addition, compared to the previous review papers, where these domains were still emerging, this paper shows many results that have been obtained very recently and how they are applied in various research fields, providing an accurate analysis of the different species selected as model organisms.

Although this review focuses on the animal kingdom, it is interesting to mention that the use of artificial devices is not only restricted to animals, but is also currently deployed in other types of organisms such as plants (Hamann et al. 2015). Furthermore, interactions between human beings and artificial entities represent an increasingly common context in human society (Ruhland et al. 2015), with promising application as therapy or assistive functions (Pennisi et al. 2016; Breazeal et al. 2016).

Figure 1 shows the scheme of the classification of the animal–robot interactions, based on the aim outlined above.

2 Methods

Scopus and Web of Science databases (accessed June 2018) have been used to survey the literature. As keywords in the bio-hybrid organism domain, we used "bio-hybrid organism", "bio-hybrid animal", "bio-hybrid system", "cyborg",

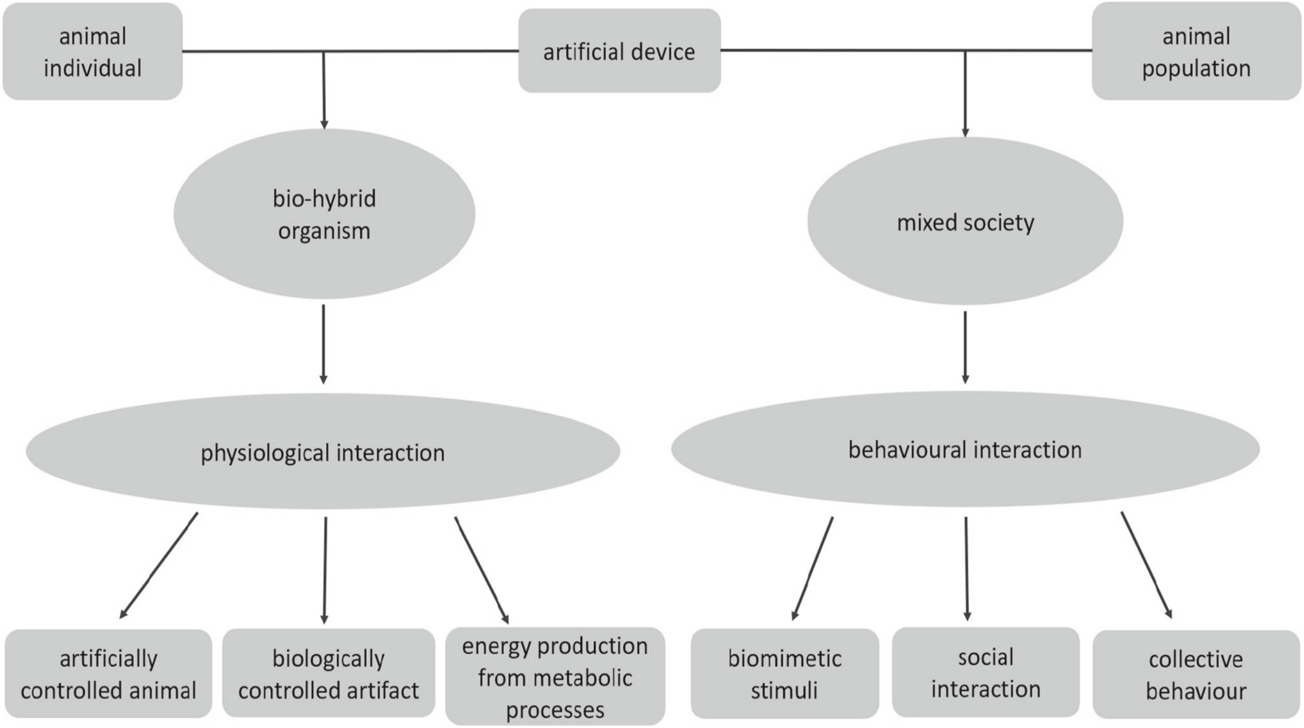

Fig. 1 Classification of animal–robot interactions in two subcategories: bio-hybrid organisms and animal–robot mixed societies, and their relative field of applications

"animal cyborg" "bio-robot", "animal–robot". Concerning the animal–robot mixed society domain, the used keywords were: "animal–robot mixed society", "mixed society", "animal–robot interaction", "animal–robot bio-hybrid society", "robotic fish", "robotic animal", "ethorobotics". We double-checked the literature by using the same keywords on these domains also in another database (Google Scholar). In addition, journals in which the articles reviewed in this work were published were defined as biology oriented, engineering oriented or multidisciplinary oriented by reading the aim and scope of the different journals on their Web platforms, as well as by searching the journals' subject areas on Scimago (scimagojr.com).

3 Research progress in bio-hybrid organisms

Bio-hybrid organisms, endowed with animal visual, audio and tactile sensory skills, represent a new category of robots showing outstanding abilities to navigate in the environment and carry out complex tasks under extreme conditions. Recently, a growing number of studies focused on bio-hybrid organisms, aiming to exploit the great potential of this research area (Fig. 2). The main tested species and research topics are detailed in Fig. 3a, b. In the following paragraphs, we examined several attempts to design controllable systems, according to different bio-artificial interactions and integrations.

3.1 Movement control

3.1.1 Controlling animal terrestrial locomotion

Generating stable and robust legged locomotion devices is still a challenging topic in the current robotics. Arthropods, despite the relatively limited number of motoneurons, outperform locomotion performances of a number of vertebrates. An early study on neural control of locomotion in lobsters was conducted, where the output of leg muscles during walking was analysed by electrophysiological analyses (Ayers and Davis 1977; Ayers and Clarac 1978). Later, a cockroach cyborg (*Periplaneta americana* Linnaeus; Blattodea: Blattidae), controlled by electrical stimuli at the antennal level routing locomotion decisions, was developed. Measurements collected on a styro-foam trackball–computer interface system allowed the record of the response to antennal electrical stimulation (Holzer and Shimoyama 1997). These data was used to establish a simple mathematical model and to design a insect cyborg by placing a series of electronic backpacks on the back of the animal. A microcontroller sent electrical stimulations to the insect that was steered on a coloured route by two photosensors.

Very recently, motion identification and localization were obtained in a cockroach species of public health importance, *Gromphadorhina portentosa* Schaum (Blattoidea: Blaberidae), equipped with a custom board with a five degrees of

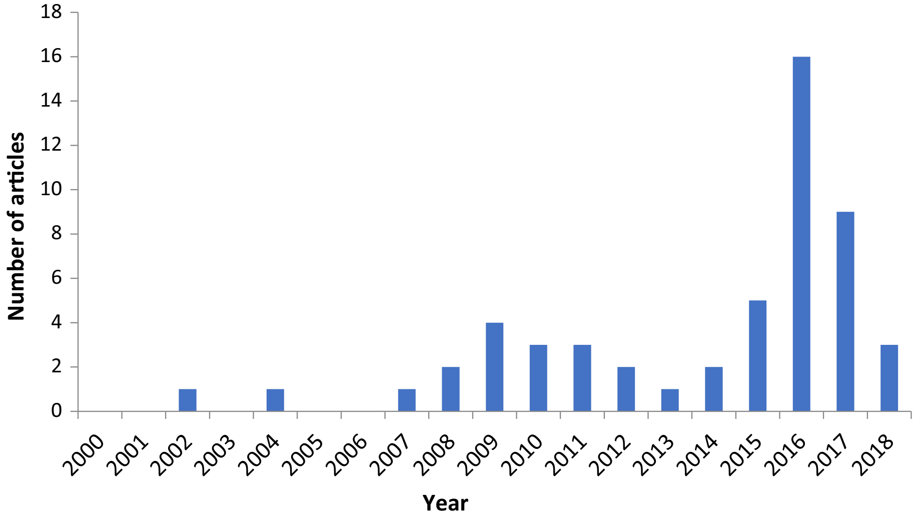

Fig. 2 Number of published research items per year (from 2000 to June 2018) focusing on bio-hybrid organisms

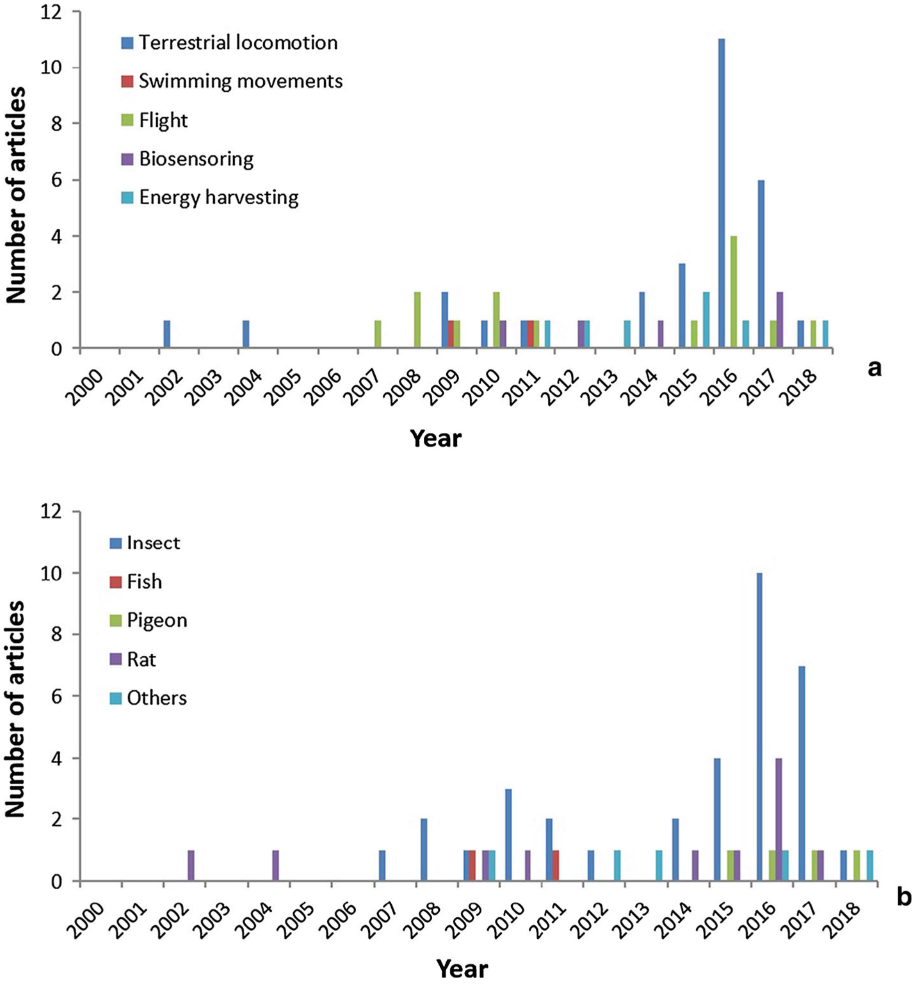

Fig. 3 Number of published research items from 2000 to June 2018 focusing on **a** animal movement control in cyborgs and **b** different species under investigation in bio-hybrid organisms

freedom inertial measurement unit, transmitting accelerometer and gyroscope data, to accurately detect the cockroach's movements (Cole et al. 2017).

Later, Zhang et al. (2016) controlled the leg motion of the beetle *Mecynorhyna torquata* Drury (Coleoptera: Scarabaeidae), by exploiting a fuzzy feedback control system that sends directly to the antagonistic pair of muscles two separate electrical stimulations. The fuzzy control system modulated its internal parameters depending on different situations. The authors were successful in moving *M. torquata* legs in a predetermined trajectory. This system was very simple, requiring a low power for hardware, and it could be used on different beetles with different responses to the same electrical stimulation. Moreover, Cao et al. (2016) electrically stimulated eight leg muscles via eight pairs of implanted electrodes in *M. torquata* producing protraction/retraction and elevation/depression motions in both forelegs of the insect. Also, a vision-based automated system for insect navigation in *G. portentosa* has been designed (Latif et al. 2016).

Notably, in another study, human intention was translated to a cyborg *G. portentosa*, remotely controlled by applying micro electrical stimulation to the nerves of its antennas, where steady-state visual evoked potential (SSVEP)-based electroencephalography (EEG) was used as a robust brain—computer interface (BCI) (Li and Zhang 2016, 2017).

Besides, in terrestrial scenarios, jumping locomotion represents a valuable strategy adopted by a number of animal species, since it can be highly functional to locate food and mates, as well as to avoid predators. It has been reported that jumping insects generate accelerations far exceeding gravitational acceleration, thus producing exceptional jumping performances. To control this ability, the jumping response was elicited in the desert locust *Schistocerca americana* Drury (Orthoptera: Acrididae), by stimulating, with constant current square wave pulses, the metathoracic T3 ganglion, which coordinates the neuromuscular activity leading to a jump (Giampalmo et al. 2011).

Furthermore, several attempts aimed at the control of terrestrial locomotion have been carried out in vertebrates. Wenbo et al. (2009) induced artificial locomotion on the lizard gecko *Gekko gecko* Linnaeus (Squamata: Gekkonidae), by stimulating the midbrain with electric signals. Electric stimulations supplied by the implanted electrodes successfully elicited several locomotor behaviours in both anesthetized and awake geckos.

Kim et al. (2016) developed a display mounted on the head of the turtle *Trachemys scripta elegans* Wied. (Testudines: Emydidae), with wireless communication, and a stimulation device that produced the turtle's escape behaviour remotely controlling its movements relying to a human brain–computer interface.

Rats (*Rattus norvegicus* Berkenhout; Rodentia: Muridae) were widely exploited to develop zoo-artifical organisms because of their well-developed motion and perceptual abilities, as well as their ease of breeding in laboratory. Movement control is generally based on the principles of "virtual award" and/or "virtual punishment" (Wang et al. 2013). Since an electrical stimulus can produce a cue or a reward, depending on the site of brain stimulation, virtual learning, involving direct stimulation of somatosensory cortices (SI) as cues and of medial forebrain bundle (MFB) as rewards, can be developed to guide the rat (Talwar et al. 2002; Xu et al. 2004). Talwar et al. (2002) used the virtual award principle, producing the first example where a rat was controlled by humans to steer, jump or climb in several environment scenarios. Huai et al. (2009) steered rats with the principle based on virtual punishment, which eliminates training in advance. The thalamic ventral posterolateral nucleus (VPL) and amygdale nucleus (AMY) of the rat were stimulated with electrical signals to affect its locomotion causing changes of direction and run-off. This method showed better outcomes in controlling rat moves with respect to the method based on virtual rewards, and a lower stimulation intensity was necessary. In order to induce a turning response in rats, Xu et al. (2016) investigated two different areas of the brain. The effect of the electric stimuli was observed in the ventral posteromedial (VPM) thalamic nucleus and the barrel field (BF) cortex. The authors demonstrated that a better control of a rat navigation can be achieved by stimulating VPM. Maintaining immobility during navigation is also crucial. Lin et al. (2010) investigated the immobile behaviour evoked by dorsolateral periaqueductal gray (dlPAG). By implanting electrodes in the brain of rats, motion and motionless states were triggered after stimulating the MFB or dlPAG during navigation. Interestingly, Yu et al. (2016a, b) developed a vision-based automatic training system, controlling a rat by brain electrical stimulation, to reduce the time-consuming training procedure.

Later on, Wang et al. (2015, 2017) designed a rat robot able to perform automatic navigation and to find target objects thanks to incorporated object detection algorithms. Furthermore, since a single stimulus was not enough to trigger rat motion, a closed-loop stimulation model giving a series of stimuli to the rat was developed allowing it to perform a motion successfully. Videos captured by a miniature camera mounted on the rat were wireless transferred to a computer developing detection algorithms that enable the rat robot to navigate automatically and find targeted objects. Remarkably, Yu et al. (2016a, b) demonstrated the principle of a "cyborg intelligence" (e.g. an animal with augmented intelligence), by producing a computer-aided rat able to quickly deal with the maze escape task. Two microelectrodes were implanted into the medial forebrain bundle of the rat's brain, and the whisker barrel fields of left and right somatosensory cortices were the areas where the other two pairs of micro electrodes were implanted. The computer-aided maze solving system sent directional stimuli to the backpack of the rat via Bluetooth. Brain–machine interface systems have been recently developed since advances on methods for the recording of neural signals have enabled to collect brain neural activities (Wu et al. 2013). The first attempt of using brain—machine interfaces for interacting with a rat was proposed by Wu et al. (2014). Researchers incorporated a speech translator module, which produces electric stimuli to the brain to control the rat locomotion by translating speech commands by humans. Virtual punishment was exploited by

Yang et al. (2015) to develop a navigation control system in pigeons (*Columba livia domestica* Gmelin; Columbiformes: Columbidae). This was obtained by stimulating the left and right nucleus dorsalis intermedius ventralis anterior (DIVA), and the periaqueductal gray (PAG) region of the brain. This system was able to send multi-mode electrical stimuli to the brain of the pigeon, which turned left or right to escape the virtual fear by stimulating the DIVA and moved forward when the PAG was stimulated. A micro-embedded computer sent remotely digital commands to a receiver microprocessor module mounted on the pigeon's head producing biphasic TTL pulses to the brain. Huai et al. (2016) proposed microelectrodes for multiple brain region synchronization, which were implanted in different regions of the brain of a pigeon at the same time. In this way, the animal control was higher, if compared to the original electrodes.

In a pilot study, liquid crystal polymers were used as depth electrodes implanted in the pigeon brain for wirelessly navigation (Seo et al. 2017).

The walking forward has been recently obtained in pigeons by microstimulating the intercollicular nucleus of this avian species that is thought to correspond to the midbrain periaqueductal gray region in mammals (Wang et al. 2018). The time and the accuracy of the response were significantly improved compared to the previous experiments in which archistriatum was stimulated. Developing a swarm of bio-hybrid organisms aimed to form a mobile sensor network would be very useful in environmental monitoring applications or to locate survivors after natural disasters (Bozkurt et al. 2016). This was the purpose of some studies that—after implanting electrodes to the antennae of Madagascar hissing cockroach *G. portentosa*—equipped them with wireless electronic backpacks and solar panels (Latif et al. 2014; Dirafzoon et al. 2017a, b). The bio-hybrid cockroaches were kept in a target area by using an automated platform, which defined virtual barriers. Furthermore, solar panels ensured automatic charging of electronic backpacks improving the duration of the cockroach searching activities. To make feasible a swarm of bio-hybrid insects, Erickson et al. (2015) investigated and quantified several electrical stimuli parameters (i.e. pulse types, amplitude, frequency and duration) eliciting controlled navigation in *G. portentosa* (Fig. 4a). In addition, locomotor response to electrical stimuli was investigated through a trackball. This study demonstrated a notable response by delivering bipolar voltage stimuli to antennae and cerci of cockroaches.

3.1.2 Controlling animal aquatic locomotion

Aquatic and underwater environments represent scenarios where bio-hybrid organisms can have successful applications, although researches on locomotion control in fishes are scarce. Artificially induced swimming behaviour was obtained in the goldfish *Carassius auratus auratus* Linnaeus (Cypriniformes: Cyprinidae) (Fig. 4b) by stimulating a small mesencephalic area via an electrode inserted in the fish brain (Kobayashi et al. 2009).The fish was free to move and was equipped with a wireless controller delivering electrical stimuli to the medial longitudinal fasciculus (Nflm), which has an important role in activating fish swimming movements (Kobayashi et al. 2009; Uematsu and Todo 1997). Forward movement was elicited by stimulating sites on and off the midline, steering fish towards the stimulated side. Thus, Nflm direct stimulation, affecting trunk and tail movements, allowed to control swing fish robot in the horizontal plane.

Peng et al. (2011) designed and controlled fish robots by delivering electric stimuli to the brain of carps, *Cyprinus carpio* Linnaeus (Cypriniformes: Cyprinidae). The authors sent stimuli through electrodes implanted into the corpus cerebelli of fish, observing that steering directions occurred in the opposite side of the stimulation sites, whereas forward and backward movements were all induced by stimulating the midline of the corpus cerebelli. Overall, these studies showed successful artificially induced locomotion in animals for the development of swimming zoo-artifical organisms.

3.1.3 Controlling animal flight

Potential roles of micro-air-vehicles and nano-air-vehicles (MAVs/NAVs) are well known by industry, commercial and military applications (Ellington 1999). Even if great advancements have been carried out to improve MAVs/NAVs performances, insects still show incomparable flight abilities. Flight-controlled insects could be directly used as MAVs/NAVs by artificially modulating the wing movements using flight muscles (Sato and Maharbiz 2010). However, the electronic system implantation is a critical phase since it can produce tissue damages and negatively affect the insect flight performances (Bozkurt et al. 2008).

Bozkurt et al. (2007) developed a method to insert a microsystem in insects at the pupal stage, named early metamorphosis insertion technology (EMIT), able to electrically stimulate wing muscles. This method exploits the fact that in holometabolous insects, the latest juvenile stage (pupa), undergoing metamorphosis, is almost motionless. The authors inserted polyimide thin-film-based flexible microprobes in the thorax of *Manduca sexta* Linnaeus (Lepidoptera: Sphingidae) pupae and, afterwards, they actuated the wing motion in emerging adults, controlling steering during tethered flight. Implanting artificial components during metamorphosis allowed a better interface and a strong attachment with the insect tissues enabling to avoid adverse effects on the insect flight performance. Furthermore, the microsystem was implanted in the moth before its emergence; thus, the insect did not consider it as a foreign object. The manipulation of flight direction changes was explored in tethered *M.*

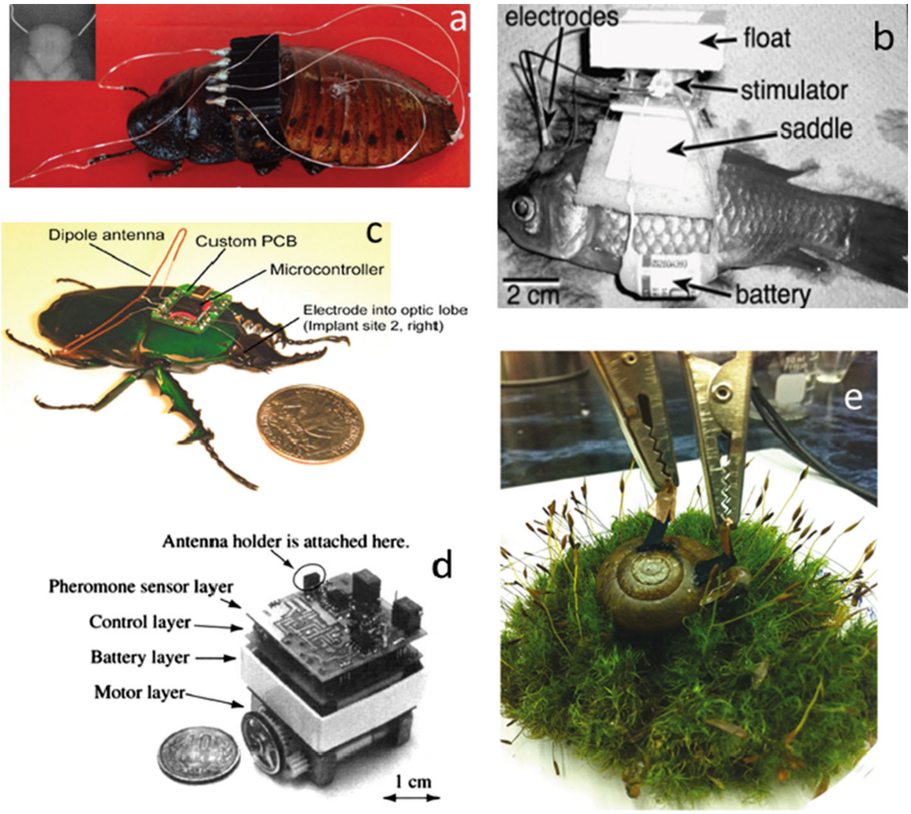

Fig. 4 Examples of animal–technology physical integration: **a** artificial-induced terrestrial locomotion in the Magascar hissing cockroach (adapted with permission from Erickson et al. 2015), **b** goldfish mounting an electric stimulator to induce swimming behaviour (adapted with permission from Kobayashi et al. 2009), **c** radiocontroller mounted on the pronotum of *Mecynorhyna torquata* controlling its flight (adapted with permission from Sato et al. 2009), **d** PheGMot-III, a mobile robot using silkworm moth antennae as pheromone sensors (adapted with permission from Kuwana et al. 1999), **e** electrodes of biofuel cells inserted in a snail to obtain electric power from metabolic compounds (adapted with permission from Halámková et al. 2012)

sexta, designing a flexible electrode to implant in the insect abdomen at pupal stage (Tsang et al. 2008). Electrical stimulation of the abdominal nerve cord in adult moths produced variations in abdomen angle that, during flight, allowed to modify the moth flight direction.

Flight control can be obtained not only in tethered insects, but also sending radio controlled signals triggering wing stroke movements (Bozkurt et al. 2009). By implanting electrodes in the brain and the thorax of *M. sexta* via EMIT, emerged adults were connected to the control electronics as well as to a plastic stick holding a helium balloon. The lifting force of the balloon relieved the weight of foreign components. A remote controller sent radio signals to a receiver mounted on the mouth and used to stimulate the neuromuscular system of the insect routing the flight.

Sato et al. (2009) guessed that insects with asynchronous flight muscles do not need to continuously send signals to the muscles for each wing oscillation; thus, less controller power would be involved over insects with synchronous flight muscles. They inserted neural probes in *M. torquata* beetles bodies at pupal stage and then mounted a radio frequency system in emerged adults (Fig. 4c). The radio controller mounted on the pronotum delivered stimuli to the brain and to the flight muscles, enabling to start and stop flight behaviour and to steer the beetle during flight.

Bao et al. (2011) selected *Apis mellifera ligustica* Spinola (Hymenoptera: Apidae) for developing a flying insect robot. After implanting two electrodes into the left and the right optic lobes, various electrical stimulus pulses were delivered to a tethered honeybee with the purpose to investigate how they condition flight behaviour. Below a specific amplitude threshold, flight did not occur. Exceeding the threshold of 3.5 V, the flight was successfully triggered and continued for a period of time without additional stimulus, since honeybees had asynchronous muscles.

Investigating which muscles are involved in grading turning in insect free flight is essential to obtain an accurate steering control. For this purpose, a group of researchers used a miniature radio system mounted on *M. torquata* for remote electrical stimulation, proving that the third axillary muscle plays a key role in regulating steering in beetles (Sato et al. 2015).

Poon et al. (2016) successfully evoked flight initiation in *M. torquata* by mounting a miniature stimulator on the insect pronotum and applying 2–4 V, 100 Hz, 20% duty cycle, biphasic square pulse trains between two electrodes implanted into the optic lobes, massive neural clusters of compound eyes. Flight cessation was obtained by a single DC pulse applied to the same sites. To turn the insect, the same pulse trains were applied to the left or right basilar flight muscle. Also, Choo et al. (2016) developed a reliable electrical stimulation protocol, obtained by analysing a pair of dorsal longitudinal muscles that elicited flight in *M. torquata*. To study insect flight, a wireless backpack was mounted on the pronotum of *M. torquata,* relying to a silver electrode implanted on the 3Ax muscle on each side of the insect (Doan and Sato 2016). During free flight, the beetle was successfully turned depending on the side where the muscle was stimulated.

Electric stimuli directed to the basalar and third axillary muscles of *M. torquata*, enabled the control of the beetle's horizontal flight (Li et al. 2018). Authors developed a controller based on a proportional derivative feedback to decrease the effect due to the insect adaptation to long-lasting electrical stimulations.

Simulation results of a novel chip architecture were obtained by Wang et al. (2016), showing that the system would work efficiently to control the flight of free honeybees.

However, avoiding the invasive implantation of electronics in animals represents the best path to follow in this research context in order to produce highly efficient systems as well as more acceptable approaches from an ethical point of view.

In this framework, Zheng et al. (2017) attempted to control the flight of bumblebees (*Bombus* spp. Hymenoptera: Apididae) in a non-invasive way. The authors proposed a control method based on real-time virtual reality and reinforcement learning that consisted in a configurable LED display system as visual stimulus that was projected on the insect compound eyes.

3.2 Controlling robots by biological inputs

The control of animal behaviour by sending artificial stimuli to their nervous and/or muscular systems is of great interest in ethorobotics. Besides, another fascinating field of research is the development of robots responding to cues produced by biological interfaces. In this context, the biological part drives the artefact, creating a reverse condition over zoo-artificial organisms discussed in the paragraphs above.

Animals have extraordinary sensorial skills to detect and recognize olfactory, auditory and light stimuli, which can be used to control machines. Kuwana et al. (1995) used the antennae of silkworm moths, *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae), to develop a bio-hybrid sensor able to measure pheromone concentration in the air, thus guiding a mobile robot towards the source of this stimulus. Two male moth antennae were fixed to a mobile robot, and their electrical activity was monitored by an electroantennograph and then used to successfully steer the robot along the pheromone trace, as naturally occurs in male moths. A similar mobile robot, mounting silkworm moth antennae, but characterized by a smaller size and a higher level of performances, has been developed (Fig. 4d), in order to test it in a wind tunnel and analyse the robot behaviour with the same experimental apparatus normally employed for moths (Kuwana et al. 1999). With the aim of locating an odour source in several downwind areas by using an insect antenna bio-hybrid sensor, Myrick and Baker (2010) combined an electroantennogram (EAG) odour detection system with a GPS and a 2D anemometer. The EAG was simultaneously recorded in two moths species, *Heliotis virescens* Fabricius and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae). In addition, by using Lagrangian atmospheric dispersion models, to describe odour parcel turbulence and Bayesian inference to quantify the uncertainty in the considered source parameters, they obtained the odour source location in a 0.2 m radius from the device.

A description of a protocol for tracking odour traces and sources using insect antennae was recently provided by Martinez et al. (2014). They also used electroantennograms to detect the response of olfactory neurons. In order to guide an autonomous robot mounting real insect antennae, they developed an interface between the electroantennograph electrodes and the robot. This robotic platform represents a valid appliance to investigate animal odour search strategies. Besides, in a very recent study, a tethered male of *B. mori* successfully routes a robot to localize a targeted odour source (Ando and Kanzaki 2017).

Biological inputs also can be obtained by photoreceptors, driving robots by light stimuli. Jadhav et al. (2012) designed flexible microfabricated neural interfaces to be inserted at level of eyes of *Zophobas morio* Fabricius (Coleoptera: Tenebrionidae) during the pupal stage. Adult beetles with these interfaces were subjected to optical stimulation experiments allowing to record their neural activity. This interface system represents a worthy attempt to simplify the production of bio-hybrid organisms exploiting animal sensory systems.

As a further step, a bio-hybrid approach was proposed by Stefanini et al. (2012), who developed a new bio-inspired robot extensively tested in aquatic environment. This robot was inspired by the lamprey *Lampetra fluviatilis* Linnaeus (Petromyzontiformes: Petromyzontidae), a vertebrate model where the neural control system for goal-directed locomotion is well documented at a cellular level (Grillner et al. 2007). This robotic platform enabled the idea of interfacing the artefact with real lampreys, to directly control the robot by the neural activity of the animal (Manfredi et al. 2013).

Lastly, rat heart muscle cells were added to a microfabricated gold skeleton and a rubber body to produce a bio-hybrid robot swimming like a ray fish (Park et al. 2016). The undulatory movements of the robot were obtained by the genetically engineered cells that gave responses to light cues allowing the robot to follow a light source.

3.3 Energy harvesting from bio-hybrid organisms

A number of efforts aimed to develop bio-hybrid organisms as new methods to harvest and manage energy from

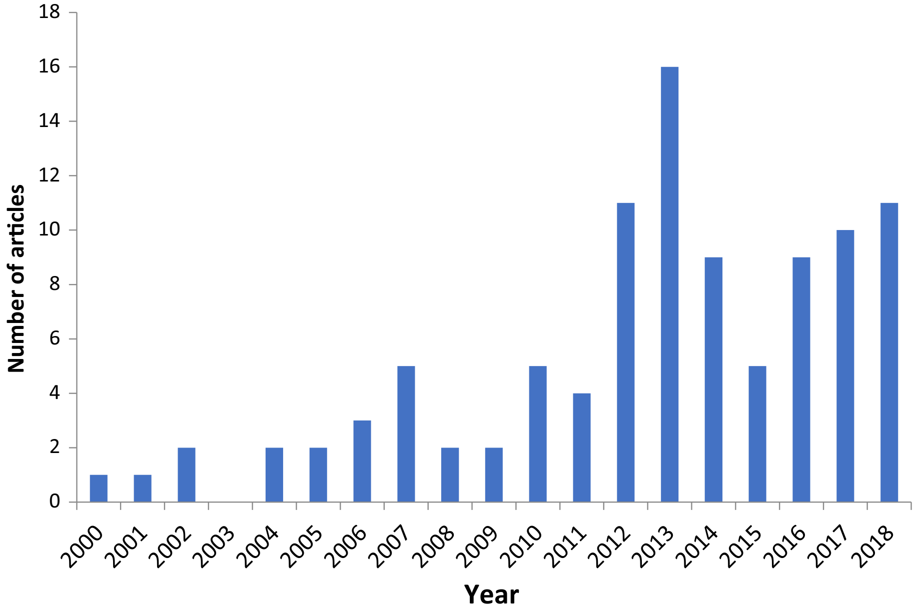

Fig. 5 Number of published research items per year (from 2000 to June 2018) focusing on mixed animal–robot societies

animal physical exertion as well as biological metabolic compounds. Aktakka et al. (2011) presented a vibration energy scavenger that can be employed during the flight of *Cotinis nitida* Linnaeus (Coleoptera: Scarabaeidae). This generator utilizing non-resonant piezoelectric bimorphs was able to convert 1–10% of mechanical energy deriving from beetle wing vibrations into electricity. One generator for each wing was placed, and electrical output was obtained from wing motions without disturbing effects on the flight of the beetle. Further studies focused on harvesting energy from internal metabolic compounds of animals. Biofuel cells were implanted in a snail, *Neohelix albolabris* Say (Stylommatophora: Polygyridae), producing electrical power over a long period of time using physiologically produced glucose as a fuel (Fig. 4e), (Halámková et al. 2012; Katz and MacVittie 2013). In addition, enzyme-based biofuel cells implanted into living American lobsters, *Homarus americanus* Milne-Edwards (Decapoda: Nephropidae), were able to activate an electrical device by generating electrical energy (MacVittie et al. 2013). Schwefel et al. (2015) developed a bio-hybrid cockroach by implanting a biofuel cell in living *Blaberus discoidalis* Audinet-Serville (Blattodea: Blaberidae). The biofuel cell had a bienzymatic anode that dissociated trehalose to glucose and then oxidized it to gluconolactone by the enzyme glucose oxidase. The cockroach, presenting a custom-designed oscillator mounted on his back powered by the biofuel cell, was capable of generating and transmitting wireless signals to an external receiver. The same experiment was successfully conducted by implanting biofuel cells in the moth *M. sexta*. Shoji et al. (2016) produced a self-powered robot represented by a *G. portentosa* endowed with a biofuel cell and a micro-wireless sensor module for environmental monitoring. The biofuel cell generated electric power from glucose in the insect haemolymph.

These self-powered bio-hybrid organisms could have biomedical applications, and they could be utilized in environmental monitoring, homeland security and military applications (MacVittie et al. 2013).

Recently, El Ichi-Ribault et al. (2018) developed a device including an enzymatic biofuel cell implanted in the abdomen of a rabbit and connected to a wireless tele-transmission system, providing an interesting achievement concerning the output obtained from an enzymatic biofuel cell integrated in a mammal.

4 Research progress in animal–robot mixed societies

In recent years, as illustrated in Fig. 5, robotic devices have been increasingly proposed to shed light on hot topics in animal behaviour and behavioural ecology, with special reference to the study of social interactions among animals and robots. Recent technology has made it possible, by developing interactive robots, which can perform complex behavioural tasks, adapting their behaviour to signals from living animals and the environment (Garnier 2011). Furthermore, these robots allow to deliver selected cues triggering animal responses or manipulating the animal collective behaviour.

Robotics devices in mixed society can be useful for several reasons, i.e. to validate in silico systems, creating a closed-loop society with real animals, or to observe and modify

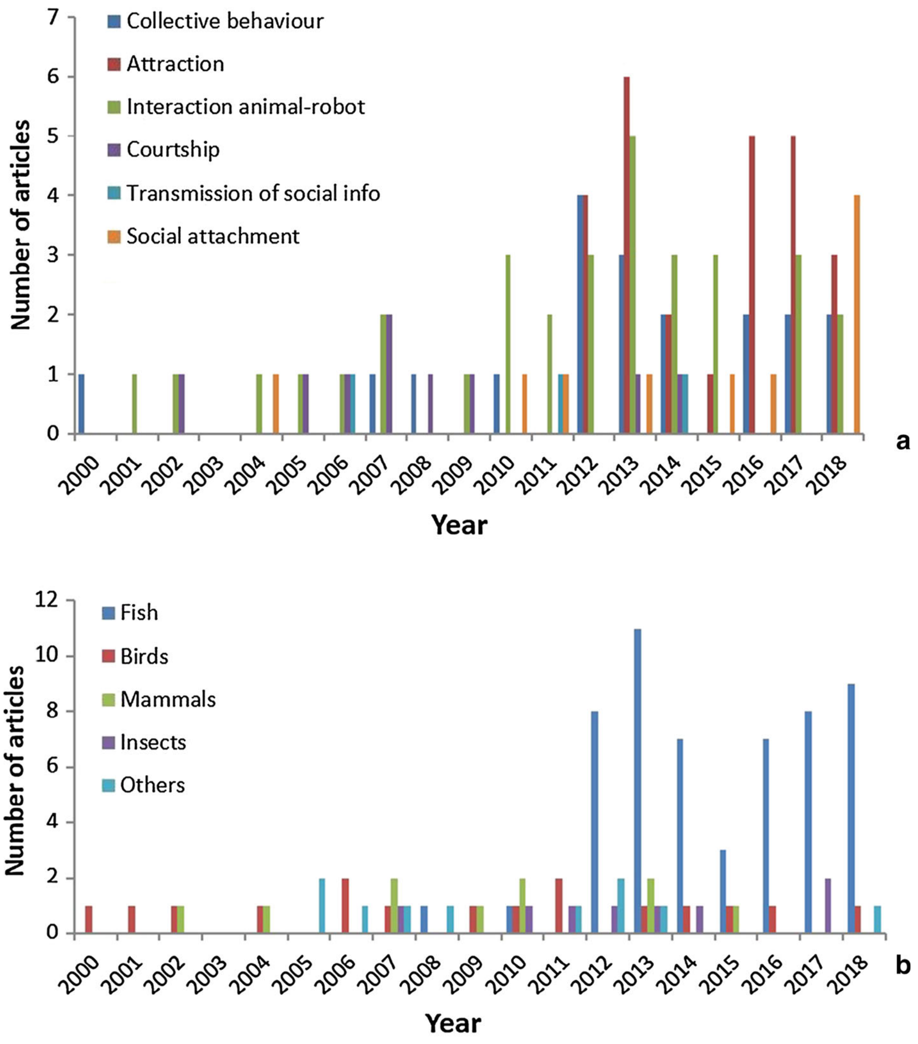

Fig. 6 Number of published research items from 2000 to June 2018 about **a** different topic of investigation in mixed societies and **b** the different species under investigation

the collective behaviour. Thus, robotics represents a sophisticated approach contributing to the study of social behaviours in animals, with potential applications in the control of animal populations in agriculture or in the improvement in animal farming conditions (Garnier 2011; Halloy et al. 2013). In the following paragraphs, we examined the main issues in these research fields.

Figure 6a, b shows the current trend of behavioural studies using robot replicates. Figure 6a shows the number of published research items per year considering the different topics. Most of the articles focus on the animal–robot interactions as well as on the level of attraction of the replica. The latest was investigated to clarify which morphological features boost the chances of acceptance by living animals, thus the possibility to influence their behaviour. Figure 6b shows the number of published research items per year according to the species under investigation. The largest number of studies is on fish species. This can be due to the easier conditions of fish maintenance in the laboratory, compared to other vertebrates, such as mammals. Besides their easy rearing, fishes allow us to investigate a number of behavioural interactions, such as the influence of swimming acceleration and speed, as well as peculiar morphological features. However, studies involving fish collective behaviour are often focused on single mechanisms acting on group dynamics that limits experimental demonstrations of these displays (Ioannou 2017). Indeed, multiple mechanisms are involved in producing improved group behaviours in fish species. In insects, collective behaviour is often demonstrated by the relatively clarity on how information and decision-making occur (Halloy et al. 2007), while in fish the stimuli transferring information in the shoal are more complex and unclear (Ioannou 2017). In addition, contrary to colonies of many social insect species, shoals of fish include unrelated subjects and thus cooperative behaviours are in several contexts eclipsed by competitive interactions (Halloy et al. 2007; Couzin 2009). Also, the heterogeneity of cognitive abilities among individuals of a shoal of fish can pose some difficulty in their study compared to other animals such as several arthropods species (Ioannou 2017).

4.1 Animal perception of robots as conspecifics

Robot features are extremely important for their acceptance by single individual and animal groups as well. In order to elicit interactions with single individuals or group, the robot as to be treated as a conspecific. This is particularly important, especially in gregarious animals. In this context, the use of robots may help to determine which features and signals are critical to trigger conspecific attraction.

Motor patterns seem to play an important role in conspecific attraction. For example, Marras and Porfiri (2012) determined the effect of tail-beat frequency of a robotic replica on individual golden shiners, *Notemigonus crysoleucas* Mitchill (Cypriniformes: Cyprinidae). They found that locomotion is a determinant cue to evoke fish preference, since *N. crysoleucas* individuals are more attracted towards the robot with tail-beat movement rather than when it is statically immersed in the water. An explanation can be because it provided considerable hydrodynamic advantages.

Another study showed that the water flow is determinant for schooling, because the coordinate swimming reduced energy expenditure (Polverino et al. 2013). A similar experiment, but in a closed-loop interaction, was performed with zebrafish, *Danio rerio* Hamilton (Cypriniformes: Cyprinidae) (Kopman et al. 2013; Bonnet et al. 2016a, b), where the tail beat was real-time controlled based on fish motion. Bonnet et al. (2016b) showed that a robotic zebrafish replica is able to attract a shoal of zebrafish inside of a circular corridor according to the speed of the device, even if this effect was not enough to guarantee a full integration. Later on, the authors developed a robotic system that achieved similar motion patterns displayed by zebrafish for direct interactions with *D. rerio*, establishing a closed-loop interaction (Bonnet et al. 2017a, b).

Another important issue in conspecific attraction is the visual information, which seems to be crucial in many species, especially colour and shape features. Interestingly, to understand how the body size affects the social behaviour in zebrafish, Bartolini et al. (2016) investigated behavioural responses of zebrafish to shoals of 3D printed conspecifics of different sizes. In addition, several experiments combined visual and motion features to demonstrate the effective attraction of the robotic replica by living animals. It was found that the variations in morphophysiological and locomotory features were determinant of attraction towards the robotic fish (Abaid et al. 2012; Polverino et al. 2012). The behavioural response of zebrafish individuals to small shoals changed according to the variation in the aspect ratio, colour pattern, tail-beat frequency and speed of the robotic fish. The attraction is maximized when the robotic fish replicated the animal counterpart in aspect ratio and colour (Bonnet et al. 2014). In contrast to the aforementioned observations on zebrafishes, results on mosquitofish, *Gambusia affinis* Baird &Girard (Cyprinodontiformes: Poeciliidae), showed that they were repelled by mosquitofish-inspired robotic replica, independently of its aspect ratio or swimming depth (Polverino and Porfiri 2013). A recent study on the acceptance of biomimetic replica of the Trinidadian guppy, *Poecilia reticulata* Peters (Cyprinodontiformes: Poeciliidae), provides a description of the effect triggered by different appearances, motion patterns and interaction modes on the acceptance of the artificial fish replica. Integration of realistic eye dummies along with natural motion patterns led to a significant improvement in the robotic replica acceptance level (Landgraf et al. 2016). Bierbach et al. (2018a) investigated which cues produced by a robotic fish evoke acceptance in fish species. Authors tested the effect of the biomimetic artefact on two populations of *Poecilia mexicana* Steindachner (Cyprinodontiformes: Poeciliidae): the first population was adapted in dark habitats (e.g. caves), while the second population was adapted to natural light conditions. In light conditions, the acceptance of the robotic fish occurred in both populations, while in dark conditions the robotic fish did not have effects, since probably other cues are needed in the absence of light (e.g. chemical cues, auditory stimuli). In addition, Bierbach et al. (2018b) studied individual responsiveness to social stimuli in *P. reticulata* individuals, by controlling a biomimetic robot, to avoid influences rising from mutual interactions among fish. Authors observed that responses to social stimuli are independent traits, not correlated with other individual behavioural displays.

Since zebrafish behaviours depend on social interactions as well as on their position in the environment, a multilevel model describing the zebrafish collective behaviours was developed to control a robot that was integrated socially in zebrafish group (Cazenille et al. 2017). In this case, optimization methods to calibrate automatically the controllers of a robotic agent according to the animal behaviour were developed (Cazenille et al. 2017).

However, the reconstruction of 3D trajectories should be considered (Macrì et al. 2017), since traditional behavioural displays observed in 2D can undermine data integrity. Ruberto et al. (2016, 2017) studied the zebrafish response to a 3D-printed conspecific replica moving along realistic trajectories (Ruberto et al. 2017).

As observed, the design of biomimetic robots able to interact with fish is complex since it should ensure a luring capability, as well as the acceptation of the robots by the animals as a conspecific. Stochastic model-based behaviours of the robot were proposed by Cazenille et al. (2018a), to integrate it in a group of *D. rerio*.

Animal–robot interactions are benefitting from progress in technology that provides more complex systems to be exploited in animal behavioural studies. Worth to be mentioned is the recent use of virtual reality to control the animal behaviour (Stowers et al. 2017). This strategy boosts detailed surveys into neural and behavioural functions through the accurate control of sensorimotor feedbacks in animals moving in 3D scenarios. Besides the study of morphological and mobility features, Worm et al. (2014) investigated the acceptance of a robotic counterpart of the weakly electric fish *Mormyrus rume* Valenciennes (Osteoglossiformes: Mormiridae). This fish is able to communicate by using electric signals, and for this reason, the dummy fish was equipped with electrodes for the generation and reception of signals. Results showed that the animals preferred to interact with the dummy fish in the presence of electric signals (Donati et al. 2016). Worm et al. (2018) observed an enhanced interaction between *M. rume* individuals and a robotic fish when the artificial agent generated dynamic echo playback of biomimetic electric organ discharges compared to inter-discharge intervals displays statically and randomly produced.

4.2 Robots for modification of behaviour through different biomimetic stimuli

Animals rely to different senses to perceive external stimuli. A complex sensory system allows to perceive the surrounding environment and its dynamic changes. Therefore, understanding how it works is a fundamental question of the animal cognition. Robots can represent an effective solution to this question, creating different combinations of perceptual cues, by modifying their features, which can elicit different responses in animal behaviour. For example, a mechanical honeybee model (Michelsen et al. 1992) was used to understand the role of dance in the transfer of foraging information to follower bees. The robot was able to separate waggle dance and sound-producing wing vibration of the dance, and the experiments demonstrated that the waggle dance is important to transmit information about distance and direction of a given food source. Close-related studies have been conducted subsequently to investigate the acceptance of the robot in the hive (Landgraf et al. 2010, 2012).

Recently, robotic agents were used in neuroethology studies focused on invertebrate brain lateralization. In particular, Romano et al. (2017b) investigated the lateralization of escape and surveillance responses in *Locusta migratoria* Linnaeus (Orthoptera: Acrididae), during predator–prey interactions with a robot inspired to a natural enemy of locusts, the Guinea fowl, *Numida meleagris* (Linnaeus) (Galliformes: Numididae). Benelli et al. (2018) developed a mechatronic device mimicking a potential host, providing a combination of visual and olfactory cues important in the host-seeking behaviour in ticks. Results showed a population-level left-biased use of forelegs in *Ixodes ricinus* L. (Acarina: Ixodidae), during climbing on the robotic host. The action performed by these artefacts enabled a high degree of standardization and accuracy of the stimuli presented, which represent crucial issues in the study of lateralization, especially in experiments involving invertebrates.

The possibility to deliver different kinds of cues separately through robots interacting with animals allows us to dissect the relative importance of the different stimuli triggering a response in living organisms. This is hard in classical natural observations because all the cues are presented together and the response to a single feature in a complex (multi-stimuli) behaviour is not measurable. Robots make possible the deconstruction of different behavioural components to measure the individual responses. For example, a study focused on display modification to visual signals in the lizard *Sceloporus graciosus* Baird & Girard (Squamata: Phrynosomatidae) used a robotic counterpart to investigate two types of headbob displays (special, typical and unusual) both in short- and in long-term assays (Martins et al. 2005). No evidence for immediate changes in signal structure or long-term differences was detected. However, the lizard was more agitated and produced highly aggressive displays when exposed to unusual headbob displays. Another study focused on the impact of different displays in *Anolis sagrei* Duméril & Bibron (Squamata: Dactyloidae) (Partan et al. 2011), showing that the social response to the movement of the robot was higher in signature pattern than alternate pattern, despite that they had a high degree of variability in signature bobbing display. In another study, the frog *Epipedobates femoralis* (Anura: Dendrobatidae) defends their territory and emits visual cues (i.e. vocal sac pulsations) and auditory cues (i.e. advertisement cues). In this study, the two stimuli have been presented with spatial disparity or/and time delay and the result showed that bistimuli with temporal overlap evoked aggressive behaviour while with lacking overlap they were ineffective. For spatial disparity, the response was the same up to 12 cm. Similar studies have been conducted on ground squirrel, *Spermophilus beecheyi* Richardson (Rodentia: Sciuridae) (Rundus et al. 2007), where the importance of infrared signal to deter rattlesnake predator was investigated. Natural observation showed that squirrels, when confronting infrared sensitive rattlesnakes, add an infrared component to their snake-directed tail-flapping signals, whereas, when confronting infrared-insensitive snakes, the tail flagging is without augmenting infrared emission. The robot squirrel simulated the same behaviour, and the results showed a greater shift from predatory to defensive behaviour of the rattlesnake when the infrared was present. In similar studies on multi-stimuli with the squirrel *Sciurus carolinensis* Gmelin (Rodentia: Sciuridae) (Partan et al. 2009, 2010), a robotic counterpart presented different alarm cues (i.e. visual and acoustic ones), in different combinations, investigating the response of wild squirrels (Fig. 7a). Multimodal signals

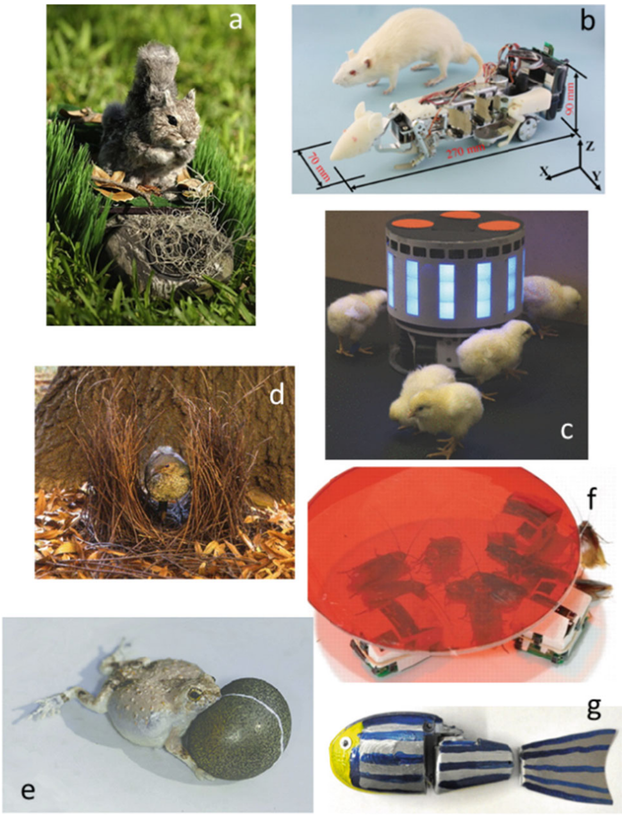

Fig. 7 Examples of robots used during animal–robot interaction studies: **a** robot squirrel used to test multiple sensory channels (adapted with permission from Partan et al. 2009), **b** rat robot WR-4 used to modulate rat behaviour (adapted with permission from Shi et al. 2013), **c** chick–PoulBot mixed group (adapted with permission from Gribovskiy et al. 2010), the robot was used to investigate the acceptance by a group of domestic chicken by using filial imprinting, **d** robotic female bower birds used to investigate courtship (adapted with permission from Patricelli et al. 2006), **e** robotic túngara frog used to study female mate choice (adapted with permission from Taylor et al. 2008), **f** robot used to investigate decision-making behaviour in cockroaches (adapted with permission from Halloy et al. 2007) and **g** robotic fish used in experiments to investigate the collective response of shoal (adapted with permission from Butail et al. 2013)

showed higher responses to cue combinations over separate signals.

Siamese fighting fish males perform stereotyped and vigorous aggressive displays towards conspecific males, related to their highly territoriality. On this basis, *Betta splendens* Regan (Perciformes: Osphronemidae) was tested as model system to investigate the effect of a robot fish eliciting aggression (Romano et al. 2017a). The authors evaluated how multiple signal systems, including a light stimulus, affect aggressive responses in this species. The efficacy of an artificial opponent eliciting aggressive behaviour in fish can be boosted by exposure to multiple signals. The optimized cue combination delivered by the robotic fish demonstrated its help to predict escalating levels of aggression.

A biomimetic soft robot able to 3D move in underwater environments was developed by Katzschmann et al. (2018), to be perceived as a fish, thus approaching and studying the aquatic life.

4.3 Robots in social interaction

Animals live in a dynamic social environment with frequent changes, and they continuously update the information they hold from conspecifics (e.g. foraging for food and mates). The transfer of information between individuals is a complex behaviour, hard to fully understand and control. The discrimination between different features of a selected cue and information processed by the individual still needs research efforts. In this scenario, robots can be extremely useful to solve this problem, allowing to control independently specific aspects of behaviour.

The relevance of social interactions was investigated in a study on dogs interacting with commercially available quadrupedal robots (Kubinyi et al. 2004), where the latter elicited either aggressive or playful responses from live dogs. Results showed that age and context influence the social behaviour of dogs. A laboratory study on learning showed that rats followed a remote-controlled electromechanical rat (WM-2) to sources of food (Takanishi et al. 1998). Live rats recognized the movement of robot and it helped them to learn response to stimulations. Later, the authors increased the complexity of the interaction by using a legged rat robot, which successfully taught the rat a lever pushing task to get food (Laschi et al. 2006; Patanè et al. 2007). Recently, Shi et al. (2010, 2013, 2015) investigated the interaction between a robotic rat and living rats (Fig. 7b), observing that rats with more active behavioural parameters are more susceptible to being adjusted by the robot.

In animal interactions, it is possible manipulate the behaviour of individuals by using animal–mimicking robots (Fernández-Juricic et al. 2006). In this context, the foraging and scanning behaviour of the house finch *Carpodacus mexicanus* Muller (Passeriformes: Fringillidae) in response to different types of behaviours from artificial flocks were studied. Finches spent more time foraging when the robots simulated body movement that could be associated with successful foraging behaviour. A comparison on social information transfer in three different bird species (i.e. European starling, house finch, brown-headed cowbird) (Fernández-Juricic and Kowalski 2011) showed that in all species, a nonlinear decrease in social information flow with increasing distance between the robots and live bird was found. This was more pronounced in species with lower visual acuity, because animals with higher visual acuity can detect changes in the behaviour of conspecifics from farther distances, which may have consequence in spatial distance between individuals within a flock. High visual acuity is due to regions of the retina with a localized high density. These regions project into a visual space, which may require, in order to detect changes, to move the body and modify the position within the group (Butler and Fernández-Juricic 2014).

Robots can be used to study the learning occurring at a particular early life stage, namely "imprinting". There is a brief receptive period, typically soon after birth or hatching, in which the animal is able of rapid learning and establishing a long-lasting response to a specific stimuli (Lorenz 1935). The input can be a visual, auditory or tactile experience that creates the attachment with a given object. Usually, in nature, this object is a parent, but in experiments other animals and inanimate objects, as robots can be used. The idea is to use the robot to fully control an animal's experience by exposing it to a single or multiple robots from the birth. It was shown that chicks, *Gallus gallus domesticus* Linnaeus (Galliformes: Phasianidae), can be imprinted on robot introduced as a surrogate hen which can spatially interact (Gribovskiy et al. 2010, 2015, 2018) (Fig. 7c). In a social experiment with the Japanese quail chick *Coturnix coturnix japonica* Temminck & Schlegel (Galliformes: Phasianidae), a mobile robot carrying a heat source was used to control the motion of quail chicks. Chicks showed better spatial abilities when raised with heated mobile robot than when exposed to an immobile heater. These experiments demonstrated that there was a measurable attachment to the robot (favoured when the chick encounters the robot early after the birth), and this attachment was also combined with a synchronization of chick and robot activity (Jolly et al. 2016). Finally, in a study assessing social cues in the Australian brushturkey *Alectura lathami* Gray (Galliformes: Megapodiidae), a series of robot chicks was built with the same characteristics of the real one, except for one made with pecking movements. Chicks preferred a pecking model over static or scanning models (Göth and Evans 2004), suggesting that social responses of chicks depend upon conspecific motion patterns.

Animal behaviour may vary post-exposure to different traits. This is particularly true for courtship behaviour, where males often differ in their courtship sequence and these differences modify the rate of success at convincing female to mate. Interactive robot can provide different combinations of mate values to evaluate their effect to investigate the relevant features in mating choice. A study on courtship in fish *Lucania goodei* Jordan (Cyprinodontiformes: Fundulidae) (Phamduy et al. 2014) measured the preference of a fertile female for male robot movements by varying aspect parameters (e.g. colour: red, yellow or blue skin) during motion of classical courtship behaviour. In satin bowerbirds *Ptilonorhynchus violaceus* Vieillot (Passeriformes: Ptilonorhynchidae) (Patricelli et al. 2002, 2006), a robotic female robot was used to study male displays adjusted to female's response and to test how and when the males reduce the intensity of their courtship displays after females starling (Fig. 7d). Another example is the study of head-bobbing movements in the lizard *S. graciosus* that can elicit both aggressive and mating displays (Martins et al. 2005). Results showed that two aspects of head-bobbing displays are independently meaningful components interpreted different by different receivers. Males are attended to posture and females to number of head bobbing, using it to distinguish male courtship. Multimodal signals during courtship were also investigated in Tùngara frogs (*Physalaemus pustulosus* Cope; Anura: Leptodactylidae) (Taylor et al. 2008) to test if females preferentially respond to multimodal stimuli (i.e. auditory plus visual cues) over a unimodal stimulus (i.e. auditory cues only). In this study, the females interacted with a robotic calling male frog, validating the theory that females positively select multimodal cue combinations over the same stimuli tested singly (Fig. 7e). Another recent example is the study of fiddler crabs (*Uca mjoebergi* Rathbun; Decapoda: Ocypodidae), arm movement in mating preferences, where four robotic male crabs were used to produce asynchronous and synchronous movements during the courtship dance (Reaney et al. 2008). A last example is the study of cricket behaviour during courtship, based on pose estimation (Kawabata et al. 2013). The real cricket interacted with a mobile robot, and the studies about behaviour were based on the animal pose. In a subsequent study (Kawabata et al. 2014), the robot robes cricket's pheromone and its position was controlled by designing motion patterns based on visual motion tracking of the both agents. Results showed a change in the behavior of crickets due to experience.

4.4 Robots in collective behaviour

A further application of robots is to understand and influence the animal collective behaviour (Mondada et al. 2013). The collective behaviour is a complex system that presents several levels of organization (i.e. hierarchical organization). One of the major challenges in mixed society is to design robots that can modulate the natural society towards a desired behaviour influencing the decision-making process. The resulted system is a closed-loop feedback system between artificial and natural animals where robots can react to sensory input triggered by the animals. To investigate the mechanism of shelter seeking in cockroaches (*P. americana*) (Halloy et al. 2007), a robot with the same behaviour of real counterpart was developed. The robots were autonomous, did not look like roaches, but were pheromone scented. It was able to recognize the shelters and to interact with real cockroaches as well as to elicit novel collective decisions. They were programmed to lead cockroaches from the favourite shelter into unsafe one in open area. Also, the Robot Sheep dog project developed a mobile robot to control a flock of ducks to go to a specific and safety place (Vaughan et al. 2000). The robot moved round behind ducks with respect to the goal and the flock moved away from the robot to the goal. When the flock reached the goal started the repulsive phase and the robot was less attracted.

Studies on collective behaviour in fish shoals are rapidly increasing. One of the reasons is the possibility to have easy access to fish and the limited space they needed. Zebrafishes is one of the main species currently studied, due to their high reproduction rate, short intergeneration time and evident shoaling tendency. For example, in Butail et al. (2013) a robot able to move at different speed varying tail beating was used to explore the fish responses (Fig. 7g). It has been showed that for group cohesion, speed is a determinant feature. Indeed, the relative distance increases with speed of the robot. Further experiments investigated the influence in shoal size and configuration (Butail et al. 2014) of fish increasing the number of robots. Stress measurements showed increased value in the presence of more robots, or with fast robot swimming alone instead of slow swimming of two robots. Other experiments focused on the collective behaviour of different fish species, including the analysis of self-organization and aggregation in Giant danios, *Devario aequipinnatus* McClelland (Cypriniformes: Cyprinidae) (Aureli et al. 2012). A similar study with robotic replica, but different species of fishes (i.e. sticklebacks and guppies) involved one robot that moved according to the position of fish detected through an external camera (Landgraf et al. 2013). Risk-taking behaviour of individual golden shiners (*N. crysoleucas*) was investigated in the presence of a self-propelled robotic fish to test the hypothesis whether the behaviour of fish can be modulated by varying the behaviour of a robotic fish and to investigate whether such response depended on fish individual boldness (Abaid et al. 2013).

Robots can be used to explore how animals select the leader and in which contests they follow it. Leadership occours when one or more individuals initiate a new direction of locomotion, followed by other group members. For example, in Faria et al. (2010) a fish-like replica was used to visually attract and drive single fishes out of a refuge and to initiate new swimming directions in both individuals and groups. Ward et al. (2008) investigated the decision-making process in a Y-maze by using a robotic replica moved on a guide line, to demonstrate the role of "quorum responses" in the movement of the fish *Gasterosteus aculeatus* Linnaeus (Gasterosteiformes: Gasterosteidae). An information-theoretic approach to infer leadership starting from positional data of fish was also proposed (Butail et al. 2016, 2017). Rashid et al. (2012) presented another work about leadership, which involved the use of mobile light sources for guiding swarms of brine shrimp larvae (*Artemia salina* Linnaeus; Anostraca: Artemiidae).

Recently, computer vision and real-time control have enabled the development of closed-loop control systems that boost the degree of biomimicry of the artefacts, by improving the attraction and the interaction of *D. rerio* with the robotic stimuli (Kim et al. 2018). These artefacts that are perceived as conspecifics can be used to study social processes and to affect collective decision in the fish (Bonnet et al. 2018). Interestingly, Cazenile et al. (2018a, b) presented a strategy for real-time calibration of behavioural models based on an evolutionary algorithm, to improve the integration of the robots into the shoal.

4.5 Robots as tools for scientific validation

Finally, robots can be useful for testing and validating models of behaviour in embodied simulators. We define this possibility "in artefacto", compared to in vivo, in vitro and in silico well-established approaches. Interactive robots can be used to assess mechanistic models and the assumption they are based on (Krause et al. 2011; Manfredi et al. 2013), allowing scientists to test hypotheses about mechanical mechanisms and interactions with external cues. They can be effective tools for validation theories and natural models, thanks to the achievement of high accessibility to the environment. It is possible to embed the biological knowledge and models directly on the robots; thus, the observations can be made on such a complete physical model interacting with the real environment.

5 Lesson learned from animal–robot interaction studies and future perspectives

Overall, our review on animal–robot interaction and integration shows the mutual contribution between robotics and biological systems in science and technology. Bio-hybrid organisms can be considered advanced engineered organisms resulting from a hot trend in biotechnology, aimed at coupling the biological and robotic worlds in one entity. This approach outlined fascinating applications for both research fields, and its potential is still minimally exploited (see Table 1). Bio-robotics researchers can boost the still clumsy adaptability of robots to the real world by exploiting animal complex architectures and their capability of negotiating unknown and unstructured environments. Furthermore, their energy autonomy is worth to be considered in long-lasting explorations. These bio-hybrid organisms could significantly outperform traditional robots used for monitoring, searching and/or rescuing activities in dangerous and intricate scenarios. Neuroscientists and biologists could rely to bio-hybrid organisms to investigate and verify neurophysiological functions and mechanisms involved in muscle activation. The latter could be exploited to carry out behavioural interactions with conspecific animals.

Table 1 Current state of the art about bio-hybrid organisms; it shows the species investigated, the scientific topic of the experiments and the biological interface with the artificial component

Animal model	Topic	Biological interface	Study
Snail	Electric energy from bio-chemicals	Metabolic compounds	Halámková et al. (2012), Katz and MacVittie
Cockroach	Terrestrial locomotion	Antennae/ganglia electrical stimulation	Holzer and Shimoyama, Sanchez et al. (2015), Li and Zhang (2016, 2017), Cole et al. (2017), Latif et al. (2016)
	Collective terrestrial locomotion	Antennae/cerci electrical stimulation	Bozkurt et al. (2016), Erickson et al. (2015), Dirafzoon et al. (2017a, b)
	Electric energy from bio-chemicals	Metabolic compounds	Schwefel et al. (2015), Shoji et al. (2016)
Desert locust	Terrestrial locomotion	Metathoracic t3 ganglion electrical stimulation	(Giampalmo et al. 2011)
	Bio-controlled artefact	Behaviorial chemical sensing	Mehta et al. (2017)
Moth	Wing motion	Muscle electrical stimulation	Bozkurt et al. (2008, 2009)
	Bio-controlled artefact	Antennae electrophysiology	Kuwana et al. (1995), Martinez et al. (2014), Ando and Kanzaki (2017)
	Electric energy from bio-chemicals	Metabolic compounds	Schwefel et al. (2015)
Beetle	Terrestrial locomotion	Muscle electrical stimulation	Zhang et al. (2016), Cao et al. (2016)
	Wing motion	Muscle electrical stimulation	Sato and Maharbiz (2010), Sato et al. (2009, 2015), Poon et al. (2016), Doan and Sato (2016), Choo et al. (2016), Li et al. (2018)
	Optical perception	Eye electrophysiology	Jadhav et al. (2012)
	Electric energy from vibrations	Wing vibration	Aktakka et al. (2011)
Honeybee	Wings motion	Brain electrical stimulation	Bao et al. (2011), Wang et al. (2016)
Bumblebee	Wings motion	Visual stimuli	Zheng et al. (2017)
Lobster	Electric energy from bio-chemicals	Metabolic compounds	MacVittie et al. (2013)
Goldfish	Swimming movements	Brain electrical stimulation	Kobayashi et al. (2009), Uematsu and Todo (1997)
Carp	Swimming movements	Brain electrical stimulation	Peng et al. (2011)
Reptile	Terrestrial locomotion	Brain electrical stimulation	Wenbo et al. (2009), Kim et al. (2016)
Pigeon	Terrestrial locomotion	Brain electrical stimulation	Yang et al. (2015), Seo et al. (2017), Huai et al. (2016), Wang et al. (2018)
Rabbit	Electric energy from bio-chemicals	Metabolic compounds	El Ichi-Ribault et al. (2018)
Rat	Terrestrial locomotion	Brain electrical stimulation	Wang et al. (2015, 2016, 2017), Talwar et al. (2002), Wu et al. (2014), Xu et al. (2016), Yu et al. (2016a, b)
	Immobile behaviour	Brain electrical stimulation	Lin et al. (2011)
	Bio-controlled artefact	Genetically engineered cardiomyocytes phototaxis	Park et al. (2016)

In addition, a visionary pathway to target is that of tightly merging a biomimetic robot with a biological nervous system (e.g. Stefanini et al. 2012; Fry et al. 2009). In this way, electrophysiological signals should control the artefacts, and conversely, sensory signals recorded by the robot could be used as feedbacks by the animals, in order to affect its behaviour.

Finally, some limitations have to be challenged to develop this scientific context. A key problem concerns the interface between living tissues and artificial devices. Here, although several studies described above were addressed to it, efforts are still needed to investigate the most suitable materials possessing good compatibility with biological tissues, as well as to reduce the difficulties for the operator to manipulate the animal model. Ethical disputes could also constrain researchers involved in this field making even more complex bio-hybrid approaches. One of the main ethical issue concerns if the contribution in science can justify the killing of animals used to develop bio-hybrid organisms. The growing sensibility in animal rights has produced many efforts to find the most suitable experimental treatment ensuring animal wellness (Lockwood 1988; ASAB/ABS 2004).

Several parameters have been exploited to define the features proving animal sentience, to understand the limit between research and moral issues (Tye 2016), and often the domain of bio-hybrid organisms is considered to go too far compared to their contribution in science (Dodd 2014). Concerning this issue, a remarkable attempt has been made by some authors, which proposed control methods based on real-time virtual reality and reinforcement learning in order to control animal locomotion in a non-invasive way (Wang et al. 2016; Zheng et al. 2017).

Regarding animal–robot mixed societies, recent technological advances made possible to control and manipulate animal behaviour. These robots are able to perceive, communicate and interact/adapt with the animals. Therefore, robots can be used to bring new capabilities producing shared collective intelligence (Halloy et al. 2013). Animal–robot interactions, in addition to new knowledge, can hold to a remarkable socioeconomic impact on our daily lives. Their application includes the control of animal populations in agriculture, the improvement in animal farming conditions, improving breeding conditions and the protection of endangered species. An interesting area for application is the use of robots as leaders, guiding animals away from a dangerous situation. Another possibility is to use robots during the imprinting phase, allowing animals to follow the robots along a suitable migration route or away from danger. A further potential application is in herding animals prior to catching or transportation. An effective herding robot must be able to interrupt ongoing animal behaviour without causing panic or flight reactions.

However, although many robots have been developed for research purposes, robots available outside of the laboratories are very rare in these contexts. A good example of artificial agents interacting with animals in the “real world” can be represented by milking robots that are becoming more and more used in cattle husbandry and dairy production (Jacobs and Siegford 2012). In this case, although robots are not biomimetic, animals directly interact with the machine without involving humans (Pastell et al. 2006). Also smart collars, wearable devices mounted directly on animals (e.g. “mounted nodes” described by Mondada et al. 2013), are very common in farms (King 2017). Furthermore, an impressive biomimetic robot (e.g. Robird) has been recently developed to be marketable as a sustainable product for bird control (e.g. to relocate birds around airports) (Folkertsma et al. 2017). This robot mimics in its appearance and flight a rapacious searching for a prey.

Service robots are having a great impact on the market in the domestic environment (Decker et al. 2011; Forlizzi and DiSalvo 2006), and innovative artificial agents used to interact/control animals would have a huge market potential as well.

The main challenge that could still limit the developing of these kinds of robots for real applications is represented by the design of biomimetic agents able to perform long-term interactions and to behave in highly unstructured scenarios (Beer et al. 1997). Further efforts on this are imperative, considering the potential that these devices would have.

Concerning ethical issues, they can rise also in this domain, since people might erroneously be concerned by the fact that scientists are building tools to potentially control all types of animals in the planet, as well as human being, using artificial devices. However, concerning potential applications of this domain should be considered that traditional strategies to manage livestock as well as to control pest and wildlife populations are often carried out by adopting unethical and non-eco-friendly methods (e.g. employment of pesticides and selection hunting). Thus, one of the aims of this domain, concerning real-world applications, is to produce advancements in animal wellness and environmental sustainability. In addition, the approach of this domain in studying animal–robot interactions is elegant and minimally stressful for animals as stated in most the works mentioned in this review. Furthermore, the use of predators in laboratory-staged predator–prey experiments and real opponents in aggressive behavioural studies are no longer ethically acceptable (Huntingford 1984; ASAB/ABS 2004). The use of robotics in studies focused on predator–prey interactions and aggressive behaviour turned out to be an excellent solution to these issues (Ladu et al. 2015; Romano et al. 2017b; El Khoury et al. 2018).

Table 2 shows the list or recent studies on animal–robot behavioural interactions. It is evident how, in the last years, the studies focused on experimental behaviour on fish, due to easy access to them and the simple breeding farm they required, although different limitations, already discussed, complicate the use of these animals as model organisms. The overlapping and managing of several communication channels, according to different behavioural contexts, as well as diverse personalities characterizing each individual, should be also considered, to ensure a high degree of reliability and biomimicry of the artefacts during the interaction.

A recent successful employment of biomimetic robots is represented by their use in neuroethology studies, with special reference to research on brain lateralization in invertebrates (Romano et al. 2017b; Benelli et al. 2018). Robots accurately provide stimuli that are fully controllable by

Table 2 Current state of the art about animal–robot mixed society; it shows the species investigated, the scientific topic of the experiments with the number of robots and the animals involved

Animal model	Topic	Robotic units	Animal units	Study
Brine shrimp	Leadership	1	> 1	Rashid et al. (2012)
Crab	Courtship	4	1	Reaney et al. (2008)
	Animal–robot interaction	1	8	Fujiwara et al. (2012)
Honeybee	Behaviour with different stimuli	1	> 1	Michelsen et al. (1992), Landgraf et al. (2011, 2012), Griparić et al. (2017)
Cockroach	Collective behaviour	4	12	Halloy et al. (2007)
Orthopterous	Courtship	1	1	Kawabata et al. (2014)
	Animal–robot interaction	1	1	Kawabata et al. (2013), Romano et al. (2017b)
Tick	Animal–robot interaction	1	1	Benelli et al. (2018)
Fish	Collective behaviour	1	> 1	Polverino et al. (2013), Worm et al. (2014), Butail et al. (2013), Aureli et al. (2012), Swain et al. (2012), Landgraf et al. (2013, 2014), Bonnet et al. (2018), Cazenille et al. (2018b)
		1 or > 1	1	Butail et al. (2014)
		1or > 1	> 1	Ward et al. (2008)
		1	1 or > 1	Faria et al. (2010), Donati et al. (2016), Butail et al. 2016, 2017), Kim et al. (2018)
	Attraction	1 or > 1	1 or > 1	Abaid et al. (2012), Bartolini et al. (2016)
		1	1 or > 1	Polverino et al. (2012), Langraf et al. (2016), Bonnet et al. (2016a, b, 2017a, b), Cazenille et al. (2017, 2018a), Katzschmann et al. (2018), Worm et al. (2018), Bierbach et al. (2018a)
		1	1	Marras and Porfiri (2012), Kopman et al. (2013), Butail et al. (2014), Spinello et al. (2013), Ruberto et al. (2016, 2017), Macrì et al. (2017), Bierbach et al. (2018b)
		1	> 1	Bonnet et al. (2014, 2016a, b), Polverino and Porfiri (2013)
	Anxiety-related behaviour	2	1	Cianca et al. (2013)
	Anti-predator behaviour	> 1	1	Ladu et al. (2015), Cord-Cruz et al. (2017), El Khoury et al. (2018)
	Risk-taking behaviour	2	1	Abaid et al. (2013)
	Courtship	1	1	Phamduy et al. (2014)
	Intraspecific agonistic behaviour	1	1	Romano et al. (2017a)
Lizard	Courtship	1	1	Martins et al. (2005)
	Behaviour with different stimuli	1	1	Partan et al. (2011), Brian Smith and Martins (2006)
Frog	Behaviour with different stimuli	1	1	Narins et al. (2005)
	Courtship	1	1	Taylor et al. (2008)
Duck	Collective behaviour	1	12	Vaughan et al. (2000)
	Animal–robot interaction	1	12	Henderson et al. (2001)
Satin bowerbird	Courtship	1	1	Patricelli et al. (2002, 2006), Savard et al. (2011)
Quail chick	Social attachment	1	1 or > 1	De Margerie et al. (2011, 2013)
		1	2	Jolly et al. (2016)

Table 2 continued

Animal model	Topic	Robotic units	Animal units	Study
Chick	Social attachment	1	> 1	Gribovskiy et al. (2010)
		> 1	> 1	Gribovskiy et al. (2015, 2018)
Bird	Social attachment	2	> 1	(Göth et al. 2004)
	Courtship	1	1	Patricelli and Krakauer (2009)
	Transmission of social info	2	> 1	Fernández-Juricic et al. (2006)
		2	3	Fernández-Juricic and Kowalski (2011)
		1	1	Butler and Fernández-Juricic (2014)
Squirrel	Behaviour with different stimuli	1	1	Rundus et al. (2007), Partan et al. (2009, 2010)
Rat	Animal–robot interaction	1	1	Takanishi et al. (1998), Laschi et al. (2006), Patanè et al. (2007), Shi et al. (2013)
		1	3	Shi et al. (2010, 2015)
		1	> 1	Ishii et al. (2013)
Dog	Animal–robot interaction	1	1	Kubinyi et al. (2004)

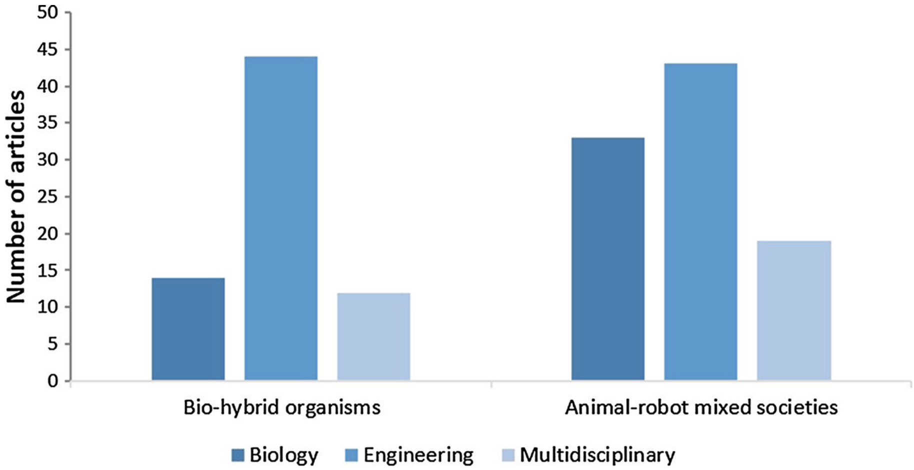

Fig. 8 Number of published research items from 2000 to June 2018 in the domains of bio-hybrid organisms and animal–robot mixed societies. Articles that were published in biology research journals, engineering research journals and multidisciplinary research journals were grouped separately

humans and that overcome limits related to the direction/orientation of cues during these experiments.

In addition, a fascinating and noble role of biomimetic robots concerns their potential use as “artificial hosts” for feeding, in laboratory conditions, haematophagous arthropods, to study their vector competence for parasites and pathogens representing a key threat for humans and animals (Romano et al. 2018).

The employment of these agents would be perfectly in line with the 3R principle “reduce, replace and refine” addressed to limit the use of experimental animals in research, thus avoiding the transmission of diseases, ethical problems regarding animal welfare and the costs of animals rearing as well.

Lastly, an issue to be discussed concerns the fact that robots can be seen as fancy devices to “sell” such studies to the public (Garnier 2011). As many of the proposed studies have demonstrated, robotics has a crucial role in both engineering and biology researches. In addition, they could also have an interesting role as tools for the scientific dissemination and for educational purposes. However, in the case of bio-hybrid organisms, a larger number of papers have been published on engineering journals/conferences compared to biology journals/conferences (Fig. 8a). This is maybe due to weight that ethical issues have on research communities, especially in biology.

Conversely, concerning animal–robot mixed societies, the number of research articles published on engineering journals is rather balanced with research papers published on biology journals (Fig. 8b), highlighting the mutual benefit that this domain ensures to both engineering and biology research communities. However, robotic devices are mainly used by few groups that are highly multidisciplinary. We should not forget the importance that traditional dummies and mirrors had in the study of animal behaviour (Tinbergen 1948). Although these tools have many limitations, they are still commonly used in laboratories, since they are easier to manufacture and to use, if compared to mechatronic devices. Further efforts are needed for improving worldwide the number of collaborations involving researchers in engineering and biology. In addition, the training of novel scientists with a multidisciplinary background would greatly advance this research field, with advantages to both engineering and biology contexts.

6 Conclusions

In our review, recent research about the most significant works on animal–technology interaction, from individual to animal population, is critically discussed. To our mind, this innovative studies can lead to the development of new applications, such as the possibility to merge the two separate fields and create mixed society with cyborgs, revolutionizing the bio-inspired design, as well as leading new biological researches in the fields of animal cognition, evolutionary ecology, conservation biology, as well as parasitology research. In addition, in this review, an accurate analysis on the animal species and abundance of articles for each research topic was carried out, summarizing the current knowledge on bio-hybrid systems and mixed society. Results show that both areas are rapidly growing. Thus, it is crucial to identify their potential future impact on bio-behavioural research. Our review aims to be a concise and timely tool to help researches in the identification and discussion of exciting research challenges in animal behaviour and bio-robotics.

Funding This work was funded by the EU project subCULTron (submarine cultures perform long-term robotic exploration of unconventional environmental niches) number 640967. The funder had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

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