**OVERVIEW** 

# Managing invasive crayfish: is there a hope?

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Abstract Given that the impact exerted by non-indigenous crayfish species (NICS) is most often severe, can occur across many levels of ecological organization, and results in the loss of native crayfish populations, the Convention on Biological Diversity approach, as complemented by the European Strategy, is viewed as an excellent framework to be followed to prevent the introduction of NICS and to alleviate or eliminate the damage they inflict. Much effort should be directed to minimize the risks of intentional introductions, as in part done by the Council Regulation No. 708/07 in force in the European Union since 2009. However, this and other regulations are not well harmonized, for instance, with those concerning both the aquarium trade and the harvest of crayfish for human consumption. To make prevention more difficult, there are many records of illegal release of NICS into the wild and of their accidental introduction as undetected contaminants in batches of regulated fish species. As a consequence, it seems necessary that post-introduction mitigation and remediation protocols and processes, such as contingency plans, are always in place to enable rapid detection and early response in order to minimize and, ideally, annul the threats posed by NICS. The aim of this review paper is to offer a synthetic view of the different methods (mechanical removal, physical methods, biological control, biocides, and autocidal methods) proposed and adopted until now to

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control NICS with a discussion of their pitfalls and potentialities. A glimpse to the ongoing research in the matter will be also given.

Keywords Invasive non-indigenous crayfish · Trapping · Biological control - Biocides - Sex pheromones - SMRT

## Introduction

Species that have been introduced outside their native range (alien or non-indigenous species) have the potential to cause irreparable ecological and economic damages. Although the fraction of the introduced species that become invasive is relatively small, the significant risks that these few organisms pose require the adoption of precautionary approaches that might, first, prevent the deliberate or accidental introduction of potential invaders and, second, design contingency plans detailing the actions needed to rapidly mitigate and remediate their negative impact (Manchester and Bullock [2000\)](#page-14-0).

The implementation of mitigation and remediation measures to face invasive alien species (IAS) was viewed as a priority by the United Nation Convention on Biological Diversity (CBD) at the 1992 Earth Summit in Rio de Janeiro: signatories to CBD called on governments ''as far as possible and as appropriate, (to) prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species'' (article 8 h). In 2002, the CBD Conference of the Parties adopted the Decision VI/23 on Alien Species that threaten ecosystems, habitats and species (COPVI, The Hague, April 2002), which stressed the urgency of prioritizing the development of strategies and contingency plans against IAS at national and regional

levels. At the same time, the CBD Conference formulated Guiding Principles for the Prevention, Introduction and Mitigation of Impacts of Alien Species that threaten Ecosystems, Habitats or Species to be promoted and implemented by governments and organizations.

This latter document set out a hierarchy of actions composed of (1) prevention of IAS introductions between and within States; (2) early detection and rapid response if an IAS has been introduced; (3) containment and long-term control measures, where eradication is not feasible. The European Strategy on Alien Species (Genovesi and Shine [2004\)](#page-13-0) has enucleated the above listed actions in a more articulated approach that also includes (4) raising awareness and disseminating information on IAS issues and on ways to tackle them; (5) strengthening a national and regional capacity and cooperation to deal with IAS issues; and (6) recovering species and restoring natural habitats and ecosystems that have been adversely affected by biological invasions.

Crayfish are the largest and amongst the longest lived invertebrate organisms in temperate freshwater environments, and often are present at high density. Most species are keystone consumers, feeding on benthic invertebrates, detritus, macrophytes, and algae in lotic and lentic waters (Nyström et al.  $1996$ ). They also constitute the main prey of several species, including otters, fishes, and birds (Gherardi  $2007a$ ). Because of their ability to integrate into the food web at many levels and to persist on the substantial energy reserves of the detrital pool, non-indigenous crayfish species (NICS) are good candidates for invading aquatic systems (Moyle and Light [1996](#page-14-0)). Once added to a system, NICS have the potential to impose ''considerable environmental stress'' and, in several instances, they may induce ''irreparable shifts in species diversity'' (Hobbs et al. [1989](#page-13-0)): the changes caused by their introduction usually affect all levels of ecological organization (e.g., Lodge et al. [1998;](#page-14-0) Nyström [1999\)](#page-14-0). The modes of resource acquisition by crayfish and their capacity to develop new trophic relationships, coupled with their action as bioturbator, may lead to dramatic direct and indirect effects on the ecosystem. When NICS replace native crayfish, their ecological effects should not be novel to the colonized community and therefore the resulting impact is expected to be weak. But their overall impact can be particularly strong if, once introduced, they are capable of reaching high densities and/or getting large size. Several NICS often occur in much higher densities than native crayfish species:  $>70$  m<sup>-2</sup> for *Orconectes limosus* in Poland,  $>20$  m<sup>-2</sup> for *Orconectes rusticus* in North America, and 30 m<sup> $-2$ </sup> for Pacifastacus leniusculus in UK. On the contrary, reported densities of the native species range from  $1 \text{ m}^{-2}$  for *Pacifastacus fortis* in California to 3  $m^{-2}$  for *Paranephrops*  planifrons in New Zealand,  $4 \text{ m}^{-2}$  for *Cambaroides japo*nicus in Japan, and  $14 \text{ m}^{-2}$  for Astacus astacus in Sweden (Gherardi [2007a\)](#page-13-0). The success of NICS also depends on several biological traits; once compared to native crayfish, most of them are characterized by higher fecundity  $($ >500 pleopodal eggs in Procambarus clarkii), protracted spawning periods, faster growth rates (50 g in 3–5 months in P. clarkii), and maturity reached at relatively small size (10 g in P. clarkii). They are also extremely plastic in their life cycle and are better at coping with changes induced by human activities that cause pollution and habitat destruction. A higher survival rate is also expected when crayfish species are introduced without a full complement of specific parasites, pathogens, and enemies. Large sizes, in turn, make crayfish both resistant to gape-size limited predators (such as many fishes) and agonistically superior in resource fights. As a consequence, NICS exert a greater direct (through consumption) or indirect (through competition) effect on the other biota, particularly on other crayfish species, mollusks, benthic fishes, amphibians, and macrophytes.

Given that the impact exerted by NICS (1) is generally severe, (2) can occur across many levels of ecological organization, and (3) results in the loss of native populations, including native crayfish species (Gherardi [2007a\)](#page-13-0), the CBD approach, as complemented by the European Strategy, is typically viewed as an excellent framework to be followed to prevent the introduction of NICS and to mitigate or eliminate the damage they inflict. Yet attempts to manage invading populations often fall short of accomplishing their objective: none of the attempts made to contain the spread of NICS in the last decade has provided a definitive methodology (e.g., Blake and Hart [1995;](#page-12-0) Frutiger and Müller [2002;](#page-12-0) Stebbing et al. [2003](#page-14-0); Peay et al. [2006\)](#page-14-0). On the contrary, invasive crayfish's management should be a priority for most governments. For instance, all Member States of the European Union are required by the Water Framework Directive to have their waterbodies achieve ''good ecological status'' by 2015 (European Parliament [2000\)](#page-12-0): the presence of invasive populations of NICS, such as P. leniusculus and P. clarkii, is a contributing factor in a waterbody failing to meet the target, so that much effort should be paid implementing measures for their management (Peay [2009\)](#page-14-0).

This review aims at offering a synthetic view of the different methods adopted until now with a discussion of their pitfalls and potentialities. A glimpse to the ongoing research in the matter will be also given. With respect to other previous reviews (Holdich et al. [1999;](#page-13-0) Peay [2009](#page-14-0); Freeman et al. [2010\)](#page-12-0), this paper will discuss new methods and will extend the analysis to the entire suite of invasive crayfish species.

#### An overview

The popular saying ''an ounce of prevention is worth a pound of cure'' reflects the conventional wisdom among invasion biologists (Vander Zanden et al. [2010\)](#page-14-0). As with other IAS, preventing the introduction of NICS is far more cost-effective and environmentally desirable than measures taken after their introduction and establishment. NICS, in fact, can be hard to detect and disperse rapidly, making eradication or control extremely difficult and expensive. In Scotland, for instance, the cost of an ongoing eradication campaign against P. leniusculus amounts to GB£ 250,000 every 5 months (S. Peay, pers. comm.). So, much effort should be directed to minimize the risks of intentional introductions, as in part done by the legislation in force in some countries (see Peay [2009](#page-14-0)). In the UK, A. astacus, Astacus leptodactylus, and P. leniusculus have been designated as pests under the Wildlife and Countryside Act; much of Britain has been declared a no-go area for the keeping of P. leniusculus and the whole of Britain for the keeping of all other NICS (except the tropical Cherax quadricarinatus). Similarly, in Japan all species of Astacus and Cherax, O. rusticus, and P. leniusculus have been deemed as Invasive Alien Species under the Invasive Alien Species Act; their import and keeping alive are banned except for scientific purposes. In the European Union, the Council Regulation No. 708/07 ''concerning use of alien and locally absent species in aquaculture'' (implemented with the Commission Regulation No. 535/08) has been in force since 2009 (European Parliament [2007](#page-12-0), [2008\)](#page-12-0); its novelty is to take a ''white list'' approach, in that only importation of species that have been appropriately screened after a thorough risk assessment analysis can be approved. This approach contrasts with the homologous regulation in the USA, which permits the importation of species unless these are on a ''black'' list (Injurious Wildlife Species, US Fish and Wildlife Service) (Lodge et al. [2000](#page-14-0)). Recently, the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK), produced by the UK Centre for Environment, Fisheries & Aquaculture Science (Cefas) [\(http://www.](http://www.cefas.co.uk/media/410780/decisiontools_description.pdf) [cefas.co.uk/media/410780/decisiontools\\_description.pdf](http://www.cefas.co.uk/media/410780/decisiontools_description.pdf)), has been adapted by Tricarico et al. ([2010\)](#page-14-0) as a screening tool for identifying potentially invasive NICS: using receiver operating characteristic (ROC) curves, FI-ISK was shown to distinguish accurately (and with statistical confidence) between potentially invasive and non-invasive species of NICS (Fig. 1).

All the existing regulations, however, seem not to be well harmonized with those concerning the aquarium trade or the harvest of crayfish for human consumption. For instance, the aquarium trade in the European Union is regulated by the Commission Decision No. 2006/656/EC concerning health conditions and certification requirements for imports



Fig. 1 Plot of FI-ISK scores for 37 crayfish species assessed for Italy. Bars denote: white native species to Italy, light gray species classed as 'low risk' at threshold  $\geq 1$ , *dark gray* species classed as 'high risk' at threshold  $\geq$ 16, *black* possible 'very high risk' species (score  $>$ 35)

of ornamental fish only. Today, it is extremely easy to buy, via aquarium trade fairs and internet sales, NICS for ornamental use, as shown in the case of both the marbled crayfish (Nonnis Marzano et al. [2009;](#page-14-0) Peay [2009\)](#page-14-0), a North American species recently identified as a parthenogenetic form of Procambarus fallax (Martin et al. [2010](#page-14-0)), and the Australian red-claw crayfish C. quadricarinatus in the UK (Peay [2009](#page-14-0)). At present, in some European countries sale of NICS is legal and an aquarium wholesaler can sell NICS to anyone in Sweden, Ireland or other countries where the species are banned. The recipients may be carrying out an illegal act in his own country by keeping the crayfish, but the sellers are not, even though they may know (as the recipients may not) that the keeping is illegal in the country to which they are exporting the crayfish (Peay [2009\)](#page-14-0).

Considerable risks of introductions are also posed by harvesting NICS and selling them alive for human consumption, through permission, discard of surplus stock or illegal stocking. Indeed, commercial crayfishing might be

an alternative and productive strategy to contain NICS populations under the philosophy of ''making the best of a bad situation''. Where P. leniusculus are present within the restricted region in UK, angling clubs can apply to remove them for purposes of fisheries management and can reduce the cost of doing this by allowing commercial trappers to take the crayfish for sale (Peay [2009](#page-14-0)). However, as shown in many instances (e.g., Edsman [2004](#page-12-0)), the assignment of a commercial value to invasive species almost inevitably results in further—often voluntary—introductions of the species. Besides, from an ecological viewpoint, the prevalent removal of large (and dominant) individuals from the population (i.e., the individuals of commercial size) might reduce their pressure on juveniles that are typically trap-shy or are discarded by fishermen, thus allowing the latter to grow and give rise to even larger populations (see the ''[Mechanical removal](#page-4-0)'' section below). To make prevention even more difficult, there are cases in which NICS have been accidentally released to the wild as undetected contaminants in batches of regulated fish species (Gherardi [2010\)](#page-13-0): the oomycete Aphanomyces astaci, lethal to European crayfish (see the ''[Biological control methods'](#page-6-0)' section below), was first introduced to Europe in 1860 via infected crayfish contaminating a batch of fishes from North America (Cornalia [1860\)](#page-12-0).

A number of approaches to managing the risks of introductions associated with human activities such as aquarium trade and commerce of live specimens have been proposed—but never applied. These include controlling the conditions and location of sale for potentially harmful species, the use of tariffs to internalize invasion costs to the industries that benefit from trade in alien species, and the substitution of alien species by native species in aquaculture, pet industry, etc. (Ewel et al. [1999](#page-12-0)).

The above reported examples and problems underline the need of post-introduction mitigation and remediation protocols and processes, such as contingency plans, being in place to enable rapid detection and response in order to minimize and, ideally, annul the threats posed by NICS. As also claimed by Vander Zanden et al.  $(2010)$  $(2010)$ , "even the most effective prevention efforts are not guaranteed to eliminate new invasions, and some portion of these new invaders will have undesired ecological, economic, and human health impacts" (pg 200). As a consequence, in the many situations where prevention fails, an early detection program could alert managers to the establishment of a new invasive species: a well coordinated eradication program could thus contain or eliminate this invader before its spread.

Eradication (meaning "tearing out by the roots") consists in eliminating the entire invading population from a given area by a time-limited campaign. As indicated by Bomford and O'Brien [\(1995](#page-12-0)), ''time-limited'' is important in this definition: eradication needs to be achieved by a fixed date, because an eradication campaign without a specified end point should be defined de facto as continuing control, i.e., harvesting or killing a proportion of a population on a sustained basis. Obviously, eradication is considered the best and least expensive remediation tool. Yet eradication programs are viewed with skepticism by many conservation biologists, particularly in Europe (Genovesi [2005\)](#page-13-0). In general, eradication is seen as an impossible goal (e.g., Bertolino and Genovesi [2003\)](#page-12-0) that might have the capacity to incur ''horrendous non-target impacts'' (Simberloff [2009](#page-14-0)). In a review published in 2005, Genovesi reported only 37 successful eradication programs against vertebrates in Europe, mostly on islands (33); no eradication of freshwater invertebrates has ever been achieved with a few possible exceptions (see the "[Mechanical removal](#page-4-0)" section below). As recently stressed by Simberloff ([2009\)](#page-14-0), part of the pessimism about controlling invasions arises from widely publicized management failures. Worse, cases of successful eradication are unpublicized or barely mentioned in the popular press or scientific literature (Simberloff [2009\)](#page-14-0). However, the fraction of invasions for which eradication is seriously attempted is minuscule, and these attempts have most often failed.

Such failures might be ascribed to the several requirements to be fulfilled in order to make eradication and continuing control effective remediation tools against IAS, including invasive NICS [see also the criteria discussed by Bomford and O'Brien ([1995\)](#page-12-0) for the eradicate/control of vertebrate pests]. First, any method designed to eradicate/ control invasive crayfish populations must remove sufficient individuals to ensure their extinction, and this must be demonstrable. A density threshold—the Allee threshold (Keitt et al. [2001\)](#page-13-0)—exists for all animal populations, below which the population will cease to be self sustaining. Thus, any method to be considered as having the potential for achieving eradication must reduce the population density to below such threshold. If the number of individuals that survive the eradication attempt exceeds this, the population will be maintained in the long-term. Second, the best opportunities for eradicating an IAS are in the early stages of invasion, in the ''lag'' phase before population increase and spread. Some NICS can remain relatively uncommon and seem harmless for long periods of time before suddenly becoming invasive, perhaps following a genetic change, local environmental change, or the arrival of another alien species which might favor the boom of its population size and spread. Early detection of new biological invasions is therefore crucial and a rapid response is required to eradicate the new invader immediately upon detection. To achieve this, the knowledge of local people should be used, and immediate reporting to local biosafety

<span id="page-4-0"></span>authorities, if any, should be encouraged, in order to detect invasions as early as possible. More user-friendly identification guides are important tools (see, e.g., Souty-Grosset et al. [2006](#page-14-0)) and new high-tech diagnostic tools need to be developed for detecting crayfish of dubious identification, such as DNA barcoding.

Rapid response teams of local experts could be formed, which might detect and evaluate new invaders at the earliest stage and make recommendations for action. In these instances, procrastination is likely to be disastrous. Third, the successful programs against well established invasive populations are only those where the area of invasion is relatively small. Where large-scale eradication/control has been attempted against well-established IAS, an extremely high failure-to-success ratio was recorded. A fourth requirement is that the ecosystem concerned should be sufficiently isolated from potential sources of recolonization by the invader. Least but not last, before starting any eradication program, managers should be fully aware that adequate funds and commitment exist to complete eradication, that monitoring of the target species is feasible in order to ascertain the effective decrease in the population size, and that eradication/control will be followed by the restoration or management of the community or ecosystem after the removal of the target species.

There are a few instances in which the above listed requirements can be met with invasive populations of NICS. One is the recently recorded population of the Australian Cherax destructor in Italy (Scalici et al. [2009](#page-14-0)): its confined distribution in abandoned aquaculture ponds, the low temperature of the surrounding waters acting as a barrier against its natural spreading, and the absence of neighboring populations make eradication still feasible and economically profitable when compared to the enormous costs that this species might inflict if allowed to spread. For most of the already established populations of invasive NICS, on the contrary, the only option is to adopt some mitigation tools that might maintain their density at a very low level and thus reduce their negative impact.

In any case, both eradication and continuing control of NICS should be socially and ethically acceptable, efficient, nonpolluting, and should not damage native flora and fauna, humans, domestic animals, and cultivars. Although all of these criteria are difficult to be met, genuine attempts should be made to do so (Holdich et al. [1999\)](#page-13-0). The type of intervention should be chosen on a case-by-case basis: it is in fact crucial to evaluate the situations for which a given intervention is biologically feasible, as well as acceptable under ecological, economic, political, and ethical viewpoints (''situationalization'', Gherardi and Angiolini [2007](#page-13-0)). Predicting the spread of NICS is an essential prerequisite, which provides relevant information regarding the areas that are likely to be invaded next. The life history of the

species and its ecological attributes, along with the vectors and pathways of introductions, should be included in forecasting models (e.g., Kolar and Lodge [2002](#page-13-0); MacIsaac et al. [2004;](#page-14-0) Verling et al. [2005;](#page-14-0) Herborg et al. [2007](#page-13-0)). Besides, because funds to manage invasions are typically limited, it is important to ask whether intervention strategies can be designed to produce greater economic benefits than the costs required to implement them (Keller et al. [2008](#page-13-0)). Finally, attempts to prevent the introduction of NICS and to mitigate their damage should be based on a thorough understanding of their threats by the general public, decision-makers, and the other stakeholders. On the contrary, as recently shown in southern Spain (García-Llorente et al. [2008](#page-13-0)), most stakeholders have a limited knowledge of what invasive species are and show different perceptions of their impacts and different attitudes toward their management. As a consequence, educating the public and the relevant stakeholders of the impact exerted by NICS and on the importance of early detection and rapid response to crayfish invasions would help build a solid support for their management (Vander Zanden et al. [2010](#page-14-0)). Education and public awareness campaigns seem thus to be an essential step that might develop the shared responsibility needed to address any intervention against biological invaders, crayfish included.

## A taxonomy of methods

Several and diversified methods have been proposed or used, alone or combined, for either eradication or the continuing control of invasive populations of NICS. We can tentatively classify them, distinguishing five broad categories as follows: (1) mechanical removal; (2) physical methods; (3) biological control methods, (4) biocides, and (5) autocidal methods. Table [1](#page-5-0) synthesizes these methods, showing a class-level evaluation of their general efficacy.

#### Mechanical removal

Mechanical removal by the use of traps of various designs (Swedish traps, Evo-traps, collapsible traps, fyke nets, seine nets, etc.; e.g., Westman et al. [1979](#page-15-0); Fjälling [1995](#page-12-0)) or by electrofishing (Westman et al. [1978;](#page-15-0) Laurent [1988](#page-13-0)) may have some effects on the population size of NICS. To cite an example, catches of an invasive population of O. rusticus in the USA declined from 6,500 to 206 after 6 weeks of continuous trapping (Bills and Marking [1988\)](#page-12-0). However, in order to get some significant results, trapping should be conducted for an extended period of time. All this means considerable costs and manpower. For instance, 900 trap nights were needed to reduce P. leniusculus populations from 4,000 to 1,500 in carp ponds in England (Rogers et al.

<span id="page-5-0"></span>Table 1 Synthesis of the different methods used to control invasive NICS showing a class-level evaluation of their general efficacy according to the following criteria: population size (number of individuals in the target population), area size (dimension of the area invaded by the target population), applicability (suitable habitats for the application of the method), species-specificity (capacity to affect the target species only), selectivity (capacity to affect a specific class of individuals in the target population), impact (potential ecological damages), time (duration of the application to be effective), cost (expenses of the method), and efficacy (capacity to control the target population)



 $+$ , low;  $++$ , medium;  $++$ , high;  $-$ , irrelevant; ?, unknown

[1997\)](#page-14-0): in the absence of continuous trapping after that period, populations have returned to their former levels within a couple of breeding seasons (Holdich et al. [1999](#page-13-0)).

With the possible exception of electrofishing (which, however, is not feasible in deep or turbid waters, can remove a modest portion of a population, and is not efficient in streams with large stones or where crayfish hide in the banks; Westman et al. [1978;](#page-15-0) Freeman et al. [2010](#page-12-0)), mechanical removal is often biased by crayfish size and sex, as shown by several studies. Catches of both O. rusticus in the USA (Bills and Marking [1988\)](#page-12-0) and P. le-niusculus in the UK (Holdich et al. [1995](#page-13-0)) and in the Czech Republic (Kozak and Policar [2003](#page-13-0)) were dominated by adult males. On the one hand, large-biased catches might depend on mesh size; for instance, Peay and Hiley ([2001\)](#page-14-0) found that small mesh traps caught crayfish across a broader size range (cephalothorax length: 19–72 mm) than Swedish traps (cephalothorax length: 38–76 mm). On the other hand, the size of the captured crayfish seems to depend on the elusive and cryptic behavior of juveniles that avoid to be cannibalized by adults (Guan and Wiles [1996](#page-13-0)). A consequence of the larger trappability of big and dominant males is the reduction of competition over juveniles, allowing the latter to grow and to produce dense populations (Skurdal and Qvenild [1986\)](#page-14-0) or the attraction of big individuals from neighboring areas. Intensive trapping of P. leniusculus in a section of the River Thames in England acted like a drain on the larger individuals in the population; the areas alongside the section being trapped were thus depleted of larger crayfish and this enhanced population expansion in these adjacent sections by reducing competition (Holdich et al. [1999\)](#page-13-0). A similar phenomenon was recently quantified by Moorhouse and Macdonald [\(2010](#page-14-0)). The authors studied four stretches of the River Windrush in Oxfordshire, UK; each was 1 km in length and was divided into three sections; a 250-m long upstream section, a 500-m middle section and a 250-m downstream section. At two sites (removal sites), P. leniusculus were trapped and removed from the 500-m middle sections; at the other two (non-removal), they were marked and returned. All crayfish captured in the upstream and downstream sections were marked and returned. The percentage of captured crayfish immigrating into the middle sections was the same (3.7%) in both removal and non-removal sites, but the mean distance that crayfish moved when immigrating was significantly greater at removal sites (239 m) than at non-removal sites (187 m). These results imply that removal of large individuals may have reduced the potential for interference competition by increasing the relative competitiveness of the immigrating individuals and <span id="page-6-0"></span>permitting them to make larger movements. Consequently, the impact of manual removal strategies on NICS populations in riparial habitats is likely not only to be reduced at the point of removal, but also to extend at least 200 m beyond the trapped length of the river.

Female crayfish, in particular ovigerous females, are less active than males (Lowery [1988\)](#page-14-0) and are thus scarcely trapped, making up between 0 and 50% of catches (Cullen et al. [2003\)](#page-12-0). In any case, removing ovigerous females might lead to feedback mechanisms so that crayfish, as with most animals, would probably respond to low numbers in the population by producing more eggs and reaching maturity earlier (Holdich et al. [1999](#page-13-0)). Freeman et al. [\(2010](#page-12-0)) report the partial results of a trapping campaign in the upper River Clyde started in summer 1999: since then, about 70,000 P. leniusculus have been removed and crayfish are now trapped in lower numbers, their average size is smaller, and crayfish reach maturity at smaller sizes.

Trapping efficacy might be increased by: improving trap design that should also avoid the capture of non-target organisms, using more attractive baits (generally freshwater or marine fish, either fresh or processed), emptying traps frequently (rate of escape is high, reaching 40%; Kozak and Policar [2003](#page-13-0)), and matching crayfish rhythms of activity (the number of trapped crayfish depends on both the time of the day and the season; Laurent [1988\)](#page-13-0). Traps may be made more attractive by the use of sex pheromones as bait, but this technique is still ineffective (see the "[Autocidal methods](#page-9-0)" section below)

#### Physical methods

Drainage of ponds, diversion of rivers, and construction of barriers may be used in the case of confined populations of NICS, but very little is known about their efficacy. Drought, for instance, cannot be effective with burrowing species, such as P. clarkii, which can survive out of water for long periods. Even species, such as P. leniusculus, which, at least in their native range, are not known to burrow, can do so extensively in suitable substrata (Holdich et al. [1999\)](#page-13-0). Rivers may be diverted via a channel or pipeline and the remaining water pumped out to isolate populations of NICS: the isolated stretch can be thoroughly searched for crayfish and burrows can be treated using biocides (see the "Biocides" section below) or crayfish could be removed from their burrows by hand. However, both Holdich and Reeve [\(1991](#page-13-0)) and Perrow et al. ([2007\)](#page-14-0) reported, in England, the unsuccessful draining of both a farm pond and a sector of River Misbourne, respectively: P. leniusculus individuals were still found alive under large stones even after several weeks of drought.

In 2006, a barrier was erected in the river Buåa at the border between Sweden and Norway to prevent migration of P. leniusculus to the Norwegian part of the river. However, the barrier did not work as expected: during July 2008, signal crayfish were found in the lower parts of the Halden trans-border watercourse in the far south-east (Johnsen et al. [2008\)](#page-13-0). On the contrary, Kerby et al. ([2005\)](#page-13-0) showed that, in California, large barriers (e.g., waterfalls) may work: a mark-recapture study indicated that P. clarkii moved both up and downstream between pools; however, barriers significantly reduced crayfish's movement between pools. Other physical methods may include electric fences, used to avoid migration of cravfish (Håstein and Gladhaug [1973](#page-13-0)), and vibrations (anecdotal evidence showed higher mortalities of crayfish possibly resulting from vibrations from aerators and pumps; Holdich et al. [1999\)](#page-13-0).

# Biological control methods

Biological control (or biocontrol) is a collective term that includes a variety of interventions based on the use of natural enemies of the invader. It is in theory preferable to biocides or other methods because it is permanent, nonpolluting, and ethical. Risks lie in that these enemies are not always specific to the target organism and may instead attack native organisms. Control agents should thus be thoroughly checked for specificity and for non-target effects before their release into the wild.

Traditional enemies of crayfish are predators, such as birds and fishes, disease-causing organisms, and microbes that produce toxins, e.g., the bacterium Bacillus thuringiensis. However, only predaceous fishes are worth considering as control agents, as we will show below. Crayfish are susceptible to various microbial pathogens and parasites (Gherardi et al. [2010\)](#page-13-0). The problem in using most of them lies in that they usually lack host-specificity. There is thus the risk that microbes and other parasites will spread to non-target organisms, including native crayfish species. Freeman et al. ([2010\)](#page-12-0) discuss extensively the potential use, as biocontrol agents, of viruses (the intranuclear bacilliform viruses, IBVs, and the white spot syndrome virus, WSSV), fungi (*Fusarium* spp. and the burn spot disease), microsporidia (including Thelohania contejeani), Rickettsia-like organisms, and the enigmatic Psorospermium spp. However, more in-depth research is needed to avoid mistakes and extreme caution is required before introducing a novel parasite to a waterbody to be sure that non-target organisms, such as other crustaceans, are not adversely affected. A well known parasite of crayfish is the oomycete A. astaci; this is the etiological agent of the most devastating disease that affects the European crayfish, known as crayfish plague, first introduced into Europe from North America in 1860 (Cornalia [1860](#page-12-0)). Since then, it has spread significantly throughout mainland Europe (Alderman [1996](#page-12-0)), leading to the disappearance of 90% of the native A.

astacus in Sweden (Edsman [2000](#page-12-0)). Susceptibility to the crayfish plague depends on the host fitness and species. Native crayfish from Europe, Japan, Australia, and New Guinea, including the commercially important *Cherax* spp., are all highly susceptible to the crayfish plague. On the contrary, the North American species are much more resistant to infection, only succumbing when stressed (Persson et al. [1987\)](#page-14-0). An experiment was carried out by Diéguez-Uribeondo and Muzquiz [\(2005](#page-12-0)) to assess its use to control wild populations of C. destructor in Spain. Cages containing individuals of either C. destructor infected in the laboratory or P. leniusculus with severe signs of infection were introduced into the invaded pond. In both cases, 100% of mortality was achieved, after 30 and 120 days, respectively. As a consequence, the oomycete might be in theory used to eradicate invasive crayfish populations susceptible to it, including A. leptodactylus. It has been also hypothesized that genetically modified strains of A. astaci might overcome the defense systems of the North American crayfish. However, the use of both the wild parasite and its genetically modified strains might generate the risk of its indirect spreading to the native crayfish populations. Finally, the use of B. thuringiensis and its varieties, such as B. t. var. israeliensis, has promises as a control agent in aquatic systems for the biocontrol of mosquito larvae (Das and Amalraj [1997](#page-12-0)). However, no crayfish-specific strain has been yet developed.

Several studies have revealed that fish predation has an impact on crayfish populations. Eels, burbot, perch, and pike are well-known predators of crayfish (Westman [1991](#page-15-0)). Eels introduced into the Rumensee in Switzerland have reduced an expanding *P. clarkii* population to less than 10% within 3 years, whereas pikes, introduced at the same time, had no obvious effect (Frutiger and Müller [2002\)](#page-12-0). In the Lower Guadalquivir Basin (Spain), before the introduction of P. clarkii, eels mostly preyed upon fish species (mosquitofish and carp): after the introduction of crayfish, eels switched to a diet mostly composed of P. clarkii reaching 67% of occurrence in their stomachs (Montes et al. [1993](#page-14-0)). There are however a number of studies showing little correlation between the presence of some fish species, such as largemouth bass and yellow perch, and crayfish abundance (e.g., Hill and Lodge [1994\)](#page-13-0). Other studies even suggested a positive effect on NICS densities by stocking with fish predators, such as brook trout in Canada (Gowing and Momot [1979\)](#page-13-0) or brown and rainbow trout, perch and carp in England (Holdich and Domaniewski [1995](#page-13-0)). Fish predation may have however some sublethal effects that, in the long-term, may reduce crayfish growth, reproduction, and survival (Holdich et al. [1999\)](#page-13-0).

Few experimental studies have been conducted with the aim of understanding, at least in the short-term, the impact of fish predation on crayfish densities. Interactions between three carnivorous species, i.e., pike (Esox lucius), perch (Perca fluviatilis) and sander (Stizostedion lucioperca), and invasive populations of two NICS (P. leniusculus and P. clarkii) have been analyzed in mesocosms, enclosures, and small ponds in France (Neveu [2001a](#page-14-0)). Pike appeared to be the most efficient predator of both crayfish species, independently of its size, whereas perch and sander were found to prey on significantly smaller crayfish. In mesocosms,  $>16$  cm-long pikes eat crayfish all year round, the maximum size of the ingested crayfish being positively correlated with pike size: pikes from 40 to 50 cm length swallow adult crayfish above 8 cm length. In enclosures and natural ponds, shelter seemed to be ineffective against pikes, the few surviving crayfish showing reduced growth and delayed sexual maturity. Interestingly, when crayfish were isolated by nets, their growth was reduced by only the sight of a perch.

In a similar experiment, interactions between 11 omnivorous resident fish species and two NICS (P. leniusculus and A. leptodactylus) were studied within enclosures and in 100 m<sup>2</sup> ponds (Neveu  $2001b$ ). Young carp (Cyprinus carpio) and 2-summer old tench (Tinca tinca) preyed on YOY (young-of-the-year) crayfish, also inducing decreased growth in the survivors. On the contrary, roach (Rutilus rutilus), rudd (Scardinius erythrocephalus), grass carp (Ctenopharyngodon idellus), crucian carp (Carassius carassius), and mosquitofish (Gambusia affinis holbrooki) had little or no effect on YOY crayfish in enclosures and the former species had even a positive effect in ponds, possibly resulting from a trophic cascade.

Inclusion/exclusion experiments were run by Aquiloni et al. [\(2010](#page-12-0)) to investigate the impact of the European eel Anguilla anguilla on an invasive population of P. clarkii in an Italian wetland. Anguilla anguilla is a good candidate for mitigating the damage produced by P. clarkii in Italy: it has a benthonic feeding habit and is able to tolerate partially deoxygenated waters, properties that match the lifestyle of crayfish and the typical habitats they occupy. Besides, eels are expected to be even more efficient as predators than other fish species because they are able to detect crayfish by odor (Blake and Hart [1995](#page-12-0)) and can enter crayfish burrows. Its introduction seems not to affect the recipient ecosystems since they do not breed in fresh waters but need to migrate to the sea. The study confirmed that A. anguilla preys on P. clarkii but, similarly to other fish species such as smallmouth bass and rock bass (Hein et al. [2006](#page-13-0)), it is gape-size limited, mostly catching small crayfish (Fig. [2\)](#page-8-0). Indeed, as confirmed by a laboratory experiment (Aquiloni et al. [2010\)](#page-12-0), eels usually avoid larger crayfish, except when they are soft-shelled, and always attack the smaller from behind. However, a limitation in the use of this species is its low consumption rate: A. anguilla was not as voracious as other fish species (e.g.,

<span id="page-8-0"></span>

Fig. 2 Number of crayfish per size class found dead or missing (i.e., preyed by eels) in control (without eel,  $n = 12$ ) and treatment cages (with eel,  $n = 11$ ). The *asterisk* denotes significant differences at  $P < 0.001$  after Mann–Whitney tests. After Aquiloni et al. [\(2010](#page-12-0)), modified

Micropterus salmoides, Rach and Bills [1989](#page-14-0); E. lucius, Elvira et al. [1996\)](#page-12-0), consuming about 1 crayfish every 4 days (ca. 1.3% of the average eel weight) possibly due to its low metabolism (Owen [2001\)](#page-14-0).

Fish predators may also modify the behavior of crayfish, inducing either a reduction of activity or a shift in its peak, and a corresponding increase in the time spent in shelter (Stein and Magnuson [1976;](#page-14-0) Stein [1977;](#page-14-0) Hamrin [1987;](#page-13-0) Blake and Hart [1995](#page-12-0); Aquiloni et al. [2010](#page-12-0)). It was also found that crayfish survival is better the greater the number of hides (Blake and Hart [1993\)](#page-12-0). The reduced activity of crayfish in the presence of a fish predator may translate into its decreased trophic activity followed by an increased mortality of crayfish due to starvation on the one hand and a decreased impact on the most affected components of the community such as macrophytes and snails on the other.

# Biocides

Biocides is a general term (synonym of pesticides) that covers all the chemicals used to control invasive and noxious organisms. Many effective maintenance management projects employ chemicals, alone or in concert with mechanical or physical methods. However, expense for chemicals, especially when used for environmental purposes over large areas, is high. Additionally, the evolution of resistance is frequent: there are no species-specific biocide and concerns arise from the possibility of its bioaccumulation and biomagnification in the food chain. As indicated by Williams ([1997\)](#page-15-0), the use of chemicals may also generate ideological opposition, either creating conflict between regulatory agencies with different and competing mandates or fostering aversion by local people against the intervention (Vander Zanden et al. [2010](#page-14-0)). However, there are some biocides registered today, that, if used properly, have far more limited non-target impacts, and in some instances they may be the only means currently available to stop irreversible damage from an invasion, at least until some other methods will be developed (Simberloff [2009\)](#page-14-0).

Biocides that have been used to control invasive NICS include organophosphate, organochlorine, and pyrethroid insecticides, rotenone, and surfactants. Since no biocides are selective to crayfish, focus has been given to chemicals that are not persistent in the environment, are readily available, and are relatively inexpensive. This research led to the discovery of two methods capable of eradicating crayfish populations from small bodies of water.

The first, some derivatives of natural pyrethrum, such as 'Pyblast' (3.0% pyrethrins plus piperonyl butoxide and alcohol ethoxylate), were found to be the most cost effective and methodologically simplistic method. Natural pyrethrum is the oldest known insecticide; it is produced primarily from the flowers of Chrysanthemum cinerariaefolium and C. cineum, as extract composed of several natural pyrethrins. It is widely used as an organic insecticide on crops, where it can be applied up to harvest. It is used in food handling premises, for control of insects for public hygiene or avoidance of nuisance and as a treatment for headlice. It was first used against crustaceans in 1947 to clear infestations of Asellus aquaticus from public water mains (Hart [1958](#page-13-0)) and is still used in this way (Peay et al. [2006](#page-14-0)). The advantages of natural pyrethrum are: low toxicity to mammals and birds, rapid breakdown in sunlight, the absence of toxic residues, and its harmlessness to plants; it is however toxic to other crustaceans, insects, and fishes (Peay et al. [2006](#page-14-0)). Pyblast was chosen for the attempted eradication of P. leniusculus in the North Esk catchment in Aberdeenshire, Scotland (Peay et al. [2006](#page-14-0)). The intervention consisted of different phases: the inflow/ outflow of water was prevented, fishes were removed, the terrestrial margins were sprayed with Pyblast to prevent any escape over land, the whole waterbody was treated with Pyblast, and the treated water was contained throughout the recovery period to prevent any adverse effects in non-target areas. Biomonitoring was carried out using the freshwater shrimp Gammarus. Crayfish mortality was high, but a single crayfish was seen shortly after initial treatment and retreatment with the pyrethrum was necessary. No crayfish were found in the following summer but some individuals were caught at the pre-treated site. Thus, monitoring is still ongoing before declaring the complete success of the treatment (Esk Rivers and Fisheries Trust [2009](#page-12-0)). The possible use of Pyblast to control an invasive population of P. clarkii has been investigated in an irrigation ditch system in Northern Italy (L. Aquiloni et al., unpublished data); first, crayfish's burrows were sprayed with the biocide and, second, isolated stretches of two canals were treated according to Peay et al.'s ([2006\)](#page-14-0) protocol. Subsequent monitoring showed that only the direct treatment of water significantly reduced crayfish density,

<span id="page-9-0"></span>suggesting that the environment-safe spraying of burrows requires much work to be improved.

The synthetic pyrethroid BETAMAX VET, followed by draining of the ponds, was used to eradicate P. leniusculus and the associated A. astaci in the Dammane area, Telemark county, Norway (Sandodden and Johnsen [2010](#page-14-0)), after the first discovery of the invader. This biocide, highly toxic to aquatic crustaceans (Haya [1989\)](#page-13-0), is a cypermethrinbased pharmaceutical developed for treatment of salmon lice (Lepeophtherius salmonis) infestation of farmed Atlantic salmon (Salmo salar). A double administration of BETAMAX VET was carried out with powerful pumps placed in a boat or on the shore. The compound was dispersed both on the surface and along the bottom of the ponds, and the ponds were subsequently drained. During and after the second treatment and draining of the ponds, no signal crayfish were discovered. A post-treatment surveillance program is ongoing to verify the success of the intervention.

Alternative approaches include the use of ammonium in the presence of high pH with prior deoxygenation, and organophosphate (e.g., fenthion and methyl parathion) and organochlorine insecticides (e.g., mirex). Cases of their application to control invasive populations of NICS have been discussed by Holdich et al. [\(1999](#page-13-0)). Among the others, Laurent's [\(1995](#page-13-0)) study is worth of being mentioned here. The author tested various organophosphate insecticides on O. limosus from Lake Geneva in France and found that Baytex PM 40 (active ingredient fenthion) was effective at low concentrations. Laboratory studies showed a 24-h  $LC_{50}$ of 46  $\mu$ g L<sup>-1</sup> and 48-h LC<sub>50</sub> of 12  $\mu$ g L<sup>-1</sup>. Total mortality was achieved after 24 h with concentrations of 90–100  $\mu$ g L<sup>-1</sup> and after 48 h with 50  $\mu$ g L<sup>-1</sup>. These levels are much less than those required to kill finfish. The author also found that the toxicity of the biocide lasted several weeks. Field trials were effective at levels as low as 60  $\mu$ g L<sup>-1</sup> (total mortality was achieved after 87 h). Fishes, frogs, mammals, many species of Rotifera, and mollusks were not affected but insects and other crustaceans were killed with the exception of Copepoda. The relatively long time needed for total mortality of crayfish is an obvious limitation of the method. Additionally, studies on the fenthion residue in the food web are lacking and comparison with the results of other studies are difficult to be made due to the large number of commercial formulations of Baytex.

Surfactants have been used to control crayfish activity by inhibiting oxygen consumption through morphological and physiological changes on the surface of the gills (Cabral et al. [1997](#page-12-0); Fonseca et al. [1997](#page-12-0)), but their application showed to have a limited effect to eradicate crayfish populations. The efficacy of another biocide, Ivermectin (a synthetic derivative of abamectin, a natural fermentation product of the actinomycete Streptomyces avermitilis), has never been investigated in the case of invasive populations of NICS (Holdich et al. [1999\)](#page-13-0). Rotenone (a toxin associated with leguminous plants, e.g., Derris, which acts as a vaso-costrictor narrowing the blood vessels in the fish gills and thus preventing oxygen uptake) might be acceptable for crayfish eradication. It is toxic to fishes and amphibians at levels lower than those needed to kill crustaceans, so these taxa would have to be removed before its use. Rotenone is widely used as a piscicide in fisheries management, but it has rarely been tested on crayfish (Bills and Marking [1988\)](#page-12-0). Holdich et al. [\(1999](#page-13-0)) reported the results of experiments conducted on A. leptodactylus and P. leniusculus showing the relative tolerance of these species to rotenone, with the former species even surviving levels of  $100 \text{ mg } L^{-1}$  for 24 h. Because of the higher tolerance of crayfish than fish, considerable cost would be involved in applying sufficient levels of rotenone to eradicate them.

Finally, a pesticide that has shown to induce molting of berried American lobsters and thus the abortion of their broods is emamectin benzoate (a second-generation avermectin) (Waddy et al. [2002\)](#page-14-0). However, no experimental trials using this pesticide has been conducted until now to evaluate its potential efficacy in invasive crayfish control, as well as its possible impacts on non-target aquatic arthropods (Freeman et al. [2010\)](#page-12-0).

# Autocidal methods

Autocidal methods include the sterile male release technique (SMRT) and the use of sex pheromones. SMRT is based on capturing or rearing, sterilizing, and releasing large numbers of males into the wild to mate females, which will then produce non-viable eggs. It has been successful in the control of some insect pests (e.g., Knipling [1955](#page-13-0); Curtis [1985\)](#page-12-0) and aquatic vertebrates, such as sea lamprey Petromyzon marinus (Twohey et al. [2003\)](#page-14-0). The potential use of SMRT for the management of invasive crayfish has been recently tested in the laboratory (Aquiloni et al. [2009a](#page-12-0)) and in the field (L. Aquiloni et al., unpublished data). This technique, although initially expensive, causes no environmental contamination or nontarget impacts. It is species-specific and offers the additional advantage that, at low density, sterile specimens may seek and mate with the remaining fertile individuals. The high meiotic rate in male gonads makes them particularly radiosensitive, so irradiation can kill cells or inhibit their growth, eventually leading to the partial or total sterility of the treated subjects (Aquiloni et al. [2009a](#page-12-0)). Risks of the treatment include a reduced competitiveness of males and thus their inability to mate in the presence of wild males, a decreased lifespan, and an affected female choice (Lance et al. [2000;](#page-13-0) Lux et al. [2002](#page-14-0)).

Information on the use of ionizing irradiation in decapods comes from studies aimed at preventing unlicensed breeding of female Palaemonetes pugio (Rees [1962](#page-14-0)), Macrobrachium rosenbergii (Lee [2000\)](#page-13-0), and male Penaeus japonicus (Sellars and Preston [2005\)](#page-14-0). Testing revealed that an irradiation dose of 20 Gy apparently did not alter either the survival or mating ability of P. clarkii males but significantly affected their reproductive success by reducing (by 43%) the number of hatchlings. The damage recorded in the tested individuals (i.e., decreased gonado-somatic index and shortened seminiferous tubules) and in their tissue (i.e., increased number of pyknosis) increased with time. Except for the number of pyknosis, the damage lasted for at least 1 year, likely affecting the subsequent reproductive season (Fig. 3). Taken together, these data suggest that the release of sufficient numbers of irradiated males can in theory decrease the size of the invasive population and that their reduced fertility might persist for more than 1 year (Aquiloni et al. [2009a\)](#page-12-0). The provisional results of a field experiment showed that the release of 287 irradiated males into two 300-m long isolated experimental stretches



Fig. 3 Gonadosomatic index (GSI) (a), maximum length of seminiferous tubules (b), and number of pyknosis (c) compared between  $C$  (control) and  $T$  (irradiated) males after different days from the treatment. Sample sizes were 21 in a, 25 in b, and 10 in c for both C and T males. One and two asterisks denote significant differences at  $P<0.05$ , and  $P<0.001$ , respectively, after the application of onetailed Student's t tests for independent data. After Aquiloni et al. ([2009a\)](#page-12-0), modified

of two canals led to a significant reduction of recruitment if compared to the control, i.e., two 300-m long isolated stretches of the same canals into which the same number of untreated, but similarly manipulated crayfish had been released (L. Aquiloni et al., unpublished data). It should be noted, however, that the about 40% sterility obtained with an irradiation dose of 20 Gy is relatively low when compared to the results  $(>90\%)$  achieved with some insect species (Bakri et al. [2005](#page-12-0)). Thus, it is unlikely that this rate of sterility will lead P. clarkii's density below the ''Allee threshold'' (see above), where dispensatory densitydependent processes may accelerate further population decline and cause the eventual extirpation of the invader (Aquiloni et al. [2009a](#page-12-0)).

Sex pheromones are widely used to control insect pests (El-Sayed et al. [2006](#page-12-0)). The release of large quantities of female sex pheromones in an area can confuse the males and prevents them from finding mates, as well as pheromones may work as attractants during the mating season. Once males are removed from the population, less mating might take place and a quick reduction in the size of the population is achieved. This procedure is environmentally sound because sex attractants are in most cases speciesspecific. An apparent limitation is that it can be applied not all year round but during the breeding season only. Crustacean decapods use similar sex pheromones as insects as shown in several species (Callinectes sapidus: Glesson [1980](#page-13-0); Carcinus maenas: Hardege et al. [2002](#page-13-0); Erimacrus isenbeckii: Asai et al. [2000](#page-12-0); Homarus americanus: McLeese [1970;](#page-14-0) Dunham [1979](#page-12-0); Cowan [1991;](#page-12-0) Orconectes virilis: Hazlett [1985](#page-13-0); P. leniusculus: Stebbing et al. [2003](#page-14-0); P. clarkii: Ameyaw-Akumfi and Hazlett [1975;](#page-12-0) Bechler et al. [1988](#page-12-0); Dunham and Oh [1992\)](#page-12-0). Therefore, in theory sex pheromones can be used for the control of decapod pests. A limitation in this effort is that, up to now, the attempts to identify the molecular structure of pheromones in decapods have had a scarce or no success, so the ongoing studies should rely on the natural sources of the putative sex pheromones (Aquiloni and Gherardi [2010](#page-12-0)). A recent advance in the purification of P. leniusculus' pheromones has been reached with the successful development of reliable bioassays (Berry and Breithaupt [2008\)](#page-12-0).

In the UK, Stebbing et al. [\(2004](#page-14-0)) used standard traps baited with gel absorbed with water that had been preliminarily conditioned by mature P. leniusculus females. This study, however, was not able to prove the efficacy of the method; control traps baited with food attracted a similar number of crayfish as the traps baited with sex pheromones. Another field study was conducted in an Italian wetland invaded by P. clarkii (Aquiloni and Gherardi [2010](#page-12-0)). In this case, standard traps had been baited with live sexually receptive individuals, either males or females, and the number, sex and size of the obtained catches were

compared with empty traps and with traps baited with food. The results of this study were contradictory. On the one hand, the traps containing receptive females attracted more males than females. This confirms that crayfish females release sex pheromones and suggests that their putative sex pheromones orient the males to the female location: in fact, as shown in laboratory studies, males rely on chemicals to recognize the other sex, whereas sex recognition by females requires both chemical and visual stimuli emitted together by a potential mate (Aquiloni et al. [2009b](#page-12-0)). A second interesting result was that the crayfish attracted by receptive individuals had a smaller body size than those captured using food as bait. Since in this species body size is related to age (Huner [2002](#page-13-0)), the ability to attract young individuals with more reproductive seasons ahead might be an advantage. On the other hand, the efficacy of the method is low mainly due to three limitations. The first is that sex pheromones attract relatively fewer crayfish than food. Indeed, confinement in traps might cause stress on the senders with the consequent reduced emission of pheromones (Hazlett [1999](#page-13-0)). Purification and concentration of the molecules involved in sexual communication will certainly improve the efficacy of the method but, as said above, the chemical nature of sex pheromones in crayfish is still unknown. Second, females do not respond to the putative male sex pheromones, so only a part of the population can be affected by catches. Third, the removal of mature crayfish may not necessarily induce an effective decrease in the invasive populations. As explained above, removing large males might reduce the pressure on the juveniles allowing them to grow and might lead to a population of individuals whose growth is stunted due to competition for resources (Holdich et al. [1999](#page-13-0)). Besides, due to feedback mechanisms, female crayfish might respond to reduced numbers in the population by producing more eggs and reaching maturity earlier (Holdich et al. [1999\)](#page-13-0).

Notwithstanding the above-listed limitations, we are confident that sex pheromones, if purified and concentrated, might be adopted at least in relatively small and confined areas as a means of early detection of new NICS invasions. Their adoption for the control of established populations, on the contrary, should be complemented by the simultaneous use of other methods (trapping, predators, and SMRT).

Finally, the use of ecdysteroids, hormones that regulate both molt and reproduction in crustacean decapods, was suggested to be a tool to control NICS (Delbecque et al. [2010\)](#page-12-0). The authors found that injections of these hormones in laboratory individuals of P. clarkii induce similar sequences of events as those observed in naturally molting animals and change the neuromodulation by serotonin, which controls aggression in crustacean decapods. However, ecdysteroids are not species-specific and the

technique to apply them to wild crayfish is neither easy nor cost-effective.

# **Conclusions**

This review has shown that mitigation and remediation options for invasive NICS have been scarcely explored, and have so far met with limited success. Few studies have reported the results of long-term control of invasive populations. An exception is the case study of O. rusticus in Sparkling Lake (northern WI, USA). In this lake, intensive trapping on adult crayfish and restriction of harvesting fish predators (smallmouth bass Micropterus dolomieu and rock bass Ambloplites rupestris) were used from 2001 to 2005 (Hein et al. [2006](#page-13-0), [2007](#page-13-0)), leading to the removal of a substantial portion of the invasive population of O. rusticus. Trapping removed larger crayfish, whereas fish predation caused a decline in population growth rate (Hein et al. [2006](#page-13-0)), so that catches decreased by 95% from 11 crayfish per trap per day in 2002 to 0.5 crayfish in 2005. Overall, five summers of intensive trapping and fisheries management practices led to the removal of 88,602 crayfish, corresponding to a biomass of 1,193 kg, and to a steady recovery of the native community (Hein et al. [2006](#page-13-0), [2007](#page-13-0)). However, this large effort was not sufficient to extirpate the population.

Unfortunately, no universal silver bullet exists: NICS are so diversified and have populated so many and various habitat types that no single strategy or universal solution is likely to be attainable (Freeman et al. [2010](#page-12-0)). Integrated pest management (IPM) using a range of control and containment techniques to suit specific sites would probably yield the best results. For instance, based on Hein et al.'s [\(2007](#page-13-0)) experience, the simultaneous recourse to the introduction of indigenous predators and intensive removal of crayfish, followed by drawdown and pyrethrum treatment, might work, at least in isolated waterbodies.

In spite of the general failure of any intervention attempted against NICS, an intensification of scientific research in bioinvasions and an increased awareness by the public of their negative impact (Gherardi [2011](#page-13-0)) are expected to catalyze the development of novel approaches for the mitigation of the damage inflicted by invasive populations of NICS and thus for the long-term conservation of native freshwater communities. No doubt, science and education will make the successful management of invasive NICS not just a hope but a reality (Gherardi [2007b](#page-13-0)).

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