Research review paper

The insulin-like androgenic gland hormone in crustaceans: From a single gene silencing to a wide array of sexual manipulation-based biotechnologies

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Abstract

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Due to the over-harvesting and deterioration of wild populations, the ever-growing crustacean market is increasingly reliant on aquaculture, driving the need for better management techniques. Since most cultured crustacean species exhibit sexually dimorphic growth patterns, the culture of monosex populations (either all-male or all-female) is a preferred approach for gaining higher yields, with the ecological benefit of reducing the risk of invasion by the cultured species. Sexual manipulations may also render sustainable solutions to the environmental problems caused by the presence of invasive crustacean species with detrimental impacts ranging from aggressive competition with native species for food and shelter, to affecting aquaculture facilities and harvests and causing structural damage to river banks. Recent discoveries of androgenic gland (AG)-specific insulin-like peptides (IAGs) in crustaceans and the ability to manipulate them and their encoding transcripts (IAGs) have raised the possibility of sexually manipulating crustacean populations. Sexual manipulation is already a part of sustainable solutions in fish aquaculture and in the bio-control of insect pest species, and attempts are also being made to implement it with crustaceans. As recently exemplified in a commercially important prawn species, IAG silencing, a temporal, non-genetically modifying and non-transmissible intervention, has enabled the production of non-breeding all-male monosex populations that are the progeny of sexually reversed males (‘neo-females’). IAG manipulations-based biotechnologies therefore have the potential to radically transform the entire industry. We review here how this proof of concept could be broadened to meet both aquacultural and environmental needs. We include the major cultured decapod crustacean groups and suggest a sustainable solution for the management of invasive and pest crustacean species. We also review the key considerations for devising a biotechnological approach that specifically tailors the molecular technological abilities to the management of each target group.

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Abbreviations: AG, androgenic gland; Cq-IAG, Cherax quadricarinatus IAG; dsRNA, double-stranded RNA; GMO, genetically modified organism; IAGs, insulin-like AG-specific factors; Mr-IAG, Macrobrachium rosenbergii IAG; RNAi, RNA interference.
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1. Preface

Crustaceans comprise a large (~50,000 species) and diverse sub-group of arthropods. Present in almost every habitat, they are, on the one hand, economically important as target aquaculture species, but on the other hand, they are also agricultural pests, invasive, and pathogenic carriers. Rampant crustacean over-harvesting and the deterioration of wild stocks has left the crustacean trade increasingly reliant on aquaculture which had an estimated market value of more than US$18 billion in 2009 (FAO, 2009). The crustacean aquaculture market relies primarily on a relatively small number of species (~40 species accounted for more than 90% of the market value in 2009), most of which are sexually dimorphic decapods. In contrast, the estimated number of invasive crustacean species is much higher with some studies reporting impacts by several hundred species (Gallil et al., 2011). In the crayfish sub-group alone, 24 invasive species have been documented, some of which inflict severe pressure on the populations of the existing natural crayfish along with a negative impact on aquaculture facilities and harvests (Gherardi et al., 2011). Solutions for enhancing the profitability of crustacean aquaculture and for management of invasive/pest crustacean species may benefit from our better understanding of the sexual differentiation process in crustaceans. With these respects, monosex non breeding population culture was raised as a highly beneficial approach. Proof of concept was recently shown with the complete and functional sex reversal through the manipulation of a male differentiating organ and, even more recently, manipulation of the hormone produced by this organ and alteration of the population sex ratio. Here we suggest broadening this approach to include major decapod crustacean groups to develop biotechnologies that could be tailored specifically to address each species and the requirements of the industry and/or ecological and environmental issues.

2. From the androgenic gland to a biotechnological proof of concept

While sexual manipulation is currently widely practiced in fish aquaculture, on-going efforts are being made to implement this approach with crustaceans. In fish, monosex culture is achieved through sex reversal mediated by steroid manipulations (Smith et al., 2009). Unlike vertebrates, sexual differentiation in crustaceans is regulated uniquely by a non-stereoidogenic male-specific androgenic gland (AG), which obstructed sporadic attempts made to induce a sexual shift through steroid administration (Ohs et al., 2006). The AG was detected in all studied malacostracan crustacean species, and its key role in male sexual differentiation was exemplified in an amphipod, an isopod, and in several decapod species, marking it as a viable target for sexual manipulation-based biotechnologies. In recent years, AG-specific insulin-like peptide (IAG)-encoding transcripts (IAGs) were identified in many crustacean species, of which several are commercially important. IAG silencing in such a decapod species proved useful in obtaining a full and functional sex reversal, leading to the production of monosex populations.

2.1. Milestones in the discovery of the androgenic gland and its hormone

The crustacean male-specific AG was first observed in the blue swimming crab Callinectes sapidus (Cronin, 1947). Later on, this gland was shown in an amphipod species to be the key regulator of male sexual differentiation (Charniaux-Cotton, 1954), and was thus termed the androgenic gland (Charniaux-Cotton, 1955). Since then, a series of studies have proved that AG implantation masculinize females and its removal feminizes males in an amphipod species (Charniaux-Cotton, 1957, 1962), an isopod species (Suzuki and Yamasaki, 1991, 1997; Suzuki et al., 1990), and in several decapod species (Abdu et al., 2002; Affalato et al., 2006; Barkli et al., 2003, 2006; Karpus et al., 2003; Khalalla et al., 1999, 2001; Lee et al., 1993; Malecha et al., 1992; Manor et al., 2004; Nagamine and Knight, 1987; Nagamine et al., 1980a, b; Sagi and Cohen, 1990; Sagi et al., 1990, 1999, 2002; Taketomi and Nishikawa, 1996).

The chemical nature of the AG hormone remained an enigma for many years. Several ultra-structural studies of the AG suggested that the AG hormone is proteinaceous (King, 1964; Okumura and Hara, 2004; Taketomi, 1986). This hypothesis was strengthened by the partial purification of an active AG-borne protein extract (Hasegawa et al., 1987; Juchault et al., 1978; Kataoka et al., 1975; Okuno et al., 1997) and finally, by the full sequence of a glycosylated protein (Martin et al., 1999) in parallel to its characterization and cDNA cloning, which was verified to be AG-specific (Okuno et al., 1999). The encoded protein was shown to comprise a linear preprohormone, organized according to the insulin-like superfamily of peptides, a signal peptide (cleaved off to give rise to the prohormone) at the N-terminus, followed by a B chain, then a connecting peptide (C peptide), and finally an A chain (Fig. 1A). As is common to all insulin-like peptides, the mature hormone (the B and A chains after cleavage of the signal and C peptides) has three disulfide bridges: two inter-chain bridges, and one intra-chain bridge (within the A chain; Fig. 1B). These three disulfide bridges stabilize the three-dimensional structure common to members of the insulin-like superfamily of peptides (Fig. 1C). The first decapod insulin-like AG-specific peptide encoding cDNA (IAG) was discovered almost a decade later (Manor et al., 2007) followed by many others in a relatively short period. To date, the IAGs of nine additional decapod species were cloned (six of which are reviewed in detail by Ventura et al. (2011b)), representing the commercially and ecologically most important decapod groups: crabs, crayfish, prawns, and shrimps. All of these sequences encode proteins that share in common linear structure and the identity of the conserved cysteine residues, which are the keystone of the insulin-like superfamily of peptides (Fig. 1A and B, respectively), and that enable the three-dimensional folding (Fig. 1C). The multiple sequence alignment of the ten known decapod mature IAGs (B and A chains) are given in Fig. 1D.

The recent utilization of RNA interference (RNAi) has revolutionized the functional assays of newly discovered genes (Dorsett and Tuschl, 2004) as applied in a number of decapod species (Lugo et al., 2006; Shechter et al., 2008). Silencing IAG expression in two decapod species led to demasculinization accompanied by a shift toward feminalness in one of the species, thus proving the key role of the IAG in male sexual differentiation for the first time in decapods (Rosen et al., 2010; Ventura et al., 2009). In another recent IAG silencing experiment, full and functional sex reversal was achieved (Ventura et al., 2012), as detailed below.

2.2. Biotechnological proof of concept: an all-male prawn population – field confirmation

In the commercially important freshwater prawn M. rosenbergii, males grow larger and faster than females (Sagi et al., 1986). A case study in India—a major producer of this species—has shown that segregation of males and the removal of the less-profitable females of the species increases the grower’s income by ~60% (Nair et al., 2006). Although AG manipulation dates back half a century (Charniaux-Cotton, 1962), a biotechnological approach for the generation of all-male populations was only recently devised, and it involves the microsurgical removal of the AG from juvenile males. When successful, such removal leads to the full and functional sex reversal of males into offspring-producing neo-females (Affalato et al., 2006). Since M. rosenbergii males are the homogametic sex, bearing two homologous sex chromosomes (ZZ) as in several other studied crayfish and shrimp species, sex-reversed males produce 100% male progeny, as first reported by Sagi and Cohen (1990). Fraught with difficulties, however, this biotechnology is hampered by the low success rate of the microsurgery (~1.3%) and by lengthy (~up to ten months) and labor-intensive progeny testing (Affalato et al., 2006). These hurdles led to the exploration for novel technologies to meet market demands.

Manipulation of the AG hormone is one such route being explored. IAG (Mr-IAG) silencing in M. rosenbergii arrested spermatogenesis and temporarily inhibited the development of a masculine appendage on a
AG insulin-like peptides (IAGs): the basic linear structure and a predicted three-dimensional model. All IAGs found thus far (in 10 decapod and 3 isopod species) have characteristics of the insulin-like super family of peptides, including the linear structure of the preprohormone composed of a signal peptide, followed by a B chain, a C peptide, and an A chain (A). The signal and C peptides may be cleaved out (dashed boxes in A) to give rise to the mature hormone, composed of the B chain interlinked by two disulfide bridges to the A chain where another intrachain disulfide bridge occurs (B). These predicted disulfide bridges enable the three-dimensional folding of the predicted mature hormone, as shown here for the predicted folding of Cq-IAG (C; Modified from Manor et al. (2007)). Multiple sequence alignment of ten decapod mature IAGs is given (D; using CLUSTAL W algorithm, manually corrected). The most conserved feature is the predicted backbone consisting of six cysteine residues (highlighted in orange), which suggests disulfide bridges (orange lines). Also, highlighted in orange are two cysteine residues in prawns that may form a third inter-chain disulfide bridge. Each decapod group is represented with a sketch (left, up to bottom): the crab Charybdis feriatus, the red-claw crayfish C. quadricarinatus, the giant freshwater prawn M. rosenbergii, and the white shrimp Litopenaeus vannamei. Species names are represented by the three initial letters of each as follows: Cal. sap., Callinectes sapidus; Por. pel., Portunus pelagicus; Che. des., Cherax destructor; Che. qua., Cherax quadricarinatus; Mac. ros., Macrobrachium rosenbergii; Mac. lar, Macrobrachium lar; Pal. pac., Paleomon pacificus; Pal. pau., Paleomon paucidens; Pen. mon., Penaeus monodon; Mar. jap., Marsupenaeus japonicus.

regenerated swimming leg in mature male prawn individuals (Ventura et al., 2009). When performed at early developmental stages, however, prior to the appearance of male sexual characteristics, Mr-IAG silencing caused the full and functional sex reversal of males into neo-females. Successfully mated with untreated males, the neo-females produced all-male progeny (Fig. 2) (Ventura et al., 2012). An all-male progeny from such a neo-female was grown in parallel with a normal mixed population, and the growth performances of the two groups were compared. The size distribution of individuals in the mixed population was similar to that of the male individuals in the all-male population, which had twice the number of male individuals (unpublished results). This marked the first field study of a monosex population derived from a single gene silencing-induced sex reversal. Because it does not involve genetic modification of the organism (GMO) under investigation, thereby bypassing the regulatory pipeline required of genetically-modified crops (Stein and Rodriguez-Cerezo, 2010), this proof of concept is a boon for monosex biotechnology. As the intervention is temporal, it is not transmissible to next generations. Indeed, this approach may be of tremendous applied merit in the aquaculture industry. Moreover, it could also form part of a sustainable solution for the management of invasive and/or pest crustacean species, where the production of non-reproducing male or female populations is sought.

3. Tailoring the biotechnology to each of the major decapod groups

The case of Mr-IAG silencing in M. rosenbergii revealed the constraints that exist and the issues that must be considered when attempting to tailor a specific biotechnology to each of the known decapod groups. Defining characteristics of a species’ life cycle, reproductive strategy, intervention timing, mode of inheritance, availability of sex markers and the desired outcome should all be thoroughly investigated to customize IAG manipulation-based biotechnology to that species. In the case of M. rosenbergii, Mr-IAG silencing resulted in full and functional sex reversal only when the intervention occurred in the early post-larval stages before the appearance of external sexual characteristics (Ventura et al., 2012), a constraint that required the prior identification of molecular sex markers (Ventura et al., 2011a). Since the desired outcome in M. rosenbergii is to obtain all-male populations, and the

Fig. 2. Mr-IAG-silencing induces full-functional sex reversal of males into all-male progeny-producing ‘neo-females’. A. Ventral view of a Mr-IAG silenced neo-female incubating eggs in its brood-chamber (arrow, with a few eggs magnified). B. Genomic validation of the neo-female and broodstock maleness; PCR products generated from intact male and intact female control individuals and from the neo-female and individuals from its progeny (upper panel) as templates, as well as a control female and individuals from its progeny (bottom panel), separated on an agarose gel. n.c. – negative control, p.c. – positive control, f.s. – female-specific marker. Modified from Ventura et al. (2012).
mode of inheritance involves homogametic males, Mr-IAG silencing (to obtain all-male progeny-producing neo-females) is the preferred sexual manipulation.

Based on the above, IAG manipulation could drive improved crustacean production. Moreover, it could form part of a sustainable solution to the problems associated with invasive and/or pest crustacean species through the production of non breeding monosex cultures. This general strategy can be customized according to sex chromosome composition and preferred sex to cover all the major groups of industrially grown, invasive, pest, and/or even pathogen carrying crustacean species. IAG expression can either be knocked down in males to obtain neo-females, as was done in *M. rosenbergii*, or administered to females to obtain neo-males. Although IAG administration was not yet shown to induce full and functional sex-reversal, administration of a recombinant hormone to the isopod *Armadilidium vulgare* showed some masculinization (Okuno et al., 2002), and AG implants were shown to induce a full and functional sex reversal in a decapod species (Malecha et al., 1992).

Four biotechnological routes to achieve monosex populations have been suggested. In species where the males are homogametic (i.e., bear two homologous sex chromosomes, ZZ), and where males are the preferred sex, as in crayfish (Parnes et al., 2003) and prawns (Malecha et al., 1992; Sagi and Cohen, 1990), IAG deprivation from males to induce neo-females will enable the production of 100% male populations, as was achieved by Ventura et al. (2012) (Fig. 3, top left). For species with homogametic males in which females are the preferred sex, as in shrimps (Gopal et al., 2010) or prawns under certain management strategies (Malecha, in press), IAG administration to females, designed to generate neo-males, will eventually enable the production of 100% female populations (Fig. 3, bottom left), as supported by findings in crayfish showing that WW females were viable and able to reproduce (Parnes et al., 2003).

The same set of IAG interventions is also feasible in heterogametic males, as is the case with crabs (Triño et al., 1999) (Fig. 3, right). However, since YY males were not shown to be viable, an all-female population production scheme is simpler to achieve than the all-male strategy. Moreover, since all-female production was shown to be economically beneficial in crabs (Triño et al., 1999), the culture of all-female populations should be the preferred approach for crabs when addressing aquaculture management issues (Fig. 3, bottom right). Since little is known regarding lobster sex chromosome composition, and lobster IAGs have yet to be identified, devising a proper monosex scheme for lobsters will be possible only after further study of this commercially valuable group.

4. From need to application

Crustaceans comprise a definite number of cultured species with a steady increase in culture volumes in recent years, from more than 703,000 tons valued at over US$3 billion in 2000 to more than 4,127,000 tons with a value exceeding US$18 billion in 2009 (FAO, 2009). This increase dictates the need for better management to support this growing field and enhance yields. Since most cultured crustacean species exhibit sexually dimorphic growth patterns, thus conferring on monosex population techniques a significant advantage, harnessing solutions such as IAG manipulation-based biotechnology has the potential to radically transform the entire aquaculture industry. Monosex biotechnologies may also contribute to the creation of relatively simple environmental solutions to the problems associated with invasive species, ranging from preventing their dissemination to the active eradication of those that are already present by releasing sterile monosex individuals.

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**Fig. 3.** Possible production schemes for IAG manipulation-induced monosex populations of the most prominent decapod groups. The IAG can be manipulated in species where males are homogametic (ZZ, left) or heterogametic (XY, right). For each scheme, representative groups of species are denoted. The IAG can be either removed from males to obtain neo-females (top) or administered to females to obtain neo-males (bottom). Sketches represent the giant freshwater prawn *M. rosenbergii* (top left), the red-claw crayfish *C. quadricarinatus* (top, second left), the crab *Charybdis feriatus* (right), and the white shrimp *Litopenaeus vannamei* (bottom left).
4.1. Crustacean aquaculture

As the global crustacean industry becomes increasingly reliant on aquaculture rather than on wild catch (FAO, 2009), new avenues for improving culture techniques must be explored. Table 1 summarizes the increase in total yields and incomes of the different cultured decapod groups during the years 2000 and 2009, with the fold-change between these years, which emphasizes the immense increase in decapod aquaculture volumes (data from FAO, 2009). Moreover, the most plausible route for monosex population biotechnology is suggested for each of the above groups based on the desired sex and mode of inheritance (Fig. 3). In all the above five major farmed crustacean groups, monosex population culture could be a preferred technology. In crabs, either all-male or all-female populations are favored over mixed populations (Triño et al., 1999), while in crayfish and prawns, males grow larger and faster and as such, all-male population cultures are desired (Nair et al., 2006; Sagi et al., 1997). In contrast, female shrimp grow larger and faster, making all-female population cultures preferable (Gopal et al., 2010). Given the economic impact of obtaining single-sex enriched crustacean cultures, the idea of generating monosex cultures based on genetic manipulation is an attractive one.

Monosex crustacean culturing offers several advantages. For instance, the fresh marketing season would be prolonged due to faster growth rates as a gender-tailored interface could be implemented (Sagi et al., 1986). Moreover, the absence of any non-planned reproduction ensures that energy is not diverted from growth and that there are no siblings competing for resources. Furthermore, selective breeding can transpire in physically separated surroundings, thereby avoiding compromise by parents or siblings drawn from the growout ponds. Monosex aquaculture, moreover, is ecologically advantageous: because no reproduction occurs in the ponds, the environmental risk of introduced aquatic species arising from juveniles invading natural water bodies is reduced. Even should invasion occur, one gender would not be able to reproduce and establish an exotic species (Beardmore et al., 2001).

Currently, IAGs are known in representatives of all the major decapod groups excluding lobsters. The enhanced yield achievable through monosex population culture has been shown in case studies in a crab species (Triño et al., 1999), in a crayfish species (Curtis and Jones, 1995; Manor et al., 2002), in a prawn species (Sagi et al., 1986)—where another case study reported about a 60% increase in net income to the growers when growing hand segregated all-male populations and discarding the females (Nair et al., 2006)—and in a shrimp species where females were shown to grow faster and reach higher weights (Gopal et al., 2010).

4.2. Environmental impact of invasive crustaceans

Crustacea is among the most abundant groups of species on earth, occupying virtually every ecological niche. As such, it is not surprising that Crustacea is the most successfully introduced phylum in marine systems, accounting for 28% of reports in marine coastal communities in North America and 56% of the faunal species found in ballast tanks in Europe (Ashton et al., 2007). Many crustacean species (estimated at hundreds world-wide (Galil et al., 2011)) have been cause for genuine concern as they are aggressive competitors with native species for food and shelter, they negatively affect aquaculture facilities and harvests, they cause structural damage to river banks, and some even carry hazardous pathogens. In a recent report that summarized the 27 most threatening alien animal species introduced into Europe for aquaculture and related activities (Savini et al., 2010), five decapod species are listed. Table 2 summarizes the number of recorded invasive species from each of the decapod groups described in Fig. 3, their endemic sources and invaded areas, and the reported impacts. The main vectors of dissemination include man-made canals, live trades, intentional introductions for other purposes, and shipping traffic. Indeed, when improperly managed, aquaculture of commercially important decapod species is a two edged sword, with its potential to disseminate invasive species whose negative impact sometimes eclipses the benefits of its aquaculture. We highlight here several such cases from each group (described in Fig. 3 and Table 2), which together indicate the need for stringent controls in the relevant aquaculture areas.

Among the more than 50 reported alien crab species, the most widely spread is the green shore crab Carcinus maenas. This European crab species was introduced globally through the live bait and sea food industries hand in hand with its substantial contribution to the demise of the commercial soft-shell clam fishery in the northeastern USA during the 1940s and 1950s (Grosholz, 2011). This notorious crab species joins a list of a total of nine crab species that are common predators of cultured mollusks (Creswell and McNevin, 2008). Another example of a globally introduced crab is the Chinese mitten crab Eriocheir sinensis, cultured in the Far East as a food delicacy. This species is thought to have been unintentionally introduced to Europe via ballast water discharge of larvae in the early 20th century, and later it appeared in the San Francisco Bay area where its burrowing activity causes siltation of the waterways and bank erosion, thus increasing the risk of flooding. While it requires saline water for breeding, it has been observed as far as 1,000 km from the sea, a fact that demonstrates its unfortunate ability to move up the river systems, and indeed, this species continues to spread throughout Europe and USA (Bentley, 2011).

In the crayfish sub-group of over 600 species, 24 invasive species were reported worldwide (Gherardi, 2011) and are known to threaten the mere existence of native crayfish species in some eco-regions (Holdich et al., 2009). Since crayfish are detritivores, they tend to persist in water bodies. Like crabs they, too, can travel long distances from their place of dispatch, making them highly successful invaders. Since many non-indigenous crayfish species can be easily purchased as ornamental crustaceans, they can also be easily disseminated (Gherardi et al., 2011).

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Table 1
Scale and scope of the increasing aquaculture industry of the sexually dimorphic decapod groups with the potentially advantageous IAG manipulation-based biotechnologies.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of species</th>
<th>Inheritance mode</th>
<th>Preferred monosex route</th>
<th>Quantity (tons) in 2000</th>
<th>Quantity (tons) in 2009</th>
<th>Fold-change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>USD** in 2000</th>
<th>USD** in 2009</th>
<th>Fold-change&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crabs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>XY/XX</td>
<td>Neo-males → all-female</td>
<td>328,005</td>
<td>820,770</td>
<td>2.5</td>
<td>1,500,451</td>
<td>4,764,468</td>
<td>3.2</td>
</tr>
<tr>
<td>crayfish&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
<td>WZ/ZZ</td>
<td>Neo-females → all-male</td>
<td>8,249</td>
<td>526,637</td>
<td>63.8</td>
<td>32,884</td>
<td>2,410,284</td>
<td>73.7</td>
</tr>
<tr>
<td>Lobsters</td>
<td>6</td>
<td>?</td>
<td>?</td>
<td>73</td>
<td>1412</td>
<td>19.3</td>
<td>1,128</td>
<td>14,140</td>
<td>12.5</td>
</tr>
<tr>
<td>Prawns&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4</td>
<td>WZ/ZZ</td>
<td>Neo-females → all-male</td>
<td>217,828</td>
<td>438,818</td>
<td>2.0</td>
<td>701,793</td>
<td>2,203,725</td>
<td>3.1</td>
</tr>
<tr>
<td>Shrimps&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8</td>
<td>WZ/ZZ</td>
<td>Neo-males → all-female</td>
<td>149,477</td>
<td>2,339,384</td>
<td>15.7</td>
<td>810,020</td>
<td>9,302,973</td>
<td>11.5</td>
</tr>
<tr>
<td>Grand totals</td>
<td>35</td>
<td>703,632</td>
<td>4,127,021</td>
<td>5.9</td>
<td>3,046,076</td>
<td>18,695,590</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Fold-change – represents the value in 2009 divided by the value in 2000. <sup>b</sup>USD** – US dollars (values are represented as 1 × 10<sup>6</sup> of the amount). 
<sup>c</sup>A commercial evaluation of mud crab monosex culture indicated all-male culture is more profitable; however, all-female culture is easier to achieve and also more profitable than mixed populations (Triño et al., 1999).
<sup>d</sup>A case study of the red claw crayfish Cherax quadricarinatus showed substantial increase in marketable yields of all-male populations compared with all-female and mixed populations (Curtis and Jones, 1995; Manor et al., 2002).
<sup>e</sup>Macrobrachium rosenbergii were shown to obtain up to twice the yield compared with mixed populations (Sagi et al., 1986) and an increase in the net income of farmers by ~60% when practiced via manual discarding non-profitable females in India (Nair et al., 2006).
<sup>f</sup>A case study in Penaeus monodon showed females grow larger than males (Gopal et al., 2010).
Marketing of monosex cultured populations could thus prevent this line of dissemination, with the added value of enhanced yields. The most extensively studied case of an invasive crayfish species is that of the red swamp crayfish Procambarus clarkii. This species, native to northeastern Mexico and south-central USA, was legally introduced into southern Spain in 1973 from Louisiana (USA) and later illegally introduced throughout Spain, France, Italy, and other countries from 1970–1990. It is known to affect native European crayfish (family Astacidae) because it out-competes them and acts as a vector for the transmission of the crayfish fungus plague, Aphanomyces astaci. It also reduces the value of invaded freshwater habitats by consuming invertebrates and macrophytes and by degrading riverbanks through its burrowing activity. It accumulates heavy metals and toxins produced by Cyanobacteria, such as Microcystis aeruginosa, and can transfer them to its consumers, including humans. It is an intermediate host of trematodes of the genus Paragonimus, which are potential pathogens of humans if undercooked crayfish are consumed. If present in irrigation structures, such as reservoirs, channels, or rice fields, it may cause significant economic losses. This is due to both its burrowing activity, which alters soil hydrology and causes water leakage, and its feeding habit, which causes damage to rice plants (Souty-Grosset et al., 2006). All of these impacts make P. clarkii one of the 100 worst invasive species in Europe and among the worst 27 invasive animal species (Savini et al., 2010).

In the case of shrimp species, the Pacific white shrimp Litopenaeus vannamei is the world’s most widely cultured alien crustacean species, and as such, it poses a dire threat through its competition with indigenous shrimp species and as a vector of viral diseases (Liao and Chien, 2011). While there is little evidence of a genuine impact from this widely cultured, introduced species, the impact of two indigenous burrowing shrimp species Neotrypaea californiensis and Upogebia pugettensis was devastating to the Pacific Northwest USA oyster aquaculture industry. These species reduce the stability of the bottom substrate where oysters are raised and cause them to be covered with sediment and die (Dumbauld et al., 2006). Forty years of using a pesticide to eradicate these fouling species is due to phase-out by 2012 (Creswell and McNevin, 2008), raising the need for a new, viable solution.

5. Epilog

Decapod crustaceans comprise a definite number of aquaculture important species that is on the rise, hand in hand with the augmentation of the detrimental effects of some of these species and many others. Through a single gene silencing, a full and functional sex-reversal has been achieved recently in a decapod (Ventura et al., 2012), enabling the development of species-specific biotechnologies for monosex population culture and the design of solutions to manage invasive and pest decapod species.

Efforts to minimize the impacts of invasive and/or pest crustacean species may benefit from sexual manipulations, as has been successfully realized with other arthropods such as pest insects, where the controlled distribution of monosex sterile specimens led to infertile populations which eventually suppressed the natural population (Nolan et al., 2011; Vilcinskas et al., 2011). As was recently suggested for the eradication of invasive fish species, stocking with high proportions of YY ‘neo-females’ (Trojan Y chromosome) may lead to a disproportionately high percentage of males in the next generations (Gutierrez and Teem, 2006). This strategy could be applicable to invasive crab species where males are heterogametic.

Clearly, the vast behavioral, morphological, anatomical, and physiological changes accompanying sex reversal could not be attributed solely to the action of a single component. Improved molecular technologies may be of great importance in putting all the pieces of the puzzle of crustacean sexual differentiation cascades into place. The discovery of these novel molecular components, combined with the already available knowledge will be applicable for many sexual interventions. For instance, as the silencing of IAG enabled a full and functional sex reversal, silencing a key component of gonad maturation to be identified and characterized will enable sterility induction. Combined, the two approaches could enable the production of monosex-sterile populations, which could provide sustainable solutions for aquaculture and for the improved management of invasive and/or pest crustacean species. This approach may prove beneficial for the aquaculture industry, since monosex-sterile populations might exhibit better growth rates due to energy allocation toward growth rather than to gonad ripening (primarily in all-female populations) and could also defend commercial lines from theft. A precise intervention aimed at the expression of a single molecule could constitute a solution preferable to gonadectomy.

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