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Androgenic hormones in crustacean aquaculture: a review

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Abstract: Increased demands of the seafood market have led to the rapid growth in crustacean aquaculture production in various parts of the world. Therefore, an expansion in the knowledge of the reproductive biology of crustaceans has become more important. Hormones released from the androgenic gland (AG) in crustaceans are liable for both the separation of primary and secondary sexual features and reproductive behavior. AG removal in male crustaceans causes a transformation of the intersex animals towards the females and a degeneration of spermatids and reproductive organs. AG implantation and injection of AG extracts are common approaches for crustacean masculinization. In this review, the importance of AG and its implication in crustacean aquaculture are reiterated, while the discovery, identification, chemical nature, and isolation of insulin-like androgenic gland hormone are also overviewed.

Key words: Decapod, reproduction, gland implantation, feminization, masculinization, shrimp, crab, crayfish

1. Introduction

To date, more than 67,000 crustacean species have been described worldwide (Zhang, 2011). The order Decapoda comprises various groups of crustaceans, consisting of all identified species of crab, crayfish, lobster, and shrimp. Some decapod species are invasive and considered pathogenic vectors, while some decapod species are artificially bred and can be kept domestically in aquariums (Sellars et al., 2010).

Production and rearing of crustaceans has become a rapidly developing industry in recent years (Wickins and Lee, 2002; Harlioğlu and Farhadi, 2017a; Harlioğlu et al., 2017a). For example, of the 19.7 kg per capita global fish consumption, about 1.8 kg is composed of crustaceans (FAO, 2020). Crustaceans accounted for 8.2% of the total fish production in 2018, accounting for 21.7% of the total economic income (FAO, 2020). In addition, it is known that the total crustacean production reached 9.4 million tons (3 million tons from aquaculture) in 2018 and the estimated sales worth of this production is 69.3 billion USD (FAO, 2020).

The successful crustacean aquaculture depends, to a large extent, on controlling their reproduction and sexual development (Nagamine et al., 1980; Manor et al., 2004, 2007; Cui et al., 2005; Ventura et al., 2012; Huang et al., 2014; Zmora and Chung, 2014; Lezer et al., 2015; Harlioğlu, 2016; Harlioğlu et al., 2017b; Harlioğlu et al., 2018, Fu et

al., 2020; Harlioğlu et al., 2020). Therefore, understanding the crustacean endocrinology and reproductive biology is of immense value (Khalaila et al., 2001, 2002; Ventura et al., 2009, 2011; Harlioğlu and Duran, 2010; Ventura and Sagi 2012; Harlioğlu et al., 2013; Farhadi and Harlioğlu, 2018, 2019; Farhadi et al., 2019). The fundamental knowledge of sexual development (i.e. the role of androgenic gland) is importantly necessary to the application of sex manipulation technology, which may become the potential solution to optimize the production of crustaceans (Hidir et al., 2021; Farhadi et al 2021a; Farhadi et al 2021b). In this article, the structure and function of androgenic gland hormone in crustaceans are reviewed.

2. Androgenic gland

Growth, behavior, and sex differentiation of male decapods are controlled by insulin-like androgenic gland (IAG) hormone released by androgenic glands (AGs). The action of AG is regulated by the gonad-inhibitory hormone arising from the X-organ-sinus gland in the eyestalk (Sagi et al., 2002; Sagi and Aflalo, 2005; Phoungpetchara et al., 2010; Sroyraya et al., 2010; Zhang et al., 2017; Shi et al., 2019). However, its absence results in feminization, which usually causes the alteration in vitellogenesis (Sagi et al., 2002). AG was first recognized by Cronin (1947) in the blue crab (*Callinectes sapidus*). It was then found to have a crucial role in gametogenesis and male separation in an

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amphipod species (Charniaux-Cotton, 1954). It is mainly associated with the subterminal part of the sperm canal in many crustacean species. However, Cronin (1947) noticed that the AG can have varying positions in different suborders or species of isopods; for instance, it is detected at the three pairs of testes in *Armadillidium vulgare* and *Porcellio scaber*. Similarly, Puckett (1998) found that the AG is located at the posterior vas deferens in crayfish, *Procambarus viaeviridis* and *Cambarus diogenes*. Charniaux-Cotton (1960) stated that it is often observed

as cords of epithelial cells that bend over each other and are surrounded by connective tissue. This formation of AG was also noticed by Fowler and Leonard (1999) in crab, prawn, and crayfish. The ultrastructural observations also revealed that the AG still remains active following the sexual maturation in males (Okumura et al., 2005) and is of crucial importance in the development of testes and governing of spermatogenesis (Nagamine et al., 1980; Okumura and Hara, 2004). Figures 1 and 2 show the external characteristics of AG.

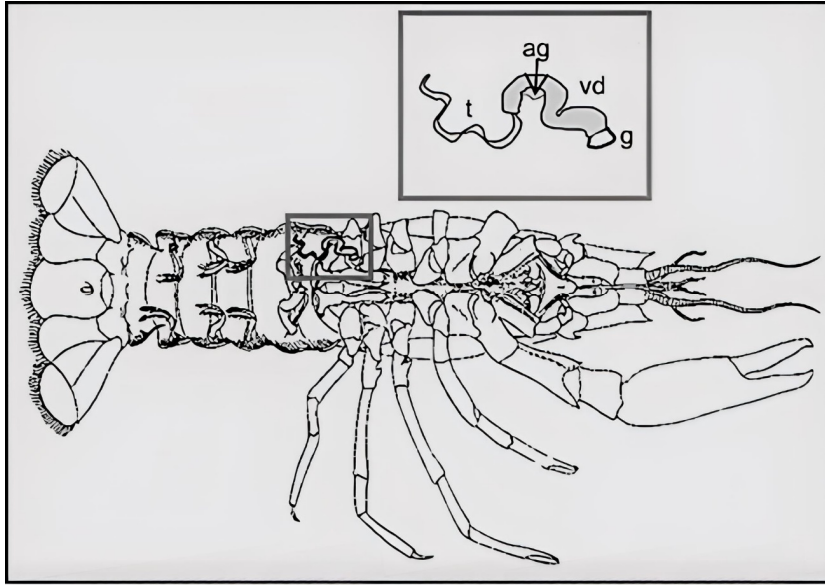


Figure 1. Male crayfish showing location of male reproductive tract accessible via the base of the fifth walking leg. ag = androgenic gland, g = gonopore, t = testes, and vd = vas deferens (adapted from Mead, 2008).

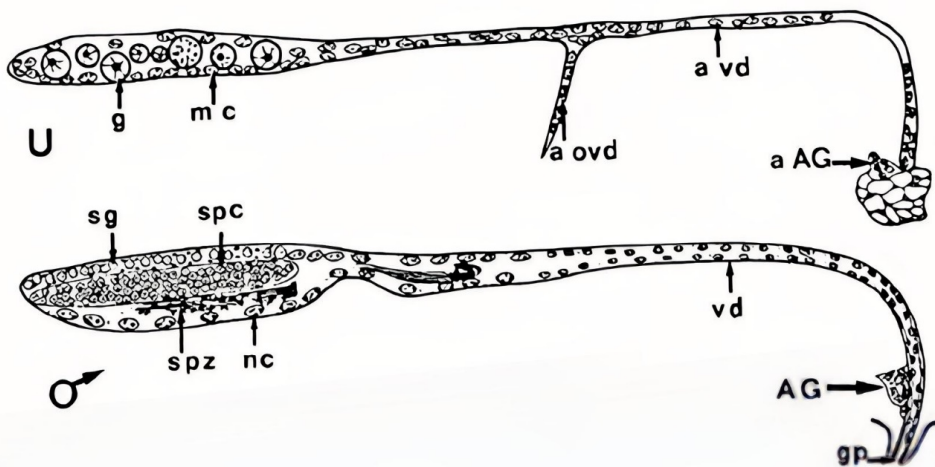


Figure 2. Organogenesis of the genital apparatus in *Orchestia gammarellus*. U, undifferentiated stage; ♂ juvenile male AG, androgenic gland; a AG, anlage of AG; a vd, anlage of vas deferens; g, protogonia; gp, genital papilla; mc, nucleus of mesodermal cell; nc, nucleus of nurse cell; sg, spermatogonia (embedded in stroma of mesodermal cells); spc, spermatocyte; spz, spermatozoa; vd, vas deferens (originally adapted from Charniaux-Cotton and Payen, 1985 and reproduced by Subramoniam, 2016).

3. Androgenic gland hormone

3.1. Chemical structure

Experimental studies have proven that AG is essential for the growth and maturation of the male primary and secondary sex traits in crustaceans. The chemical structure of the insulin-like androgenic gland hormone (IAG) was clarified long after its effective act in sex differentiation was recognized (Subramoniam, 2016). By ultrastructural investigations of AG in the shore crab (*Pachygrapsus crassipes*), King (1964), with ultrastructural investigations of AG in the coastal crab (*Pachygrapsus crassipes*), stated that IAG may be a protein. Moreover, the biochemical investigations in *Carcinus maenas* showed that the IAG is lipoidal in nature (Férézou et al., 1978). It was then refined and identified to be a farnesylacetone. On the other hand, the amino acid structure of AG was proven by amino acid sequencing in the terrestrial isopod *Armadillidium vulgare* (Katakura et al., 1975; Okuno et al., 1997).

Hasegawa et al. (1987) investigated the amino acid compositions of two protein structures exhibiting AG activity and found the molecular weight as 17 kDa. The full sequences of IAG in *A. vulgare* were described by Martin et al. (1999). They additionally found the glycosylation areas in this glycopeptide.

The mature hormone is found in insulin-like peptides (ILP) (Okuno et al., 1999; Martin et al., 1999), with one intrachain in the B chain and four disulfide bonds, two interchain disulfides, and one intrachain in the A chain. This peptide is similar to the insulin-like growth factor/ relaxin family peptides in amino acid sequence, backbone positions, and predicted cleavage patterns (Ventura et al., 2011). Additional observations showed that the chain constitutes three cysteine residues at the identical scene of the IAG in other crustaceans (Table 1).

3.2. Isolation and regulatory mechanism of insulin-like androgenic gland hormone (IAG) gene

It is assumed that the IAG is a gender separation element in decapods. However, the function of the IAG peptide was not yet fully understood (Katayama et al., 2014). IAG was synthesized from the prawn, *Marsupenaeus japonicus* and its role has been evaluated by Katayama et al. (2014). They found that the IAG with an insulin-type disulfide bond, but not a disulfide isomer, showed biological activity and therefore suggested that the natural IAG peptide had an insulin-structure disulfide.

Shi et al. (2019) isolated the cDNA of IAG (PcIAG) from the red swamp crayfish (*Procambarus clarkii*). PcIAG was commonly found in the tissues of males and females. They also detected the PcIAG protein in the nervous and reproductive organs of the mature male crayfish. However, there was a dramatic increase in the amount of PcIAG suddenly after the inoculation of 5 µg/g and 10 µg/g (higher doses) of PcIAG-dsRNA, which can stimulate the maturity and secretion of sperm.

Zhang et al. (2014) identified the cDNA coding the IAG from *Scylla paramamosain*, namely Sp-IAG. The protein, which is predicted to have a molecular organization similar to other IAG factors, has been reported in Decapoda, which encodes a signal peptide, B chain, C peptide, and A chain. Furthermore, it was found that removing the eyestalk causes a high proportion of Sp-IAG; therefore, it was concluded that the Sp-IAG in this species was adversely dominated by the X-organ. Multiple sequence alignment of deduced primary amino acid sequences of decapod IAGs are presented in Figure 3.

Li et al. (2015) duplicated the 5' flanking chain of IAG and the full-length DNA chain of gonad-inhibiting

Table 1. IAG accession numbers of crustaceans, along with the % identity to *A. vulgare* (adapted from Subramoniam, 2016).

Species	Accession no	Systematics	Identity (%)
<i>Armadillidium vulgare</i> (Arv)	Q9U8R2.1	Isopoda; Oniscidea; Armadillidiidae; <i>Armadillidium</i> ; <i>vulgare</i>	100
<i>Porcellio scaber</i> (Pos)	AAO11675.1	Isopoda; Oniscidea; Porcellionidae; <i>Porcellio</i> ; <i>scaber</i>	65
<i>Porcellio dilatatus</i> (Pod)	Q86SA8.1	Isopoda; Oniscidea; Porcellionidae; <i>Porcellio</i> ; <i>dilatatus</i>	64
<i>Cherax quadricarinatus</i> (Cq)	ABH07705.1	Decapoda; Parastacoidea; Parastacidae; <i>Cherax</i> ; <i>destructor</i>	36
<i>Portunus pelagicus</i> (Pp)	ADK46885.1	Decapoda; Brachyura; Portunidae; <i>Portunus</i> ; <i>pelagicus</i>	36
<i>Fenneropenaeus chinensis</i>	AFU60546.1	Decapoda; Dendrobranchiata; Penaeoidea; Penaeidae; <i>Fenneropenaeus</i> ; <i>chinensis</i>	31
<i>Litopenaeus vannamei</i> (Lv)	AIR09497.1	Decapoda; Dendrobranchiata; Penaeoidea; Penaeidae; <i>Litopenaeus</i> ; <i>vannamei</i>	30
<i>Penaeus monodon</i> (Pm)	ADA67878.1	Decapoda; Dendrobranchiata; Penaeidae; <i>Penaeus</i> ; <i>monodon</i>	29
<i>Macrobrachium rosenbergii</i> (Mr)	ACM181171	Decapoda; Caridea; Palaemonidae; <i>Macrobrachium</i> ; <i>rosenbergii</i>	24
<i>Macrobrachium nipponense</i>	AJQ31851	Decapoda; Pleocyemata; Caridea; Palaemonoidea; Palaemonidae; <i>Macrobrachium</i> ; <i>nipponense</i>	23
<i>Cherax destructor</i> (Cd)	ACD91988.1	Decapoda; Parastacidae; <i>Cherax</i> ; <i>destructor</i>	22

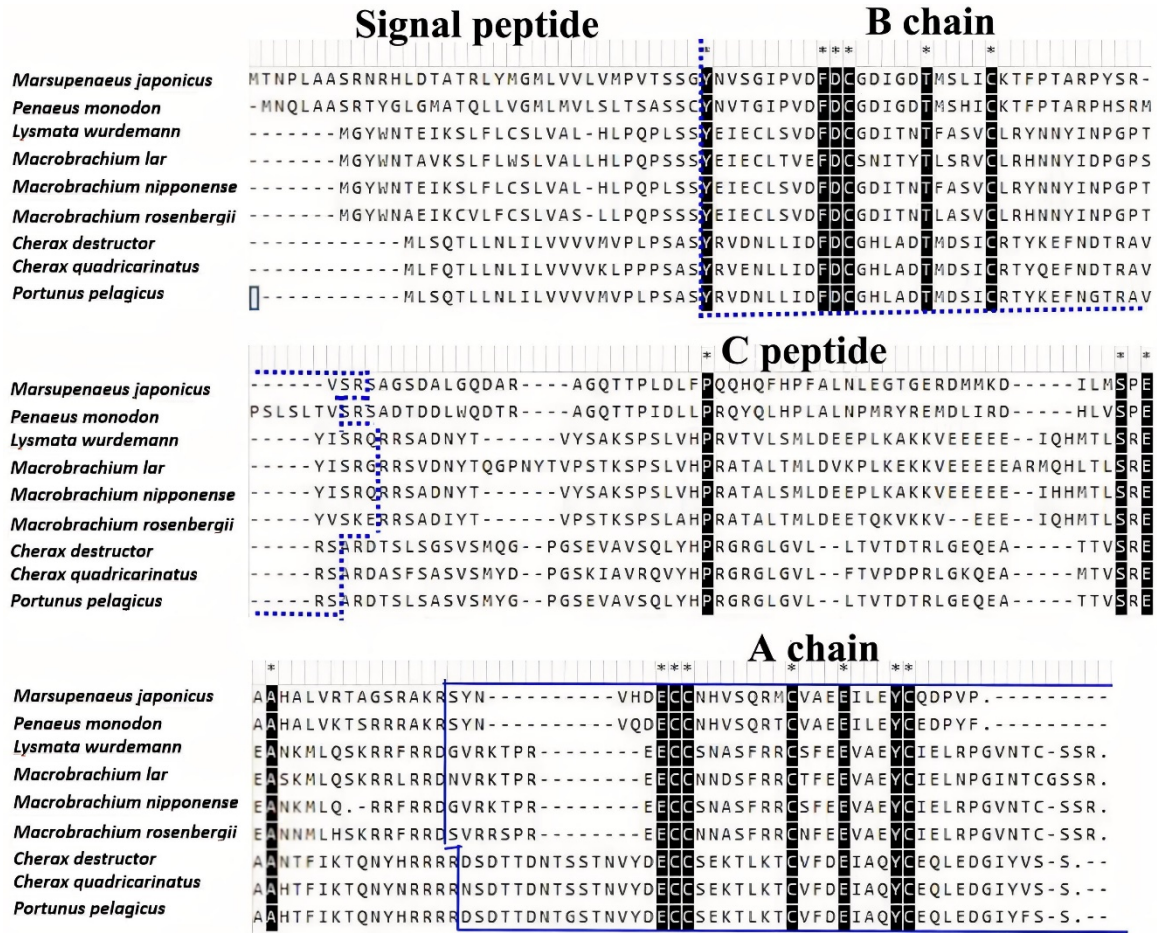


Figure 3. Multiple sequence alignment of deduced primary amino acid sequences of decapod IAGs using Clustal W. *Cherax quadricarinatus*: ABH07705.1; *Cherax destructor*: ACD91988.1; *Macrobrachium rosenbergii*: ACJ38227.1; *Portunus pelagicus*: HM459854.1; *Penaeus monodon*: ADA67878.1; *Marsupenaeus japonicus*: BAK20460.1 *Macrobrachium lar*: BAJ78349.1; *Macrobrachium nipponense*: AGB56976.1. The boundary between signal peptide and B chain is represented by a dotted vertical line. Conserved amino acids are indicated in different colors. B and A chains are marked with dotted and solid lines, respectively. The asterisk indicates the positions of conserved Cys residues in all species.

hormone (Mn-GIH), molt-inhibiting hormone (Mn-MIH), and crustacean hyperglycemic hormone (Mn-CHH) in *Macrobrachium nipponense*. They diagnosed the transcription-factor linking places in the 5' flanking sequence of IAG and realized that every CHH superfamily gene contained two introns dividing three exons. In addition, Mn-MIH and Mn-GIH proportioned the equal intron inclusion places, which were different from Mn-CHH. Furthermore, Li et al. (2015) supplied DNA-level indicators for the variety depiction and recognized two cAMP outcome elements in the 5' untranslated area. Moreover, they also examined the regulatory interactions among Mn-GIH, Mn-MIH, Mn-CHH, and IAG by double stranded RNA (dsRNA) injection at the transcription level. They observed that IAG transcription remarkably

rose to 660.2%, 472.9%, and 112.4% of control amounts in the Mn-GIH dsRNA, Mn-MIH dsRNA, and Mn-CHH dsRNA groups, respectively. Therefore, Li et al. (2015) concluded that Mn-MIH and Mn-GIH adversely modulate the appearance of the IAG gene, but not Mn-CHH. CHH negatively regulates the expression of IAG and serves to enrich the "eyestalk-androgenic gland-testis" endocrine axis involved in IAG signaling (Tang et al., 2021; Farhadi et al., 2021c).

In addition, Li et al. (2018) also stated that FcDsx gene could be involved in the sexual differentiation process of *Fenneropenaeus chinensis*. Liu et al. (2021) studied the role of IAG (LvIt-IAG1) in shrimp *Lysmata vittata*, a species with a rare protandric concurrent hermaphroditism (PCH) reproductive structure. LvIt-IAG1 was solely

indicated in the androgenic gland. The qRT-PCR showed that the level of mRNA definition was comparably denser in the functional male stage but reduced strongly in the following euhermaphrodite stage. Liu et al. (2021) found that Lvit-IAG1 adversely managed both the gonad-inhibiting hormone (LvIt-GIH) and crustacean female sex hormone (LvIt-CFSH) definitions in the eyestalk ganglion in both short-term and long-term silencing researches. In addition, it was also detected that Lvit-IAG1 gene elimination delayed appendic masculine development and male gonopores by suppressing germ cells in the primary spermatocyte phase. Furthermore, inhibiting of the Lvit-IAG1 gene stopped ovarian growth in *L. vittata*. This resulted in tiny vitellogenic oocytes and diminished definition of vitellogenin and vitellogenin receptor genes in the hepatopancreas and ovarian, respectively. Overall, Liu et al. (2021) concluded that Lvit-IAG1 modulates male sexual separation in PCH species *L. vittata*; in addition, this study showed an adverse feedback on LvIt-GIH and LvIt-CFSH genes definition in the species' eyestalk ganglion.

Ohira et al. (2003) observed the homogeneity of two AG-specific protein chains in the isopods *P. dilatatus* and *P. scaber* with ILP. The chains showed every similarities, containing the entire linear chain, the fourth intrachain disulfide bridge, and an assumed glycosylation place in the A chain. On the other hand, regarding decapods, AG-specific ILP was first found in the crayfish *C. quadricarinatus* by Manor et al. (2007). They noticed a subtractive cDNA library from the AG of this crayfish. This library showed a gene, which is expressed only in the males (Subramoniam, 2016). It was named Cq-IAG (insulin-like AG factor). In situ hybridization of Cq-IAG illustrated its special expression in AG. It was suggested that Cq-IAG broadens the viability of sperm in *C. quadricarinatus* (Manor et al., 2007). Ventura et al. (2009) observed ILP in *Macrobrachium rosenbergii* by employing a SSH cDNA library of the AG. Table 1 shows IAG accession numbers of crustaceans.

In mature males, the IAG expression remains elevated to manage male morphotype separation. This finding supports definitive verification that the IAG is the male sex hormone, affecting precociously sex variation and male phenotypic variation in *M. rosenbergii* (Ventura et al., 2011). Moreover, the sex hormone of female crustacean (CFSH) is involved in the development of reproductive phenotype (Jiang et al., 2020b). In the mud crab (*Scylla paramamosain*), the determination of sexually dimorphic traits demonstrated that sex can be discerned from juveniles in the third stage. They further analyzed the level of Sp-cfsh expression in early juveniles of *S. paramamosain* and found that the expression levels differed between juveniles after first-stage and second-stage molt. It was also

found that those crabs at their third stage has a significantly different expression pattern in the two sexes after molting, suggesting that Sp-cfsh could be included in sex separation in early juveniles. It was, therefore, concluded that CFSH has a function in managing sex differentiation in *S. paramamosain* (Jiang et al., 2020b).

ILP and insulin binding proteins were first discovered by Chandler et al. (2015) in the eastern rock lobster (*Sagmariasus verreauxi*) Sv-ILP1 and eight binding proteins were diagnosed by Chandler et al. (2015). The wide statement of Sv-ILP1 clearly indicated that ILPs had an action beyond masculinization in crustaceans. Therefore, it was concluded that the role of these new peptides could be exploited in potential applications in the development of reproduction and rearing of commercially important decapod species (Chandler et al., 2015). In addition, Chandler et al. (2017) employed molecular modeling approaches and assessed the structural, more importantly, the physicochemical nature of the insulin-like growth factor binding protein _N terminal. (IGFBP_N) in *S. verreauxi*. They also suggested that these physicochemical characteristics are at the core of the IGFBP_N' divergences across species.

Zhang et al. (2017) studied to ascertain whether an IAG has a role in gender determination in the marine shrimp (*Lysemata wurdemanni*) by cloning the IAG gene cDNA sequence. Zhang et al. (2017) found that the IAG contained an open reading frame of 528 bp, communicating to 176 amino acids, which contains a signal peptide, B chain, C peptide, and A chain. In addition, the IAG gene was indicated in both male and euhermaphrodite stages, but the expression level was significantly higher in the male phase than in the euhermaphrodite phase. At the end of the study, Zhang et al. (2017) suggested that the IAG gene could be a sex determining aspect in *L. wurdemanni*, and that the euhermaphrodite stage is governed by decreased gene assertion, i.e. the existence of the AG entirely hinders ovarian growth in the male stage, and insufficient deterioration of the AG in the euhermaphrodite stage brings about concurrent hermaphroditism.

4. AG manipulation and factors affecting AG

The effects of different methods of AG manipulation and factors affecting AG are presented in Table 2.

4.1. Eyestalk ablation

Panouse (1943) suggested, for the first time, the efficiency of eyestalk removal in promoting the sexual development in crustaceans. It was also reported that eyestalk removal gave rise to hypertrophy of the AG cells and early sexual development in the shrimp *Pandalus hypsinotus*. In addition, eyestalk ablation has stimulated AG activity, triggered an increase in the IAG level, and accelerated spermatogenesis in *P. hypsinotus* (Okumura et al., 2005).

Table 2. The effects of different methods of AG manipulation and factors affecting AG.

Method	Species	Effect	Reference
Eyestalk ablation	<i>P. hypsinotus</i> ; <i>Pandalus platyceros</i> ; <i>P. hydrodromous</i> ; <i>Macrobrachium nipponense</i> ; <i>C. quadricarinatus</i>	Caused hypertrophy of the AG cells, stimulates AG activity and increased IAG level accelerates spermatogenesis activity	Okumura et al. (2005); Hoffman, 1968; Adiyodi, 1984; Kim et al. 2002; Khalaila et al. 2002
AG ablation	<i>C. quadricarinatus</i>	Caused a shift in the behavior of the intersex animal towards the female	Barki et al. (2006)
AG ablation	<i>L. vannamei</i>	Caused a complete regeneration of appendices, including pereopods and pleopods; lower growth rate	Alfaro Montoya et al. 2016
AG implantation	<i>C. quadricarinatus</i>	Caused the development of male secondary characteristics and the inhibition of female secondary characteristics and vitellogenesis	Khalaila et al., 2001
AG implantation	<i>C. quadricarinatus</i>	Higher growth rate; lower vitellogenic cross-reactive protein in the hemolymph	Manor et al. (2004)
Dietary androgenic gland extract (AGE)	<i>C. quadricarinatus</i>	Induces growth induction	De Bock and Greco, 2010
AG-specific insulin-like factor silencing	<i>C. quadricarinatus</i> ; <i>M. rosenbergii</i>	Feminize male-related phenotypes	Rosen et al. 2010; Ventura et al. 2012
Injection of suspended hypertrophied androgenic gland cells	<i>M. rosenbergii</i>	Causes fully functional sex reversal of females into “neo-males”	Levy et al., 2016
Exposure to pollutants	<i>Neohelice granulata</i>	Imbalances in the male reproductive function, an inhibition on the secretion and/or transduction of IAG	Canosa et al., 2019

AG hypertrophy in eyestalk-ablated animals was also reported by Hoffman (1968) in *Pandalus platyceros*, by Adiyodi (1984) in *Paratelphusa hydrodromous*, by Kim et al. (2002) in *Macrobrachium nipponense*, and by Khalaila et al. (2002) in *C. quadricarinatus*.

Phoungpetchara et al. (2010) studied the AG formation and differences in the AG and IAG-creating cells after eyestalk removal in the blue swimmer crab (*Portunus pelagicus*). Following bilateral eye removal, it was found that AG suffered from hypertrophy and enlarged about 3-fold in size on day 8. The ratio of type I cells enlarged more than double in contrast to controls, whereas type II cells displayed a relative reduction.

Chung et al. (2011) isolated the entire cDNA encoding a candidate IAG, named Cas-IAG, from the blue crab *C. sapidus*. The predicted Cas-IAG protein was encoded as a precursor, which, in turn, consisted of a signal peptide, A, B and C chains. As in other decapods, AG was the principal provider of Cas-IAG, while the hepatopancreas of male *C. sapidus* exhibited little Cas-IAG. Eyestalk removal affirmed the existence of a feasible endocrine axis between the ganglia of the eyestalk and AG, suggesting that Cas-IAG expression was adversely governed by substance found in the ganglia of the eyestalk (Chung et al., 2011).

Phoungpetchara et al. (2011) found that AG of *M. rosenbergii* has three cell types. In addition, they also

noticed in spermatogonia, nurse cells, and epithelium lining of the spermatid duct. Therefore, Phoungpetchara et al. (2011) concluded that the role of IAG in these tissues has not been elucidated yet, but the dispersal points to a powerful function in the reproduction of males.

4.2. AG ablation

Barki et al. (2006) noted that AG is very crucial in establishing male crayfish behavior, and exclusion of the AG gave rise to an alteration in the behavior of the intersex animals towards that of the females. They observed that mating duration was considerably shorter in the AG-removed intersex crayfish, while their fighting time was between those of the males and females. Additionally, the AG-removed crayfish were unexpected to fight with the males and could not demonstrate copulation with the females. Moreover, exclusion of AG forced female morphological and physiological features and also permitted vitellogenin gene transcription and the imitation of secondary vitellogenesis in crayfish. Therefore, Barki et al. (2006) stated that AG manages and arranges the behavior of males in crustaceans.

Exclusion of the AG provoked an inclusive regeneration of extremities, such as walking legs and pleopods, in *L. vannamei*. The AG-removed males demonstrated a lesser development ration and shorter petasmas and appendices masculinae in comparison to the control males. Alfaro-

Montoya et al. (2016) discovered that in the AG-removed males spermatogenesis was effective with decomposition of spermatids and sexual tissues.

Sagi et al. (2002) found that AG ablation in the intersex crayfish *C. quadricarinatus* caused a permanent transition of the reproductive system from an active male to female. The hepatopancreas of AG-ablated intersex crayfish was involved in the inducing of vitellogenin gene implying that the AG inhibits transcription of this gene in intersex crayfish. Sagi et al. (2002) concluded that the experimentally inducible sex change in *C. quadricarinatus* presents a particular and administered representative structure for the research of sexual separation and flexibility at the physiological and molecular extent.

4.3. AG implantation and dietary androgenic gland extract (AGE)

Khalaila et al. (2001) found that AG transplantation leads to the growth of male secondary features as well as the repression of female secondary features and vitellogenesis, as shown by the slightly below gonadosomatic index (GSI) and the little oocyte size in *C. quadricarinatus*. In a different study, it was found that the growth rate of the juveniles of *C. quadricarinatus* with the inserted AG was remarkably bigger than that of the control. Likewise, the amount of vitellogenic cross-reactive protein in the hemolymph of the AG-inserted juveniles was remarkably lower (Manor et al., 2004). De Bock and Greco (2010) found that dietary AGE accelerated the growth rate in *C. quadricarinatus*.

4.4. AG-specific insulin-like factor silencing

Recent investigation has revealed that silencing insulin-like androgenic gland hormone (IAG) can feminize male-related phenotypes in crustaceans. Ventura and Sagi (2012) also reported that IAG silencing is an effective technique for obtaining unisex populations in crustaceans. Silencing an insulin-like gene in *C. quadricarinatus* demonstrated a reduction in sperm reproduction, significant testicular deterioration, vitellogenin gene expression, and deposition of yolk proteins in the growing oocytes (Rosen et al., 2010). Moreover, *M. rosenbergii* IAG silencing prevented spermatogenesis and impermanently blocked male sexual features development. Additionally, *M. rosenbergii* IAG silencing wedged spermatogenesis and temporarily inhibited male sexual characteristic growth (Ventura et al., 2009).

Ventura et al. (2012) reported an entire gender conversion from male to neofemale in *M. rosenbergii*, silencing IAG before physical gender features emerged. It was observed that IAG removing in males can be utilized to obtain neofemales from males, as was carried out in *M. rosenbergii*, while AG administration produces neomales (Ventura et al., 2012).

Priyadarshi et al. (2017) examined the specific role of the hormone IAG in morphotype separation in *M. rosenbergii*. They found, for the first time, that

IAG enhancement in orange claw (OC) males caused remarkably more conversions from OC to blue claw (BC) when prawns were kept in groups. Therefore, Priyadarshi et al. (2017) reported that this idea can be improved as a reduced pressure, feeding or holding process to control the heterogeneous individual development of *M. rosenbergii* males in crustacean farming.

5. AG manipulations in aquaculture

5.1. Production of all-male population of *M. rosenbergii*

Ventura et al. (2009) found that silencing through RNA interference (RNAi) of the IAG gene at a specific time in the larval development period of male *M. rosenbergii* produced neofemales. These neofemales were genetically males with all the physical characteristics of females meaning that they could reproduce and mate with a normal male giving rise to an all-male progeny. Males normally grow faster and reach a bigger size compared to the females in *M. rosenbergii*. Additionally, it is highly valued as food delicacy and is, therefore, exploited in large quantities in aquaculture around the world (Aflalo and Sagi, 2014). Development of an entire male population will enable the farmers to expand the efficiency of their production facilities in aquaculture. This technology is already used in many countries such as India and Thailand (Aflalo and Sagi, 2014; Savaya-Alkalay and Sagi, 2016). The use of RNAi procedure in crustacean aquaculture seems safe due to its transient nature (Lezer et al., 2015).

5.2. All-female monosex culture in *M. rosenbergii*

It is obvious that the all-female populations are preferred for penaeid shrimps such as *L. vannamei* (Moss and Moss, 2006) and *Penaeus monodon* (Gopal et al., 2010) since the females develop quicker and attain a bigger size at crop than the males. Nevertheless, for *M. rosenbergii*, male to female monosex production is not very common because the males are bigger at the end of the growing period (Nair et al., 2006). On the other hand, it was noted that the all-female monoculture may be the exercise of preference, as the females are less combative and show a partly homogenous development model (Malecha et al., 2010; Malecha, 2012; Harlioğlu and Farhadi, 2017).

Levy et al. (2017) carried out an investigation on all-female and combined *M. rosenbergii* populations in earthen ponds under both extensive and intensive stocking systems. The all-female population was generated by applying a new biotechnology on the basis of only one injection of suspended hypertrophied AG cells. They found that the all-female cultures had higher viability ration and harvest per hectare than the mixed cultures under both intensive and extensive systems. In addition, the intensively maintained whole female ponds exhibited higher feed conversion ratios than the combined ponds. Moreover, although the average size of *M. rosenbergii* did not show a significant difference between the two treatments, the populations of all-female

showed remarkably greater uniformity of size. At the end, Levy et al. (2016) concluded that female monosex farming is a feasible technique to harvest a homogenous product for *M. rosenbergii* (see also Levy et al., 2017).

AG ablation caused sex reversal in the immature male *M. rosenbergii* and produced an all-female population. On the other hand, implanting AG into the immature females resulted in the growth of the male reproductive structure. The sex-reversed *M. rosenbergii* individuals were capable of mating with the normal prawns and produce offsprings (Sagi and Aflalo, 2005).

Lysmata wurdemanni is a protandric concurrent hermaphrodite shrimp species. It experiences a male phase before the female phase and becomes a concurrent hermaphrodite (PCH) before the sex reversal. The latter has an externally female phenotype, but retains a decreased male reproductive structure with both male and female reproductive functions. Previous studies demonstrated that the AGs, whose hormones encourage the growth of male features in decapods, are not present in the female stage of entirely protandric species (Bortolini and Bauer, 2016).

Bortolini and Bauer (2016) tested the hypothesis of the persistence of AG in PCHs by histology of the ejaculatory ducts in *L. wurdemanni*. Although this gland was in a variably atrophied form, it was observed in PCHs. The AGs of *L. wurdemanni* MPs were compact and filled with well-grown cells with great extremely stained (hematoxylin-eosin) nuclei as in males of the gonocoric and protandric species. The AGs of concurrent hermaphrodites were apparently more reticulate because of the obvious disintegration and disappearance of cells, causing in vacuolated zones, or bare areas in the gland neighbored by connective tissue fibers or cell residues. Furthermore, it was found that all PCHs had large numbers or at least a few conceivably functional cells and the most excellent atrophy of AGs was found in the largest (i.e. oldest) PCHs. Moreover, the ovo testes of all PCHs maintained a tiny testicular part with well-grown ejaculatory ducts encompassing sperm. Therefore, Bortolini and Bauer (2016) suggested that the decreased AGs of *L. wurdemanni*'s female stage concurrent hermaphrodites permit them to retain male reproductive activity following sex alteration.

Rotem-Dai et al. (2021) demonstrated that *M. rosenbergii* primary cells infected with the Mr-IAG

lentiviruses are capable of transcription, translation, and secretion of Mr-IAG in culture. These findings provided a new platform, which produces easy-to-harvest cells in abundance, could replace the AG cells used in the first step of the biotechnology for all-female aquaculture and, importantly, pave the way for producing monosex populations in other edible crustacean aquaculture species.

5.3. Invasive species control by nonreproductive crustaceans

Prawns of the genus *Macrobrachium* are regarded as potential bio-control agents over snails (Sokolow et al., 2014). The all-male prawns have been efficient in mitigating the epidemiological problems posed by the parasitic disease schistosomiasis, which is often observed in Africa (Southgate et al., 2001). Freshwater snails of the genera *Biomphalaria* and *Balinus* serve as the intermediate hosts for the parasites causing schistosomiasis (Southgate et al., 2001). Although these snails are normally consumed by a native prawn species, human activities have caused a remarkable decrease in *Macrobrachium vollenhovenii* population. The introduction of whole male populations of *M. vollenhovenii* is tested in Senegal River basin that suffer from high infection rates hopefully decreasing the appearance of this disease (Alkalay et al., 2014).

6. Conclusion

The androgenetic gland (AG) regulates sexual differentiation in crustaceans and plays a central role in masculinization through the inhibition of female differentiation. Production of all-male monosex prawn populations by AG administration has been economically feasible. However, there are still gaps in the knowledge of some aspects such as the effects of the hormonal injections (e.g., 17 α methyltestosterone and methyl farnesoate), environmental factors (e.g., photoperiod and temperature), and nutrition (e.g., dietary lipids, protein, amino acids and vitamins) on the AG functionality. Further studies are required in order to develop the AG-related biotechnology for applications in crustacean aquaculture. For example, all-female monoculture by using novel biotechnology (on the basis of only one injection of suspended hypertrophied AG cells) may be beneficial for penaeid shrimps, such as *P. monodon* and *L. vannamei*.

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