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JUVENILE HORMONE-LIKE COMPOUNDS AND REPRODUCTION IN MALE AND FEMALE CRUSTACEANS: WITH IMPLICATIONS FOR AQUACULTURE

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H. Laufer and A. Sagi (1991) Juvenile hormone-like compounds and reproduction in male and female crustaceans: with implications for aquaculture. *Bull. Inst. Zool., Academia Sinica, Monograph* **16**: 541-551. Juvenile hormone (JH) has central roles in the regulation of insect development and reproduction. A JH-like compound has been recently analyzed in the hemolymph of several crustacean species. Methyl farnesoate (MF) is the unepoxidated form of JH III, and was found to be synthesized by the crustacean mandibular organ. The possible roles of methyl farnesoate in crustaceans, both as a morphogen and a gonadotropin, are discussed in the light of two of the problems that confront todays crustacean aquaculture, *i.e.*, the need to control female reproduction in stringently controlled species and the wide size distribution in polymorphic species.

In female, *Libinia emarginata*, the *in vitro* secretory rates of MF by mandibular organs were closely related to the stage of ovarian growth. Methyl farnesoate rate was lowest in juvenile females and previtellogenic adults. The secretory rate was significantly higher in vitellogenic animals and dropped to intermediate levels in females in which ovarian development was being completed.

Three different types of males were analyzed in *L. emarginata* males. Large abraded males, possesses significantly larger reproductive systems, also possess larger mandibular organs that synthesizes significantly more MF *in vitro* per hour than the large unabraded males and small males. The highest amount of MF in the circulatory system occurs in the large abraded males. These are the males which demonstrate most active reproductive behavior.

These data strongly support the conclusion that MF may be a crustacean hormone that has a role in the regulation of reproduc-

tive growth and morphogenesis in male crustacea. The relation between secretion of methyl farnesoate by the mandibular organ and vitellogenesis in female *L. emarginata* is suggested to be a cause-and-effect response, since it was previously shown that implant of mandibular organs into juvenile females stimulated ovarian development.

Key words: Juvenile hormone, Methyl farnesoate, Crustacea, Reprodction, Aquaculture research.

AQUACULTURAL PROBLEMS AND JUVENILE HORMONE-LIKE COMPOUNDS

At the hatchery level, one of the main problems in the culture of crustaceans is the maintenance of a steady supply of larvae. This problem is more noticeable in the production of seasonal species or species that do not reproduce easily and regularly in captivity. In the recent years the mariculture industry has experienced an increasing shortage in gravid females of several panaeidae shrimp species (NOAA, NMFS, 1988). This shortage is noticeable in particular species of importance to the industry, such as *Penaeus vannamei*, in which most of the hatcheries depend on an unpredictable supply of wild seed (Liao, 1990). One strategy to alleviate this problem is to stimulate reproduction by unilateral eyestalk ablation. This results in the production of more nauplii, however, these are deemed to be of inferior quality to those caught in the wild or those produced by intact females in captivity (Choy, 1987). Eyestalk ablation also causes irreversible damage to the female. Further, a lack of spontaneous breeding does not allow for genetic selection programs to improve the breeding stock.

Another practical problem, this one is encountered in the growout phase, is the phenomenon of differential growth in several species of crustaceans. Variation in the size of the product at the end of the growout period may be very pronounced, such variation is well illustrated by what happens in the cultured freshwater prawn *Macrobrachium rosenbergii* (Cohen *et al.*, 1981; Sagi *et al.*, 1986) where there are three different male morphotypes differing in size. Similar manifestations are also evident in other crustaceans such as crayfish, crabs and lobsters, and could be the result of a number of factors. These could include: 1) Bimodal, sex-specific and growth rates; 2) Cessation of growth

at a maturational molt or a differentiating molt accompanied by the commencement of intense reproductive maturation; 3) The coexistence of different reproductive types, in the cultured population, which either reach a terminal molt or interact with each other and affect each other's growth.

In crustacea the phenomena mentioned above are, at least in part, hormonally mediated. Possible agents likely to be regulating these processes are juvenile hormone-like substances. If that is the case, such juvenile hormone-like compounds are likely to be agents instrumental in the solution of aquacultural problems.

Juvenile hormones are well known and extensively studied in insects where they play several regulatory roles both as gonadotropins in adults (Herman and Bennett, 1975) and morphogens during development (Wigglesworth, 1970). Juvenile hormones are also responsible for the onset of reproduction after periods of reproductive diapause (De Wilde, 1983). Furthermore, polymorphic forms among social insects such as termites, locust and aphids (Luscher, 1960; Lees, 1983 Penner, 1983) are mediated by juvenile hormones. As early as 1958, Schneiderman and Gilbert reported that extracts from a number of crustaceans possessed juvenile hormone activity in insect cuticle bioassays, however the active compounds were not identified in those experiments nor the role of these substances investigated in crustaceans. Among the earliest reports that juvenile hormones effect crustaceans are the results of Paulus and Laufer (1982) and Paulus (1984) that have demonstrated an ovarian enlargement following an injection of methoprene, a juvenile hormone analog, in female crabs *Carcinus meanas*. However, juvenile hormones were not found in crustaceans, excluding one juvenile hormone-like substance, methyl farnesoate (Laufer *et al.*, 1987), which has juvenile hormone activity in bioassays, but has no clear biological function in insects (Feldlaufer *et al.*, 1982; Bruning *et al.*, 1985).

Juvenile hormone-like substances are promising aquacultural agents since methyl farnesoate occurs in crustaceans at critical reproductive stages in both males and females. Juvenile hormones are available commercially. The compounds are relatively labile and biodegradable, thus their administration to cultured

species is feasible without contaminating the environment.

METHYL FARNESOATE, A JUVENILE HORMONE-LIKE COMPOUND IN CRUSTACEANS

Laufer *et al.*, (1987) identified methyl farnesoate as the secretory product of the mandibular organ (Fig. 1) of crustaceans. It has since been discovered in many crustaceans including crabs (Laufer *et al.*, 1987; Tobe *et al.*, 1989), lobsters (Laufer and Borst, 1988), crayfish (Landau *et al.*, 1989) and prawns (Sagi *et al.*, 1991a). Methyl farnesoate is the unepoxidated form of the insect

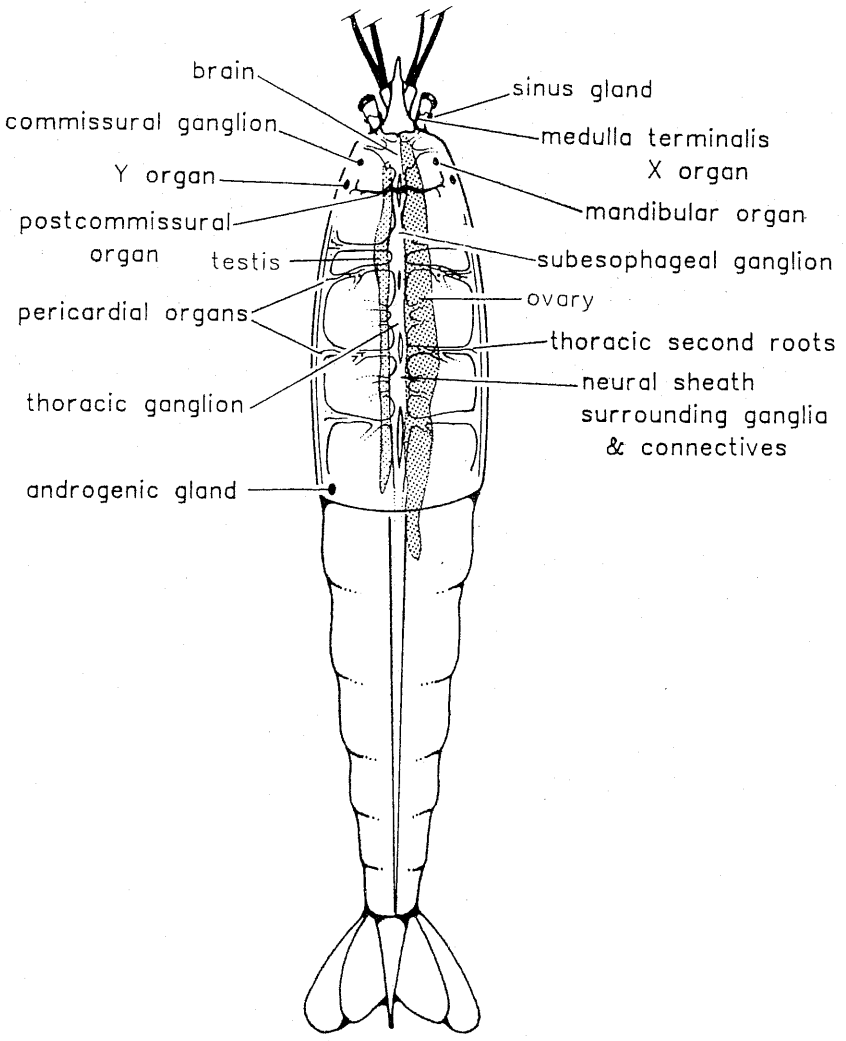

Fig. 1. The endocrine system of a generalized crustacean. The left showing the male system including the testis and androgenic gland. On the right is the female system with an ovary.

juvenile hormone III and is suggested to be a crustacean hormone. The secretion of methyl farnesoate by the mandibular organ is tissue specific, and methyl farnesoate is circulating in the blood (Laufer *et al.*, 1987; Tobe *et al.*, 1989). Its circulating levels are variable and are correlated with the reproductive states of males (Homola *et al.*, 1991) and females (Laufer *et al.*, 1987). Specific methyl farnesoate binding proteins were identified in the hemolymph of lobsters (Prestwich *et al.*, 1990) and crabs (Li and Borst, 1991). These proteins may be functionally analogous to the hemolymph juvenile hormone-binding proteins, which serve as hormone carriers in the circulation of insects. An increase in protein synthesis in integument tissue of crabs was noted following an *in vitro* exposure of this tissue to physiological levels of methyl farnesoate (Paulson and Skinner, 1988). When injected into reproductively active crab females, methyl farnesoste caused a small, but significant, increase in blood vitellogenin levels (Vogel and Borst, 1990). The production and secretion of methyl farnesoate is controlled by an inhibitory factor originating from the sinus gland X-organ (Fig. 1), in the same manner gonadal activity is controlled by the eyestalk (Laufer *et al.*, 1986). Future studies are required in order to establish the exact function and mode of action of this recently discovered hormone with respect to crustacean reproduction and morphogenesis, and particularly with respect to its possible aquacultural application.

METHYL FARNESOATE MAY INDUCE FEMALE REPRODUCTION IN STRINGENTLY CONTROLLED SPECIES

The spider crab *Libinia emarginata* is used in our laboratories as a representative crustacean. It is readily maintained in the laboratory and in captivity, and continuously reproductive throughout the year. This organism seems particularly well suited for the study of the role of methyl farnesoate in the regulation of gonadal development. Laufer *et al.* (1987) have shown that the apparent synthesis rates of methyl farnesoate *in vitro* by mandibular organs from *L. emarginata* females were closely related to the stages of ovarian growth. Methyl farnesoate synthesis and secretion were lowest, about 0.50 ng/hr/gland, in inter-molt juvenile females and in adult previtellogenic females. The

apparent synthesis and secretion rates were significantly increased to more than 3.30 ng/hr/gland in females in which oocyte development and vitellogenesis were occurring. This is more than a six fold increase. The synthesis and secretion rates of methyl farnesoate dropped in females in which ovarian development was completed. The relationship between synthesis of methyl farnesoate by the mandibular organ and vitellogenesis is in all likelihood a cause-and-effect response, since Hinsch (1980) reported that mandibular organs implanted into immature females *L. emarginata* caused noticeable enlargement of the ovaries accompanied with several morphological and ultrastructural signs of active vitellogenesis. The shortage of gravid females of cultured panaeidae shrimp could be solved by the administration of gonad stimulating substances. These results suggest that methyl farnesoate is possibly such a gonadotrophic agent.

METHYL FARNESOATE IS RELATED TO PHENOTYPIC DIFFERENTIATION AND REPRODUCTION OF MALE CRUSTACEANS

Aquacultured and fished crustacean species such as crayfish, prawns, lobsters and crabs display differential growth patterns.

Both the cessation of somatic growth the commencement of reproductive activity, and the coexistence of different reproductive types of males were described in *Libinia emarginata* (Hartnoll, 1963; Homola *et al.*, 1991; Sagi *et al.*, 1991b). The differences in morphology and reproduction were used to study the role of methyl farnesoate in morphotype induction and in the regulation of reproductive activity and behavior. Levels of methyl farnesoate in the blood and its relative rates of synthesis by the mandibular organ were investigated to determine whether this compound is correlated with the differences in morphology and reproductive states of distinct types of males existing in the population of the spider crab (Homola *et al.*, 1991). Three male types were examined (Sagi *et al.*, 1991b): 1) Abraded males, with relatively large propoduses (claws) and worn exoskeletons, 2) Unabraded males, with relatively large propoduses and velvety exoskeleton, and 3) Males with small propoduses. All males examined had sperm. Large-clawed unabraded males, identical in propodus and body size to abraded

males, had significantly smaller reproductive systems (Gonad Index =1.19% compared to 2.81%). Small-clawed male also possessed a small reproductive sytem (0.71%). Abraded males, with large repoductive systems, possessed larger mandibular organs, containing almost twice the total protein than did the mandibular organ from unabraded males. Mandibular organs from abraded males synthesized significanly more methyl farnesoate *in vitro* than did the other types of males. Circulating levels of methyl farnesoate, in the hemolymph of the abraded males, were more then twice as high as the levels detected in any of the other type of males (67.2 ng/ml compared to 29.6 ng/ml in unabraded and 10.7 ng/ml in small-clawed males). Both the abraded and unabraded have undergone their terminal molt. The degree of abrasion appears to indicate the time from the terminal molt. The unabraded state may last for as long as a year. Behaviorally, the abraded male is not only more aggressive in feeding but also in behavior relating toward females such as holding and mating. These data suggest that the abraded crabs are more active reproductively while the unabraded ones are reproductively inhibited or in a reproductive diapause. They also suggest that methyl farnesoate may play a significant role in the regulation of reproduction and morphogenesis in crustaceans.

Several major questions regarding methyl farnesoate remain which may have a bearing on our understanding of its biological action. For instance, does methyl farnesoate act directly on the reproductive organs, in both males and females, and does it do so exclusively? Experiments *in vitro*, using short term organ culture could answer such a question. If the primary target of methyl farnesoate is the gonad, then ovary and testis fragments should respond to methyl farnesoate stimulation in culture. Further, if the primary targets of methyl farnesoate are the reproductive organs, other organs should not respond. However, Paulson and Skinner (1988) have already shown that epidermal fragments in culture do change in their pattern of protein synthesis following exposure to physiological concentrations of methyl farnesoate. Thus methyl farnesoate may act on several target tissues, and may indirectly as well as possibly directly affect reproductive organs and behavior. Its increase in the circulation during crucial reproductive

events, and the increase in blood vitellogenin levels following methyl farnesoate injection into reproductively active female crabs (Vogel and Borst, 1990), may suggest an indirect effect acting through the hepatopancreas. Since the hepatopancreas was identified as a site of vitellogenin synthesis (Paulus and Laufer, 1982, 1987; Paulus, 1984), the role of methyl farnesoate in effecting hepatopancreas activity is particularly relevant.

Does methyl farnesoate act alone as a gonadotropin? Probably not. The neuropeptides Gonad Stimulating Hormone (GSH) and Gonad Inhibiting Hormone (GIH) from the Central Nervous System (Fig. 1) and the Sinus Gland (Fig. 1), respectively (Van Harp and Payen, 1991 this proceedings) may also affect the reproductive system directly, as was demonstrated in ovary fragments in culture (Eastman-Reks and Fingerman, 1984; Quackenbush and Keeley, 1987). These factors may be synergistic or antagonistic to methyl farnesoate or may affect different steps in the gonads' maturational process. Experiments conducted in our laboratory demonstrated that peptidic factors from the eyestalk have an inhibitory effect on the synthesis of methyl farnesoate by the mandibular organ *in vitro* (Laufer *et al.*, 1986). These results raise the following questions: Is the GIH, which comes from the sinus gland X-organ complex (Fig. 1), related to the mandibular organ inhibiting-factor? Also, is the GSH from the thoracic ganglion (Fig. 1) and brain (Fig. 1) related to a mandibular organ-stimulating factor? The GSH and GIH may have multiple targets including gonads, hepatopancreas and mandibular organs, or there may be multiple neuropeptides.

Is methyl farnesoate a morphogen in males? The androgenic gland (Fig. 1) determines maleness in crustacea, for in its absence a male becomes sex reversed if an andrectomy is performed early enough (Sagi and Cohen, 1990). If the andrectomy is performed in an adult prawn it has dramatic effects on the differentiation into the distinct male morphotypes (Sagi *et al.*, 1990). Thus the androgenic gland is involved in morphotypic differentiation, but the evidence, *i.e.* the differences in methyl farnesoate titers in the different male morphs of the spider crab, also appears to suggest a role for methyl farnesoate.

Is there an interaction between

ecdysteroids and methyl farnesoate with respect to reproduction? Both are certainly present in the reproduc tive tissues, embryos (Laufer and Deak, 1990) and in the circulation (Laufer *et al.*, 1988; Chang, 1989) and seem to be needed for reproduc tion. There are suggestions, from our experiments (Laufer *et al.*, unpublished), that ecdysteroids may act along with or at different stages of the reproductive cycle than methyl farnesoate.

The mode of methyl farnesoate will certainly have to be investigated further. How is its titer controlled during different phases of reproduction in both males and females, and how does it interact with other endocrine factors? These questions need to be addressed before we have a clearer understanding of crustacean reproduction. However, it can be concluded that methyl farnesoate may be a juvenile hormone of crustacea, and an understanding of its action is of primary significance for an understanding of the biology of reproduction of an important resource for aquaculture.

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