Unifying Concepts Learned from Methyl Farnesoate for Invertebrate Reproduction and Post-Embryonic Development

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Synopsis. Since the discovery that methyl farnesoate (MF), the unepoxidated form of the insect juvenile hormone (JHIII), is produced by mandibular organs of numerous crustaceans, extensive evidence has accumulated that this compound appears to perform similar functions in the Crustacea as JH performs in insects. A major function of MF appears to be in enhancing reproductive maturation. This was first shown by indirect experimentation with eyestalk ablation, which augmented MF production. Subsequently, direct treatments of several species of crustacea with MF showed that reproductive maturation was enhanced.

A second function of MF, similar to that of the JH of insects, is in the maintenance of juvenile morphology. This is especially true in the late larval transformations into juveniles, where MF plays an inhibitory role, as well as during the transformation of juveniles into adults. These results were inferred from eyestalk removal experiments. In the case of the larval-juvenile transition, inhibitory results were also obtained with MF by direct hormone treatments. However, the transition from very early larval stages, such as one nauplius stage proceeding to the next, which in many cases also involves morphogenetic changes, may be occurring in the presence of MF. Indeed, MF appears to be stimulatory to early postembryonic larval stages of Crustacea. Again, this function of MF in Crustacea appears to be similar to functions of JH in early postembryonic insects. However, it should be pointed out that there are many more “early” stages in Crustacea than there are in insects, and very few of these cases have been investigated.

When considering the animal kingdom and larval metamorphosis, the question may be raised whether there are other members of the JH family regulating metamorphosis and reproduction. One plausible example appears to be among certain annelids. The trochophores of Capitella respond to various juvenoids, but are most responsive, within one hour, to MF and eicosatrienoic acid. This latter compound is present also in adult annelids, where it has been named “Sperm Maturation Factor,” since it seems to function in the maturation of sperm in Arenicola. Therefore, eicosanoids perform in annelids two functions performed in insects by JHs.

In conclusion, it seems that there are morphogenesis promoting responses to JHs in early larval development in crustaceans, annelids, and possibly other forms, which differ from those MF effects in later larvae of Crustacea where MF retards morphogenesis. Such early responses as noted here have recently also been described for insects. Furthermore, it is clear that the polyunsaturated 8,11,14-eicosatrienoic and arachidonic acids seem to be juvenoids, and appear to function as such in annelids, and may also be functionally active in insects and crustaceans. It seems reasonable to conclude therefore that new and novel juvenoids exist, while others still await discovery.

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INTRODUCTION

The existence of juvenilizing factors in insects that prevent metamorphosis to the adult stage was first demonstrated in *Rhodnius prolixus* by Wigglesworth (Wigglesworth, 1934, 1936) and later by Williams (1956, 1961) as well as others. Juvenilizing factors were isolated and characterized as juvenile hormones (JHs) (Röller and Dahm, 1970), and were identified as mostly derived from terpenoids (reviewed by King, 1983; Laufer and Borst, 1988) (Fig. 1). Major functions attributable to these hormones in insects include control of morphogenesis, metamorphosis, and reproductive maturation in adults. However, the diverse functions and roles of this expanding family of compounds are still being elaborated and elucidated. The possibility of the existence of other juvenoids present in animals, and additional species responding to juvenoids, is discussed in this review. While this review is by no means complete, we present supporting evidence for the views and conclusions reached.

The suggestion that many different species may contain juvenoids was presented by Schneiderman and Gilbert (Schneiderman and Gilbert, 1958; Gilbert and Schneiderman, 1961), who prepared ether extracts from animals representing different phyla, including crustaceans, such as *Uca pugilator* and *Homarus americanus*, and annelids, *Nereis virens* and *Glycera dibranchiata*. These extracts showed evidence of juvenile hormone activity by the formation of pupal patches in adult insect cuticle assays, where pupae were allowed to metamorphose into adults following the application of a test sample, usually in wax, to an epithelial wound.

Another indication of the presence of JH-active substances in crustaceans came from eyestalk ablation studies. Removal of the eyestalk leads to a stimulation of ovarian maturation. Over 50 years ago, Panouse (1943) and Brown and Jones (1947, 1949) demonstrated that eyestalk ablation of female shrimp, crayfish, and fiddler crabs results in the stimulation of ovarian maturation. This result suggested that the eyestalk contains an inhibitory factor or hormone, which represses reproductive maturation. Additional evidence for eyestalk inhibitory factors were provided by Costlow (1968), who showed that removal of eyestalks from zoea larvae of the blue crab and the mud crab *Rhithropanopeus* resulted in the production of zoeal-megalopa intermediates instead of megalopa produced by intact controls. Thus, the eyestalk contained inhibitors that controlled morphogenesis. Furthermore, the removal of eyestalks from juvenile female *Libinia emarginata* resulted in their remaining juveniles, following their next molt, instead of molting into adults, as did the intact controls (Hinsch, 1972). In fact, some of these female *Libinia* could molt once more, but again they failed to mature into the adult form. We interpreted the results of these various experiments on crustaceans, involving disruption of larval or juvenile metamorphosis into adults, and accelerated ovarian maturation in adults, to indicate that destalking releases hormonal inhibition (Laufer et al., 1987, 1997b). Since the same processes seemed to be affected in Crustacea as they were in insects, we considered this hormone to be JH-like.

Besides eyestalk ablation, the application of exogenous juvenoids has also been found to affect the development and reproduction of crustaceans. The JH-analog methoprene (Fig. 1) was demonstrated to interfere with reproduction of *Daphnia magna* and to cause sterility in this species (Templeton and Laufer, 1983). Treatment of *Artemia salina* third stage nauplius larvae with methoprene slowed down development, while application of methyl farnesoate at some concentrations accelerated molting and development (Ahl and Brown, 1990). The injection of methoprene into adult *Carcinus maenas* females caused enlargement of ovaries (Paulus and Laufer, 1987; Paulus, 1984). The treatment of late *Homarus* larvae with JH caused the formation of intermediates instead of allowing them to complete metamorphosis (Charmantier et al., 1988). Similar results were found by Hertz and Chang (1986) who showed that treatment of *Homarus americanus* larvae with JH III delayed larval development and caused morphogenic abnormalities. JH analogs also retarded the development of lar-
Fig. 1. Some variants of juvenile hormone active compounds found in insects. Methyl farnesoate, while present in insects, appears to be a dominant juvenoid among the crustaceans. Several naturally occurring compounds which have been found to have juvenile hormone activity in some organisms include cis 8,11,14-eicosatrienoic acid and cis 5,8,11,14 eicosatetraenoic (aracidonic) acid which are present in annelids and bring about *Capitella* larval metamorphosis and settlement (Biggers and Laufer, 1992). Eicosatrienoic acid has been identified as the sperm maturation factor of *Arenicola* (Pacey and Bently, 1992). Nemec *et al.* (1993) showed that retinoic acid can replace JH in stimulating ovary maturation in two insects, while thyroxine has been demonstrated to have JH activity and has been implicated as replacing JH (Davey and Gordon, 1996).

vae of the mud crab *Rhithropanopeus harrisi* (Christiansen *et al.*, 1977). McKenney and Celestial (1993) showed that although JH analogs inhibited early larval and postlarval development of the shrimp *Palaeomonetes pugio*, the JH-analogs actually enhanced development of the premetamorphic stages.

Positive effects of juvenoids on crustacean metamorphosis were also encountered in barnacles early on. The remarkable results obtained with cyprid barnacle larvae were considered to be aberrant, curious, and contrary to expectations with the insect models in existence, in that juvenoids stimulated precocious metamorphosis instead of inhibiting it, as they do in most decapod *Crustacea* and insect larvae. Studies by Gomez *et al.* (1973) and Ramenofsky *et al.* (1974) showed that JH-1 and the JH-analog
ZR-512 (hydroprene) (Fig. 1) are able to induce most of the manifestations of precocious metamorphosis of the barnacle Balanus galeatus from the swimming cyprid stage to metamorphosed juvenile. The metamorphosed individuals however underwent incomplete metamorphosis since they did not attach properly to a substrate, as is usually found during normal settlement and metamorphosis. Similar results were reported for the effects of JH analogs on larvae of the barnacle Eliminus modestus (Tighe-Ford, 1977) (Fig. 2).

These experiments taken together suggested that there was ample evidence for a JH in Crustacea, and it remained to be identified and its actions elucidated.

**DISCOVERY OF METHYL FARNESOATE, SYNTHESIS BY MANDIBULAR ORGANS, AND REGULATION OF MF**

Le Roux (1968) described mandibular organs (MOs) and suggested that they may have some developmental function. This was followed by an experiment in which implantation of MOs into juvenile female Libinia emarginata stimulated an increase in ovarian size (Hinsch, 1980).

After years of searching for possible juvenile hormones in crustaceans by several investigations, the discovery of methyl farnesoate (MF) (Fig. 1) by Laufer et al. (1987), ushered in a new era in comparative endocrinology of juvenile hormones. Methyl farnesoate, an unepoxidated form of JH III, was first found in the spider crab Libinia emarginata, and has since been found in over 30 crustacean species (Table 1, Fig. 1). MF is produced by the mandibular organs of crustaceans (Laufer et al., 1987), and regulation of MF synthesis by MOs is negatively regulated by neuropeptide hormones termed mandibular organ inhibitory hormones (MOIHs), produced by the X-organ sinus gland complex, found in the eyestalk (Laufer et al., 1987; Landau et al., 1989). MOIHs were first identified as members of the CHH family of neuropeptides in Libinia by Liu and Laufer (1996). The hormones have 72 amino acids, and one of these MOIHs has been sequenced and cloned (Liu et al., 1997). Additional MOIHs have been identified and characterized by Wainwright et al. (1996) from the crab Cancer pagurus. After production by the MOs, MF is transported through the hemolymph to target tissues by MF binding proteins (Prestwich et al., 1990; Takac et al., 1998).

Since the initial discovery of MF in crustaceans by Laufer et al. (1987), the functions of MF in fulfilling its role as a juvenile hormone (JH) in crustaceans has been examined extensively. Despite an abundance of evidence, Homola and Chang (1997) still raise the question of whether
**Table 1.** Presence of methyl farnesoate in crustaceans.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>MF in hemolymph</th>
<th>MF synthesis by MO</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decapods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Libinia emarginata</td>
<td>adults, larvae</td>
<td>+</td>
<td>+</td>
<td>5, 9 etc.</td>
</tr>
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<td>Libinia dubia</td>
<td>adults</td>
<td>+</td>
<td>+</td>
<td>1</td>
</tr>
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<td>Homarus americanus</td>
<td>adults, larvae</td>
<td>+</td>
<td>+</td>
<td>6, 9</td>
</tr>
<tr>
<td>Homarus gamarus</td>
<td>larvae</td>
<td>+</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Macrobrachium rosenbergii</td>
<td>adults, larvae</td>
<td>+</td>
<td></td>
<td>4, 8, 13</td>
</tr>
<tr>
<td>Cambarus bartonii</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Cancer borealis</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Carcinus maenas</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Cancer pagurus</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Cancer magister</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>7</td>
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<td>Callinectes sapidus</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>1</td>
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<td>+</td>
<td>+</td>
<td>1</td>
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<td>Penaeus duorarum</td>
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<td>+</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>Penaeus monodon</td>
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<td>+</td>
<td></td>
<td>1</td>
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<td>Penaeus setiferus</td>
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<td>Penaeus chinensis</td>
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<td>+</td>
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<td>11</td>
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<td>Uca pugnax</td>
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<td>+</td>
<td></td>
<td>12</td>
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<td>Chionoecetes bairdi</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>12</td>
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<tr>
<td>Loxorhynchus grandis</td>
<td>adults</td>
<td>+</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Orconectes virilis</td>
<td>adult</td>
<td>+</td>
<td></td>
<td>6</td>
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<tr>
<td>Sicyonia ingentis</td>
<td>adult</td>
<td>+</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Ovalipes ocellatus</td>
<td>adult</td>
<td>+</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Procambarus clarkii</td>
<td>adult</td>
<td>+</td>
<td>+</td>
<td>15, 16</td>
</tr>
<tr>
<td>Cherax quadrilineatus</td>
<td>adult, larvae</td>
<td>+</td>
<td>+</td>
<td>14</td>
</tr>
</tbody>
</table>

**Non-decapods**

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>MF in hemolymph</th>
<th>MF synthesis by MO</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanus amphitrite</td>
<td>adult</td>
<td>+</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Balanus nubilis</td>
<td>adult</td>
<td>+</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Artemia salina</td>
<td>adult</td>
<td>+</td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>


MF may be a hormone in search of a function. Wilder et al. (1995), among others, remain without definitive conclusions regarding a role for MF because of the complex female reproductive process in Macrobrachium rosenbergii which is interrupted by a molting. This problem does not exist in Libinia females which are only reproductive as terminally molted adults (Laufer et al., 1993, 1997b). Over the past two decades much evidence has emerged, both direct and indirect, supporting a role for MF as a hormone in Crustacea. MF, at the very least, regulates both reproduction and morphogenesis, similar to the function of JH in insects, and MF may in time be found to regulate additional processes.

**ROLE OF METHYL FARNESOATE IN CRUSTACEAN REPRODUCTION**

As previously mentioned, eyestalk ablation of female crustaceans has been known for over 50 years to stimulate ovarian maturation (Panouse, 1943; Brown and Jones, 1947, 1949). More insight into how regulation of crustacean reproduction may be achieved was suggested by Le Roux (1968), who noticed that the mandibular or-
gan (MO) of crustaceans is anatomically homologous to the insect corpora allata (CA) which produces JH. He speculated further that MOs may also be functionally similar to the CA. Hinsch (1980) tested this hypothesis and showed that ovarian development in *Libinia* could be moderately stimulated by implanting mandibular organs from adults into juvenile females. Laufer and colleagues using *Libinia emarginata* showed that MF is synthesized by MOs *in vitro*, and that MF increases in the hemolymph with the onset and development of ovarian maturation, indicating a hormonal role for MF in reproduction (Laufer et al., 1986, 1987).

MF synthetic activity of the MO was found to be lowest in nearly mature juveniles and previtellogenic females and highest in females during vitellogenesis. It declines prior to oviposition. The MF concentration in the hemolymph is also elevated in *Libinia* that are eyestalk ablated compared with non-eyestalk ablated animals (Laufer et al., 1987; Ahl and Laufer, 1996; Jo et al., 1999). MF has also been correlated with increased reproductive behavior and increased reproductive system size in male *Libinia* which exhibit different morphotypes. In *Libinia*, large-clawed old shell males which are approximately one-year or more in this molt stage, and which have abraded exoskeletons, have higher circulating levels of MF, and increased reproductive activity, and larger reproductive systems than their nonabraded, new-shelled counterparts (Homola et al., 1991; Sagi et al., 1993; Laufer et al., 1993; Ahl et al., 1996).

In direct experiments, the injection of MF into eyestalk-less *Libinia* resulted in a barely significant stimulation of the reproductive system, detected by an increase in circulating vitellogenins (Vogel and Borst, 1989). The strongest direct evidence that MF is a reproductive hormone in crustacea, came from maturation trials with the commercial shrimp *Penaeus vannamei*, where MF administration enhanced egg production and resulted in a near doubling of the number of larvae produced (Laufer, 1992; Laufer et al., 1997a). Ovarian development and maturation was also enhanced in the crayfish *Procambarus clarkii*, and the crab *Oziotelphusa senex* (Reddy and Ramamurthi, 1998) with direct MF treatments compared with controls, as shown by significantly increased ovary weights and corresponding increases in gonadosomatic indices (Laufer et al., 1998). These last experiments provide quite direct and fairly strong evidence for MF being a reproductive hormone in Crustacea.

**ROLE OF METHYL FARNESOATE IN CRUSTACEAN MORPHOGENESIS**

**Larval crustaceans**

In insects, the general view is that juvenile hormone maintains larval and nymphal characteristics in developing insects and suppresses metamorphosis into adults. Thus it has gained the name of “status quo hormone” (Riddiford, 1996). In crustaceans, MF appears to display similar effects on morphogenesis in late larval and juvenile stages. As previously mentioned, eyestalk ablation results in increased MF concentrations in the hemolymph (Laufer et al., 1987, 1997a, b). This may help interpret the earlier experiments of Costlow (1968), who showed that eyestalk ablation of larvae of *Callinectes sapidus* results in the formation of larval intermediates. Others have also found that eyestalk ablated larvae of *Homarus americanus* formed intermediates (Hertz and Chang, 1986; Snyder and Chang, 1986). Eyestalk ablation of mud crab larvae *Rhiithropanopeus harrisii* and shrimp *Palaemon macrodactylus* and *Palaemonetes varians*, results in extra larval stages and a consequent delay in metamorphosis (Little, 1969; Freeman and Costlow, 1980). Charmantier et al. (1985) found similar results, in that eyestalk ablation of *H. americanus* larvae before molt stage D1 always produced intermediate larval forms, but ablation after this period did not affect metamorphosis, indicating that larvae respond differently before a critical period of morphogenetic determination than afterwards. Similar results were obtained by Borst et al. (1987) who showed that exogenous MF can retard molting and metamorphosis in late larval stages of *H. americanus*.

In direct experiments recently carried out...
by Abdu et al. (1998) with late larvae of the prawn Macrobrachium rosenbergii, exogenous administration of MF caused the formation of larval intermediates (Fig. 3). In these studies, larvae were treated with different amounts of MF, and endogenous MF was analyzed in the larvae at several larval stages. Raising the concentration of MF administered to the larvae, resulted in an increased abundance of larval intermediates, and brought about a general retardation of larval development.

In studies on barnacles, Yamamoto et al. (1997) have shown that MF also affects the development of the barnacle Balanus amphitrite. In these experiments MF induced metamorphosis, instead of inhibiting it, similar to the effects observed using other juvenoids on barnacle metamorphosis, as mentioned previously (Gomez et al., 1973; Ramenofsky et al., 1974; Tighe-Ford, 1977) (Fig. 2). These novel results have remained unexplained in the literature for many years, but should now be reexamined. A most reasonable explanation seems to lie in differences in the regulatory mechanism existing in different types of arthropod larvae, while they undergo development. Early larvae may undergo MF-dependent differentiation while late larvae and juveniles undergo MF-independent differentiation.

Juvenile and adult crustaceans

Experiments using eyestalk ablation on terminally juvenile female Libinia, resulted in females that remained juvenile after molting instead of becoming adults (Laufer et al., 1997b). Moreover, these eyestalk ablated females were larger juvenile females, instead of becoming adults, as found for control eyestalk intact animals. These morphological differences were also accompanied with high circulating MF concentrations in the eyestalk-ablated animals, indicating that MF maintains juvenile morphological characteristics.

Studies with male Libinia emarginata also point towards an involvement of MF in regulating adult crustacean mating behavior. As previously mentioned, adult male morphotypes have characteristically different MF concentrations. Adult males with abraded or old-shell exoskeletons have larger reproductive systems, larger claws, and higher MF hemolymph concentrations than non-abraded, new-shell males, showing a relationship between MF concentrations and morphotypes. Moreover, male reproductive behavior seems to be enhanced by high MF concentrations (Homola et al., 1991; Sagi et al., 1993; Laufer et al., 1993). A second male morphotype, which is small clawed, small carapaced, and old-shelled, is an active male in isolated mating behavior trials. These males have large gonad indices, and high MF concentrations in the blood, and in competition with large dominant males will “sneak mate” with females. This exceptional situation, completely unexpected, seems to provide strong support for MF being a reproductive hormone in Crustacea.

Effects of MF and JH-active fatty acids on metamorphosis and reproduction of annelids

Since methyl farnesoate is known to affect the larval metamorphoses of crustaceans, the effects of MF on the settlement and metamorphosis of larvae of the marine polychaete annelid Capitella capitata sp. 1 were also investigated (Biggers and Laufer,
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Fig. 4. The development of Capitella through two swimming larval stages is followed by settlement and metamorphosis to crawling worm. This transition can be stimulated by juvenoids in one hour or less. The process of larval maturation without juvenoid stimulation normally takes several days (from Biggers and Laufer, 1996).

1992) (Fig. 4). In nature, metatrochophore larvae of this species respond to chemical cues in the marine sediments, in which they settle and metamorphose (Butman et al., 1988). The surprising results (Biggers and Laufer, 1992; Biggers, 1994) showed that instead of inhibiting settlement and metamorphosis of the larvae in response to a sediment cue, MF was a potent stimulator by itself of settlement and metamorphosis in the absence of sediment. The juvenile hormones, JH I, II, and III, JH-analogs, and polyunsaturated fatty acids (PUFAs) which have JH-activity in Galleria mellonella insect bioassays, were also found to stimulate settlement and metamorphosis of Capitella larvae (Biggers, 1994). One of these PUFAs, 8,11,14-eicosatrienoic acid, has a very high settlement inducing activity on larvae. Since PUFAs are also present in marine sediments, there is a strong possibility that these compounds act as natural settlement cues in the marine environment. Naturally occurring PUFAs in marine sediments have also been identified as larval settlement inducers for other polychaete species as well (Pawlik, 1990).

As previously mentioned, results by Schneiderman and Gilbert (Schneiderman and Gilbert, 1958; Gilbert and Schneiderman, 1961) showed that ether extracts prepared from the polychaete species Glyceria dibranchiata and Nereis virens possessed juvenile-hormone activity in insect bioassays. Biggers (1994) investigated the nature of this activity. Chemical analyses of the extracts by gas chromatography/mass spectrometry failed to show the presence of JHI, JHII, JHIII, or MF. Instead, the JH-activity was found to be due to the presence of relatively high quantities of polyunsaturated fatty acids. Two fatty acids present in particular are, 8,11,14-eicosatrienoic acid and arachidonic acid. These also have the most JH-activity in insect cuticle bioassays. Slama (1962) has also previously reported this high JH-activity of PUFAs. Interestingly, 8,11,14-eicosatrienoic acid has been implicated as a hormone in the marine polychaete Arenicola and is the sperm maturation factor produced by the prostomium (Bentley, 1985; Bentley and Pacey, 1990); both arachidonic and 8,11,14-eicosatrienoic acids were found to induce spawning and sperm maturation in this species (Pacey and Bentley, 1992). These results indicate that these fatty acids have juvenile hormone-like effects in annelids and can be classified as juvenoids, as eicosanoids and arachidonic acids appear to perform similar functions in the two major roles of JH; that is, in larval metamorphosis and in adult reproduction.

At this point, it is worth noting the similar biological functions shared by juvenoids and eicosanoids. As reviewed by Stanley-Samuelson (1994) and Stanley and Howard (1998), eicosanoids help regulate the reproduction and development of nu-
merous invertebrate species. In addition, Leitz et al. (1994) suggest that arachidonic acid and eicosanoid metabolites may function in metamorphosis of a coelenterate Hydrozoan, *Hydractinia echinata*. As another example, prostaglandins induce vitellogenesis and ovulation in crayfish (Spaziani et al., 1995). Of further particular interest to aquaculture, several PUFAs, including arachidonic acid, appear to be the dietary components that help induce ovarian maturation in penaeid shrimp (Middleditch et al., 1980). Thus, the same compounds responsible for sexual maturation and metamorphosis in annelids are used by crustaceans as critical nutritional factors for their sexual maturation and reproduction. Now it looks as though eicosanoids can also be classified as juvenoids, based on their high JH-activity. Other compounds also have JH-like effects. Dennis (1976) reported that a farnesoate derivative increased egg laying and prevented the larval-adult molt in a nematode *Nematospiroides dubius* at low concentrations. Davey and Gordon (1996) suggest that thyroid hormone exhibits JH activity in insect follicle cells, while the effects of thyroxine on ascidian larval settlement and metamorphosis have been known for sometime (Patricolo et al., 1981).

**MECHANISM OF ACTION OF JUVENILE HORMONES AND JH-ACTIVE CHEMICALS**

*Involvement of membrane receptors and protein kinase C*

The mechanisms of action of juvenile hormones are still under investigation in a number of laboratories, and the research results point towards several different mechanisms, not just one (reviewed by Laufer and Biggers, 1992). In one mechanism of action, it appears that JH and JH-active compounds can bind to membrane receptors and activate protein kinase C (PKC), resulting in a signal-transduction cascade leading to cellular effects. Yamamoto et al. (1988) found that the stimulatory effect of JH on protein synthesis in male accessory glands of *Drosophila* is mediated by PKC and calcium. In *Rhodnius prolixias*, JH 1 induces patency in ovaries by binding to a membrane receptor, which stimulates PKC and subsequently activates Na/K ATPase (Sevala and Davey, 1989; Davey et al., 1994; Sevala et al., 1995). Recently, the vertebrate morphogens, triiodothyronine (T3) and thyroxine, members of the thyroid hormone family have been found by Davey and Gordon (1996) to have JH-activity in stimulating patency in follicle cells of *Locusta migratoria* (Fig. 1). Thyroxine bears a considerable structural resemblance to fenoxycarb and pyriproxyfen (Fig. 1) two artificial JH-active compounds. Interestingly, in this respect, thyroxine is known to regulate tail fin regression during the metamorphosis of frog tadpoles. Studies by Petcoff and Platt (1992) have demonstrated that the activation of PKC is involved in this process. In our studies, we have found that MF, juvenile hormones, and also JH-active PUFAs induce settlement and metamorphosis of polychaete annelid larvae through the activation of PKC and subsequent modulation of potassium and calcium ion channels (Biggers and Laufer, 1999). In these studies, we found that these juvenoids can directly activate in vitro both purified rat brain PKC and also a PKC-like enzyme present in *Capitella capitata* sp.I. Previous studies by Shearman (1989a) have also shown that PUFAs, including the eicosanoid fatty acids can directly activate bovine PKC in vitro. Since PKC is known to cause modulation of ion channels through phosphorylation (reviewed by Shearman et al., 1989b), it is of interest that vitellogenin uptake into oocytes, which is stimulated by JH, of the cockroach is inhibited by calcium channel blockers. PKC activation appears also to play a role in crustacean morphogenesis and reproduction. Recent studies by Soroka et al. (2000) have documented changes in the occurrence of PKC isotypes during vitellogenesis of the crayfish *Cerax quadrarcarinatus* during ovarian maturation. MF also induces settlement and metamorphosis of the barnacle *Balanus amphitrite* through PKC activation (Yamamoto, 1997). These results may point to the further involvement of PKC and subsequent modulation of transporters and ion channels in mediating the
actions of juvenoids in reproduction and morphogenesis. It is possible that JH or MF can regulate gene activity through the activation of PKC. PKC is well known to cause activation of nuclear factor-kB (NF-kB) (Ghosh and Baltimore, 1990) and cause activation of the mitogen activated protein kinase pathway (MAP kinase) (Hoshino et al., 1998; Soltoff et al., 1998). It is therefore possible that JH and MF may regulate gene activation during metamorphosis and reproduction through the activation of PKC.

Involvement of nuclear receptors

There is also much evidence supporting a mechanism of action of JH similar to that of steroid hormones which bind nuclear receptors and cause gene activation. Nuclear binding proteins for JH have been detected in a number of insect species (Laufer and Biggers, 1992; Riddiford, 1996), although the functionality of these binding proteins is not known. Recent studies by Jones and Sharp (1997), however, have shown that JH III binds to the *Drosophila* ultraspiracle (USP) receptor, the retinoic acid RXR receptor homolog of insects, promoting USP homodimerization and inducing USP-dependent transcription in a reporter gene assay: this suggests that USP may be a functional nuclear juvenile hormone receptor. Mammalian RXR receptors have also been demonstrated to be activated by the JH-analog methoprene (Harmon et al., 1995) (Fig. 1). It is possible that RXR homologs similar to USP that bind methyl farnesoate may also be found in crustaceans.

A New Look at Larval Metamorphosis and the “Status Quo Hormone”

We arrive here at an interesting juncture. The JH has been called by some the “Status Quo” hormone of insects (Riddiford, 1996). That is, not much is supposed to happen in terms of form changes throughout larval life until the end, when metamorphosis occurs in holometabolous forms, or when wings sprout out of preformed wing pads in hemimetabolous nymphs (Truman and Riddiford, 1999). However, if one looks carefully at larval development of particular insects, each instar is morphologically distinct from every other in several respects. Differentiation is even more distinct when one looks at developmental stages of larval crustaceans, many of which hatch from nauplii; and which undergo continued alterations throughout larval life. Each larval stage is distinct and differentiated from its predecessors. For example, common shrimp have 6 naupliar stages, 3 zoal stages and 3 mysid stages before reaching the post-larval stage (Fig. 5, Table 2). Furthermore, some of these early stages have been found to be unaffected by juvenile hormone treatments, making them resemble the pro-larva or pro-nymphal stages of insects described by Truman and Riddiford (1999). Abdu et al. (1998) showed that treatment of late larval stages of *Macrobrachium rosenbergii* with MF results in the formation of larval-juvenile intermediates (Fig. 3). So one might infer that the same or similar regulatory rules apply in late larval Crustacea as in late larval insects. When we examine barnacle metamorphosis, however, which passes from a cyprid to a juvenile sessile stage, we find it to be stimulated by juvenoids or MF. This presents a somewhat anomalous and unexplained phenomenon. That is, a metamorphosis in this case is promoted by the addition of a juvenoid. This phenomenon was described by Ramenofsky et al. (1974), Gomez et al. (1973), and Tighe-Ford (1977) (Fig. 2). Furthermore, when we examine the situation in *Capitella* settlement and metamorphosis, here too, this is stimulated by juvenoids and JH-active eicosanoids. Here the effect is one of stimulation of metamorphosis, rather than metamorphosis being retarded by a “Status Quo” hormone.

We propose to clarify these events, with an examination of and comparison with early insect larval development, or, rather, pro-larval and pro-nymphal development as recently summarized by Truman and Riddiford (1999). These authors point out the differential responses of early, post-egg emergent stages of hemimetabolous and holometabolous insects. In these stages, early post-embryo insects, molt and differentiate, undergoing progressive larval and nymphal stages in the presence of high concentra-
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Fig. 5. Postembryonic development of larval shrimp (from Treece and Yates, 1993) with many metamorphic changes seen in the early larval. (with permission of the author).

We expand their proposal and extend the concepts limited to insects by Truman and Riddiford (1999) to early Crustacea, and annelids, and perhaps still other larval forms, since early Crustacea larval such as cyprids of barnacles, and trochophores of annelids, appear to respond positively to juvenoids, in that metamorphosis is stimulated or promoted and is not inhibited as in later crustacean larvae. While late larvae of decapod crustaceans are inhibited during metamorphosis by administration of JHs and MF similarly to the situation in stages of late larval insects.

CONCLUSIONS

Some of the studies cited here suggest that there may be additional compounds found among the invertebrates which act as juvenoids in their development, metamorphosis, and reproduction. As also mentioned, eicosanoid fatty acids and the vertebrate hormones T3 and thyroxine have been found to have JH-activity in insects.
### Table 2. Differential regulation by JHs on reproduction and development of insects, crustaceans, and annelids.

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<td>hemimetabolous</td>
<td>eggs</td>
<td>pro-nymph</td>
<td>nymph 1,2,3,4</td>
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<td>holometabolous</td>
<td>eggs</td>
<td>pro-larva</td>
<td>larva 1,2,3,4,5</td>
<td>adult (JH)</td>
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<td>pupa</td>
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<td><strong>Crustacea</strong></td>
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<td>shrimp</td>
<td>egg</td>
<td>nauplius* 1,2,3,4,5,6</td>
<td>zoea 1,2,3*</td>
<td>adult (MF)</td>
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<td>prawns</td>
<td>egg</td>
<td>early larvae* 1,2,3,4,5,6,7,8,9,10,11</td>
<td>late larvae</td>
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<td>crabs</td>
<td>egg</td>
<td>zoea 1,3,3,4*</td>
<td>megalopa</td>
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<td>zoea 3</td>
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<td>larval juveniles</td>
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<td>zoea*</td>
<td>juvenile 1,2,3*</td>
<td>adult (MF)</td>
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<td>cypris</td>
<td>adult (MF)</td>
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<td>metamorphosis to juvenile (MF)</td>
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<td><strong>Polychaete Annelids</strong></td>
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<td><strong>Capitata</strong></td>
<td>egg</td>
<td>trochophore</td>
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<td>metatrochophore</td>
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<td>juvenile (eicosanoids)</td>
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<td><strong>Arenicola marina</strong></td>
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<td>sperm maturation factor</td>
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* not tested.
Retinoic acid (Fig. 1), a retinoid known to regulate vertebrate morphogenesis, has also been found by Nemec et al. (1993) to have juvenilizing effects on developing insects and to stimulate vitellogenesis and egg deposition. Therefore it appears that thyroid hormones, retinoids, juvenile hormones, and eicosanoids all can act as juvenoids and have JH-like effects. It is no wonder that Schneiderman and Gilbert found JH-activity in extracts of so many species! The molecular basis for the JH-activity of these other juvenoids still needs to be elucidated however. Do all of these compounds bind to a common receptor? Are nuclear hormone receptors, which have a close similarity to steroid hormone receptors and bind to similar response elements involved? This topic has been the subject of much investigation in the past, and will certainly lead to further exciting research discoveries in the future.

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