Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Aquaculture 317 (2011) 251–254

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

The exogenous methyl farnesoate does not impact ecdysteroid signaling in the crustacean epidermis in vivo

Michelle Felterman, Enmin Zou *

Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310, USA

ARTICLE INFO

Article history:
Received 7 January 2011
Received in revised form 28 February 2011
Accepted 5 April 2011
Available online 12 April 2011

Keywords:
N-Acetyl-β-glucosaminidase
Ecdysteroid signaling
Methyl farnesoate
RXR
Uca pugilator

ABSTRACT

Methyl farnesoate (MF) produced by the mandibular organ is a crustacean terpenoid hormone involved in the regulation of larval development, reproduction and male morphogenesis. But the receptor for MF has remained unresolved. In view of the fact that MF can bind to crustacean retinoid X receptor (RXR) and that the terpenoid mimic, pyriproxyfen, is capable of altering the expression of crustacean RXR gene, crustacean RXR has been proposed to be a candidate receptor for MF. It is well known that ecdysteroids signal through the ecdysteroid receptor (EcR), which heterodimerizes with the RXR in Crustacea. This study was aimed to investigate whether the exogenous MF impacts ecdysteroid signaling in vivo using *N*-acetyl-β-glucosaminidase (NAG) mRNA from epidermal tissue as a biomarker for ecdysteroid signaling. The NAG mRNA from the model crustacean *Uca pugilator* injected with 0, 0.2, 1, 5, and 20 ng/g wet weight of MF was quantified using quantitative real-time PCR (qRT-PCR). An assay of epidermal NAG activity in crabs injected with 0, 20, and 2000 ng/g wet weight of MF was also performed. The administration of the exogenous MF was found to have no effects on epidermal NAG gene transcription or NAG activity in *U. pugilator*. These results clearly show that MF is not capable of affecting epidermal ecdysteroid signaling in the fiddler crab, *U. pugilator*. Our data are not supportive of the notion that MF signals through the RXR in Crustacea.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Methyl farnesoate (MF) is a crustacean terpenoid synthesized and secreted from the mandibular organ, a homologue of the insect corpora allata (Nagaraju, 2007). The production of MF by the mandibular organ is negatively controlled by the mandibular organ inhibitory hormones produced in the X-organ sinus gland complex in the eyestalk (Landau et al., 1989; Laufer et al., 1987). Insects contain a group of terpenoid compounds, known as juvenile hormones, which regulate metamorphosis and gametogenesis (Laufer et al., 1987). MF is the unepoxidated form of the insect juvenile hormone III (JHIII) (Abdu et al., 1998), and has been shown to exhibit insect JH-like activities in crustaceans. Larvae of the lobster, *Homarus americanus*, showed an increase in time to metamorphosis when exposed to the exogenous MF (Borst et al., 1987). In the freshwater prawn, *Machrobrachium rosenbergii*, exogenous MF resulted in the delay of larval development and morphogenesis and caused an increase in intermediate specimens (Abdu et al., 1998). MF has also been implicated in the regulation of crustacean reproduction, capable of stimulating ovarian maturation and testicular development. An increase in the hemolymph titer of MF was found to coincide with ovarian development in *Libinia emarginata* (Laufer et al., 1987), and injection of the exogenous MF stimulated ovarian maturation and testicular development in *Oziotelphusa senex* (Reddy et al., 2004). Recently, MF has been found to be a crustacean morphogen, involved in controlling adult male morphogenesis in *Procambarus clarkii* (Laufer et al., 2005) and male differentiation in *Daphnia magna* (Olmstead and LeBlanc, 2002). As a result of the endogenous production of MF as well as the evidence of its involvement in larval development, reproduction, and male morphogenesis, MF has been recognized as a genuine crustacean hormone (Laufer and Biggers, 2001; Nagaraju, 2007).

Despite voluminous evidence of MF's hormonal roles in Crustacea, its receptor has not been definitively recognized. In view of the fact that MF can have high affinity binding with the *Drosophila* ultraspiracle (USP), a vertebrate retinoid X receptor (RXR) ortholog (Jones et al., 2006), and that the juvenile hormone mimic pyriproxyfen can alter the expression level of the RXR mRNA in *D. magna*, RXR has been suggested as a candidate receptor for MF in Crustacea (Wang et al., 2007). This suggestion seems to be supported by the results of a receptor binding study. Hopkins et al. (2008) showed that MF can not only bind to the *Uca pugilator* RXR (UpRXR) with high affinity but also influence the binding to an ecdysteroid of the *U. pugilator* ecdysteroid receptor (UpEcR)/UpRXR dimer. But the results of a recent study utilizing luciferase reporter assays run counter to the notion that MF acts through RXR since MF, but not 9-*cis* retinoic acid which is the natural ligand for RXR, failed to transactivate the reporter gene in

* Corresponding author. Tel.: +1 985 448 4711; fax: +1 985 493 2496.
E-mail address: em.zou@nicholls.edu (E. Zou).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.04.009

Author's personal copy

human hepatocellular carcinoma cells (HepG2) transfected with daphnid RXR construct (Wang and LeBlanc, 2009).

It is known that at the cellular level crustacean ecdysteroids signal through binding to the ecdysteroid receptor (EcR), which then heterodimerizes with the RXR (Chung et al., 1998; Durica and Hopkins, 1996). The EcR/RXR heterodimer binds to the DNA response elements of the genes regulated by the molting hormone. Among the products of the genes regulated by ecdysteroids are hydrolytic enzymes necessary for the deconstruction of the old exoskeleton. *N*-Acetyl-β-glucosaminidase (NAG), a chitinolytic enzyme found in the epidermis, is involved in degradation of exoskeletal chitin. There is plenty evidence that the genes encoding epidermal chitinolytic enzymes, including chitinase and NAG, are under control of the molting hormone in arthropods. Injection of 20-hydroxyecdysone significantly increased the enzymatic activity and mRNA of both chitinase and NAG in the epidermis of the tobacco hornworm, *Manduca sexta* (Fukamizo and Kramer, 1987; Kramer et al., 1993; Zen et al., 1996). In crustaceans NAG activity has been found to be correlated with the hemolymph titer of ecdysteroids in *U. pugilator* (Zou and Fingerman, 1999a). Injection of the molting hormone stimulated chitinolytic activity in the integument of the barnacle *Balanus amphitrite* (Freeman, 1980) and elevated the activity of both chitinolytic enzymes in the epidermis of *U. pugilator* (Zou and Bonvillain, 2004; Zou and Fingerman, 1999b). Recent gene expression studies have shown that the administration of 20-hydroxyecdysone significantly upregulated the transcription of NAG gene in the epidermis of *U. pugilator* (Meng and Zou, 2009a,b). Clearly, epidermal chitinolytic enzymes are the products of the genes regulated by the molting hormone in arthropods. The expression of NAG gene in the epidermis of *U. pugilator* constitutes the terminal event of the actions of ecdysteroids, and epidermal NAG mRNA represents a biomarker for ecdysteroid signaling in the epidermis. Therefore, ecdysteroid signaling as measured by NAG mRNA in the epidermis of the fiddler crab is an excellent model for studying the signaling of ecdysteroids as well as MF, a putative ligand for crustacean RXR. The present study was designed to test the notion that MF acts through the RXR using *U. pugilator* as the model crustacean. Our reasoning was that should MF signal through the RXR, the ecdysteroid signaling in the epidermis of the fiddler crab would be impacted after administration of the exogenous MF. Our emphasis was placed on two lines of in vivo evidence, the transcription of NAG gene and the activity of NAG.

2. Materials and methods

Female fiddler crabs were purchased from the Gulf Specimen Marine Laboratories (Panacea, FL). The crabs were distributed into tanks containing artificial seawater made with Instant Ocean synthetic sea salt (Aquarium Systems, Mentor, OH). The animals were maintained under the natural light regime and at a temperature of 19–21 °C. The animals were allowed to acclimate to laboratory conditions at least 5 days before use in an experiment. Since NAG activity in the epidermis varies during the molting cycle (Zou and Fingerman, 1999a), only intermolt crabs were used in the experiment. Molt-staging was performed using the setogenic technique which is based on epidermal retraction and setogenesis in pleopods of female crabs (Vigh and Fingerman, 1985). Since in male crabs the pleopods are modified into copulatory appendages, which makes molt-staging impossible, male crabs were not used.

Quantitative real-time PCR (qRT-PCR) was used to quantify epidermal NAG mRNA from *U. pugilator*. RNeasy mini kit (Qiagen, Valencia, CA) was used to extract the total RNA from epidermal tissues followed by DNase digestion of RNA and RNA cleanup using protocols described in the RNeasy Mini Handbook. RNA concentration was determined by absorption spectrophotometry at a wavelength of 260 nm. Quality of isolated RNA was determined by the ratio of absorbance at 260–280 nm. First strand cDNA was synthesized using the Qiagen QuantiTech Reverse Transcription Kit (Qiagen, Valencia, CA). Two pairs of primers were designed to obtain a PCR product of about 150 base pairs. 18S rRNA gene primers (forward primer: 5 -CTCGGTTCTATTTTGTCGGTTT-3 ; reverse primer: 5 GGCAAATGCTTT-CGCAGTAGT-3), bracketing a sequence of about 150 base pairs, were designed according to the conservative region of the fiddler crab, *Uca princeps* (GenBank accession no. 177767). NAG mRNA primers (forward primer: 5 -GGCATGGCAGCTGCTACA-3 ; reverse primer: 5 -GCGAGCGCTCCAAATTTGT-3) were designed based on the DNA sequencing result (Meng and Zou, 2009a). qRT-PCR was performed according to the protocols described in the QuantiTech SYBR Green PCR kit (Qiagen, Valencia, CA) on the Applied BioSystems StepOne Real-Time PCR System. 18S rRNA gene was used as endogenous control to indicate the NAG induction level in epidermal tissues. Amplification started with 50 °C for 30 min, first denaturing at 95 °C for 15 min, and followed by 40 cycles of amplification (94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s). The sample was increased to 95 °C for 15 s followed by a reduction to 60 °C for 1 min. The melting curve was increased from 60 °C to 95 °C at a rate of 0.1 °C/s. The sample remained at 95 °C for 15 s then was reduced to 40 °C for 15 s. The melting curve ensured that the correct DNA fragments were amplified. The PCR products of 18S rRNA and NAG mRNA have a melting temperature of 80.5 °C and 82.5 °C, respectively.

For the dose–response experiment, 5 intermolt crabs were randomly selected and injected with 0 (control), 0.2, 1, 5, and 20 ng/g wet weight MF, respectively. This experiment was repeated 7 times involving a total of 35 crabs. MF was first dissolved in ethanol, and then one volume of this MF solution in ethanol was mixed with 9 volumes of crab saline to get the injection solution. Control crabs were injected with an appropriate amount of crab saline with 10% v/v ethanol. One and half hours after injection, crabs were sacrificed and epidermal tissues were taken from beneath the carapace. The timing of sample collection was based on the previous finding that NAG gene transcription in the epidermis of *U. pugilator* is responsive to the molting hormone 1.5 h after hormonal treatment (Meng and Zou, 2009a). The total RNA of epidermal tissues from each crab was extracted and NAG mRNA quantified using the method described above. The relative quantity values of NAG mRNA were calculated using $2^{\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

To investigate whether the exogenous MF affects NAG activity, three groups of 25 intermolt crabs each were injected with 0 (control), 20, and 2000 ng/g wet weight MF, respectively, on day 0. On day 2, crabs were injected again with the same dosage of MF. After an additional 2 days, all survivors were sacrificed and epidermal tissue from beneath the carapace was extracted and homogenized on ice in 0.15 M pH 5.5 citrate-phosphate buffer containing proteinase inhibitor cocktail (Sigma, St. Louis, MO). After centrifugation at 10,000 *g* for 3 min, 20 μL of supernatant was incubated with 100 μL of 2 mM 4-nitrophenyl *N*-acetyl-β-D-glucosaminide (Sigma, St. Louis, MO), a specific substrate for NAG, at 25 °C for 20 min. The reaction was stopped by the addition of 0.9 mL 0.5 M NaOH. The liberated nitrophenol was quantified at 405 nm with the Beckman DU730 Life Science UV/VIS Spectrophotometer. Protein concentrations in the supernatant were determined using the Bradford method. Enzymatic activity was expressed as μmol nitrophenol liberated (μg protein)$^{-1}$ (20 min)$^{-1}$ (Zou, 2009).

One-way analysis of variance (ANOVA) was used to determine the impacts of hormonal treatment on epidermal NAG mRNA and enzymatic activity. A probability value of less than 0.05 was deemed significant.

3. Results and discussion

No statistically significant difference was observed in NAG mRNA from the epidermis of control crabs and those injected with 0.2, 1, 5, or 20 ng/g MF ($p = 0.948$, Fig. 1), suggesting that the exogenous MF does

Author's personal copy

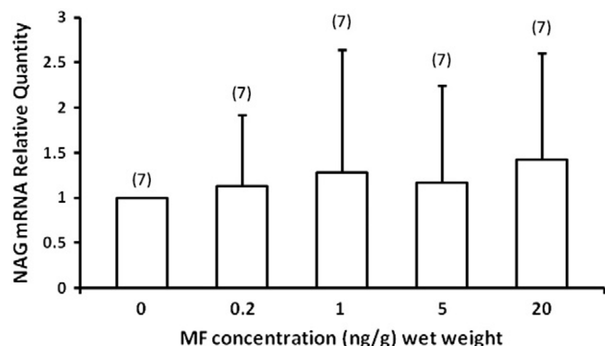

Fig. 1. Effects of methyl farnesoate (MF) on the mRNA of N-acetyl-β-glucosaminidase (NAG) in the epidermis of *Uca pugilator*. NAG mRNA is expressed in relative quantity. Sample size is shown in parenthesis. Error bars represent standard deviation.

not affect epidermal NAG mRNA. At the enzymatic level, no significant difference was seen between NAG activities in the epidermis of control crabs and those receiving two injections of 20 or 2000 ng/g MF either ($p = 0.430$, Fig. 2).

In the present study we show that the exogenous MF does not impact epidermal ecdysteroid signaling as measured by NAG mRNA in the fiddler crab. The validity of our finding depends, to a certain degree, on whether the MF doses used for injection are physiologically relevant. To our knowledge, there is still no data on MF titers in the hemolymph of *U. pugilator*. The selection of MF injection doses was based on the range of MF concentrations, <0.4–140 ng/mL, in the hemolymph of various decapod crustaceans (Nagaraju, 2007). Since the blood volume of various decapods varies between 10 and 50% of the wet weight and the density of the blood in various decapods ranges from 1.025 to 1.052 (Maynard, 1960), a mean blood volume/wet weight ratio of 30% and a blood density of 1.0 can be used to estimate the total blood volume of *U. pugilator*. For instance, a crab of 2.0 g wet weight is estimated to have a blood volume of 0.6 mL. An injection dose at 1 ng/g wet weight would give rise to an MF concentration of about 3.3 ng/mL in the hemolymph. The injection doses of 0.2, 1.0, 5.0 and 20 ng/g wet weight for qRT-PCR experiments would give rise to estimated MF hemolymph concentrations of 0.7, 3.3, 17 and 67 ng/mL, respectively, well overlapping the reported MF range for decapods. The greater MF dose, 2000 ng/g wet weight, used for NAG activity induction experiment, which would result in an estimated MF concentration far exceeding the physiological range, was chosen to examine whether treatment with an excessive amount of MF would elicit any pharmacological effects on epidermal ecdysteroid signaling. The fact that none of the MF injection doses impacted NAG mRNA in the epidermis or affected epidermal NAG activity strongly indicates that MF has no bearing on epidermal ecdysteroid signaling as measured by NAG mRNA in *U. pugilator*.

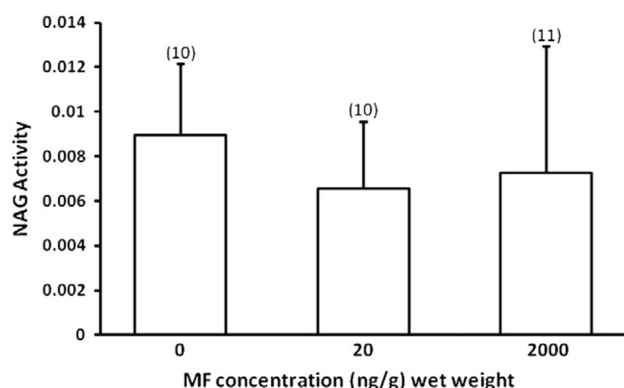

Fig. 2. Effects of methyl farnesoate (MF) on activity of N-acetyl-β-glucosaminidase (NAG) in the epidermis of *Uca pugilator*. Enzymatic activity was expressed as μmol nitrophenol liberated (μg protein)$^{-1}$(20 min)$^{-1}$. Sample size is shown in parenthesis. Error bars represent standard deviation.

Although it is well known that MF functions as a hormone in Crustacea, its receptor remains equivocal. As mentioned above, because of the high affinity of MF for crustacean RXR and effects of a terpenoid mimic on the dynamics of daphnid RXR gene expression, crustacean RXR has been proposed to be a candidate receptor for MF (Wang et al., 2007). In vertebrates, RXR, also known as the 9-*cis* retinoic acid receptor, can form homodimer RXR/RXR as well as heterodimers with dimerizing partners, such as retinoic acid receptors (RARs), thyroid hormone receptors (TRs) and peroxisome proliferator-activated receptors (PPARs) (Chambon, 1996; Laflamme et al., 2002; Mangelsdorf et al., 1995). The formation of RXR homodimer can be triggered by 9-*cis* retinoic acid (Zhang et al., 1992), and this homodimer can bind to the promoter and induce gene activation *in vivo* (IJpenberg et al., 2004). These results suggest that the RXR/RXR dimer can mediate the signaling of 9-*cis* retinoic acid in vertebrates. The question here is: does crustacean RXR mediate the signaling of MF? The reporter gene assays performed with HepG2 cells transfected with the daphnid RXR have shown that MF, but not 9-*cis* retinoic acid, is inactive in transactivating the reporter gene, suggesting crustacean RXR alone cannot mediate MF signaling (Wang and LeBlanc, 2009). Given that the presence of MF is known to be capable of altering the affinity of the EcR/RXR for the molting hormone (Hopkins et al., 2008), the next question becomes: can MF signal through the heterodimer EcR/RXR in Crustacea? The *in vitro* results seem to suggest that MF may signal through the EcR/RXR heterodimer. Wang and LeBlanc (2009) found that MF at 10 μM or 2500 ng/mL, but not at 1 μM, can enhance gene transactivation induced by 20-hydroxyecdsone in HepG2 cells transfected with both daphnid EcR and daphnid RXR. However, our *in vivo* data clearly show that MF does not impact epidermal ecdysteroid signaling. The discrepancy between our results and those of Wang and LeBlanc (2009) may lie in the fact the reporter gene assays were performed with human cancer cells, whose molecular make-up may be different from that of crustacean cells. Additionally, two RXR isomers, UpRXR (−33) and UpRXR (+33), have been discovered in *U. pugilator* (Wu et al., 2004), and the presence of MF can enhance the affinity of UpEcR/UpRXR (−33) for ponasterone A but reduce the affinity of UpEcR/UpRXR (+33) for such an ecdysteroid (Hopkins et al., 2008). It is likely that the cellular effects mediated by these two heterodimers would cancel out each other when MF is present, which could account for the absence of a significant effect of MF on epidermal ecdysteroid signaling. Even if MF were capable of affecting ecdysteroid signaling through its interaction with crustacean RXR in the EcR/RXR heterodimer complex, the RXR should still not be regarded as the cognate receptor for MF, and MF is at most a modifier of molting hormone signaling. Therefore, the results of the present study, along with the finding that MF cannot activate RXR-mediated gene transcription (Wang and LeBlanc, 2009), are not supportive of the notion that crustacean RXR is the receptor for MF.

If MF does not signal through the nuclear receptor RXR, then what receptor possibly mediates the MF signaling? It is generally known that hydrophobic hormones, such as steroids, render their biological effects through interactions with intracellular receptors, while water-soluble hormones signal through the receptors in the plasma membrane. It has recently been discovered that steroid hormones, such as progestin, estrogen and androgen, can signal through their respective membrane-bound receptors in vertebrates (Thomas et al., 2006). There is also evidence pointing to the existence of the membrane receptor for the molting hormone in insects (Elmogy et al., 2006; Srivastava et al., 2005). So, if hydrophobic steroid hormones can signal through membrane receptors, why limit the search for the terpenoid hormone receptor to the nuclear receptor?

Author's personal copy

Acknowledgements

This investigation was supported by the National Science Foundation EPSCoR Pfund grant NSF(2010)-PFUND-208 and the Louisiana sea grant Undergraduate Research Opportunities Program (UROP).

References

Abdu, U., Takac, P., Laufer, H., Sagi, A., 1998. Effect of methyl farnesoate on late larval development and metamorphogenesis in the prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae): a juvenoid-like effect? Biol. Bull. 195, 112–119.

Borst, D.W., Laufer, H., Landau, M., Chang, E.S., Hertz, W.A., Baker, F.C., Schooley, D.A., 1987. Methyl farnesoate and its role in crustacean reproduction and development. Insect Biochem. 17, 1123–1127.

Chambon, P., 1996. A decade of molecular biology of retinoic acid receptors. FASEB J. 10, 940–954.

Chung, A.C.K., Durica, D.S., Clifton, S.W., Roe, B.A., Hopkins, P.M., 1998. Cloning of crustacean ecdysteroid receptor and retinoid-X receptor gene homologs and elevation of retinoid-X receptor mRNA by retinoic acid. Mol. Cell. Endocrinol. 139, 209–227.

Durica, D.S., Hopkins, P.M., 1996. Expression of the genes encoding the ecdysteroid and retinoid receptors in regenerating tissues of the fiddler crab, *Uca pugilator*. Gene 171, 237–241.

Elmogy, M., Terashima, J., Iga, M., Iwami, M., Sakurai, S., 2006. A rapid increase in cAMP in response to 20-hydroxyecdysone in the anterior silk glands of the silkworm, *Bombyx mori*. Zoolog. Sci. 23, 715–719.

Freeman, J.A., 1980. Hormonal control of chitinolytic activity in the integument of *Balanus amphitrite*, in vitro. Comp. Biochem. Physiol. Part A 65, 13–17.

Fukamizo, T., Kramer, K.J., 1987. Effect of 20-hydroxyecdysone on chitinase and β-N-acetyl-glucosaminidase during the larval–pupal transformation of *Manduca sexta* (L.). Insect Biochem. 17, 547–550.

Hopkins, P.M., Durica, D., Washington, T., 2008. RXR isoforms and endogenous retinoids in the fiddler crab, *Uca pugilator*. Comp. Biochem. Physiol. Part A 151, 602–614.

IJpenberg, A., Tan, N.S., Gelman, L., Kerten, S., Seydoux, J., Xu, J., Metzger, D., Canaple, L., Chambon, P., Wahli, W., Desvergne, B., 2004. In vivo activation of PPAR target genes by RXR homodimers. EMBO J. 23, 2083–2091.

Jones, G., Jones, D., Teal, P., Sapa, A., Wozniak, M., 2006. The retinoid-X receptor ortholog, ultraspiracle, binds with nanomolar affinity to an endogenous morphogenetic ligand. FEBS J. 273, 4983–4996.

Kramer, K.J., Corpuz, L., Choi, H.K., Muthukrishnan, S., 1993. Sequence of a cDNA and expression of the gene encoding epidermal and gut chitinases of *Manduca sexta*. Insect Biochem. Mol. Biol. 23, 691–701.

Laflamme, L., Hamann, G., Messier, N., Maltais, S., Langlois, M.-F., 2002. RXR acts as a coregulator in the regulation of genes of the hypothalamo-pituitary axis by thyroid hormone receptors. J. Mol. Endocrinol. 29, 61–72.

Landau, M., Laufer, H., Homola, E., 1989. Control of methyl farnesoate in the mandibular organ of the crayfish *Procambarus clarkii*: evidence for peptide neurohormones with dual functions. Invert. Reprod. Dev. 16, 165–168.

Laufer, H., Biggers, W.J., 2001. Unifying concepts learned from methyl farnesoate for invertebrate reproduction and post-embryonic development. Amer. Zool. 41, 442–457.

Laufer, H., Borst, D.W., Baker, F.C., Carrasco, C., Sinkus, M., Rueter, C.C., Tsai, L.W., Schooley, D.A., 1987. Identification of a juvenile hormone-like compound in a crustacean. Science 235, 202–205.

Laufer, H., Demir, N., Pan, X., Stuart, J.D., Ahl, J.S.B., 2005. Methyl farnesoate controls adult male morphogenesis in the crayfish, *Procambarus clarkii*. J. Insect Physiol. 51, 379–384.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., et al., 1995. The nuclear receptor superfamily: the second decade. Cell 83, 835–839.

Maynard, D.M., 1960. Circulation and heart function. In: Waterman, T.H. (Ed.), The Physiology of Crustacea, Metabolism and Growth, vol. 1. Academic Press, New York, pp. 161–226.

Meng, Y., Zou, E., 2009a. A molecular biomarker for disruption of crustacean molting: the N-acetyl-β-glucosaminidase mRNA in the epidermis of the fiddler crab. Bull. Environ. Contam. Toxicol. 82, 554–558.

Meng, Y., Zou, E., 2009b. Impacts of molt-inhibiting organochlorine compounds on epidermal ecdysteroid signaling in the fiddler crab, *Uca pugilator*, in vitro. Comp. Biochem. Physiol. 150C, 436–441.

Nagaraju, G.P.C., 2007. Is methyl farnesoate a crustacean hormone? Aquaculture 272, 39–54.

Olmstead, A.W., LeBlanc, J.A., 2002. Juvenoid hormone methyl farnesoate is a sex determinant in the crustacean *Daphnia magna*. J. Exp. Zool. 293, 736–739.

Reddy, P.R., Nagaraju, G.P.C., Reddy, P.S., 2004. Involvement of methyl farnesoate in the regulation of molting and reproduction in the freshwater crab *Oziotelphusa senex senex*. J. Crust. Biol. 24, 511–515.

Srivastava, D.P., Yu, E.J., Kennedy, K., Chatwin, H., Reale, V., Hamon, M., Smith, T., Evans, P.D., 2005. Rapid, nongenomic responses to ecdysteroids and catecholamines mediated by a novel *Drosophila* G-protein-coupled receptor. J. Neurosci. 25, 6145–6155.

Thomas, P., Dressing, G., Pang, Y., Berg, H., Tubbs, C., Benninghoff, A., Doughty, K., 2006. Progestin, estrogen and androgen G-protein coupled receptors in fish gonads. Steroids 71, 310–316.

Vigh, D.A., Fingerman, M., 1985. Molt staging in the fiddler crab *Uca pugilator*. J. Crust. Biol. 5, 386–396.

Wang, Y.H., LeBlanc, G.A., 2009. Interactions of methyl farnesoate and related compounds with a crustacean retinoid X receptor. Mol. Cell. Endocrinol. 309, 109–116.

Wang, Y.H., Wang, G., LeBlanc, G.A., 2007. Cloning and characterization of the retinoid X receptor from a primitive crustacean *Daphnia magna*. Gen. Comp. Endocrinol. 150, 309–318.

Wu, X., Hopkins, P.M., Palli, S.R., Durica, D.S., 2004. Crustacean retinoid-X receptor isoforms: distinctive DNA binding and receptor-receptor interaction with a cognate ecdysteroid receptor. Mol. Cell. Endocrinol. 218, 21–38.

Zen, K.-C., Choi, H.K., Krishnamachary, N., Muthukrishnan, S., Kramer, K.J., 1996. Cloning, expression, and hormonal regulation of an insect β-N-acetylglucosaminidase gene. Insect Biochem. Mol. Biol. 26, 435–444.

Zhang, X.K., Lehmann, J., Hoffman, B., Dawson, M.I., Cameron, J., Graupner, G., Herman, T., Tran, P., Pfahl, M., 1992. Homodimer formation of retinoid X receptor induced by 9-*cis* retinoic acid. Nature 358, 587–591.

Zou, E., 2009. Effects of hypoxia and sedimentary naphthalene on the activity of N-acetyl-β-glucosaminidase in the epidermis of the brown shrimp, *Penaeus aztecus*. Bull. Environ. Contam. Toxicol. 82, 579–582.

Zou, E., Bonvillain, R., 2004. Chitinase activity in the epidermis of the fiddler crab, *Uca pugilator*, as an in vivo screen for molt-interfering xenobiotics. Comp. Biochem. Physiol. Part C 139, 225–230.

Zou, E., Fingerman, M., 1999a. Chitobiase activities in the epidermis and hepatopancreas of the fiddler crab, *Uca pugilator*, during the molting cycle. Mar. Biol. 133, 97–101.

Zou, E., Fingerman, M., 1999b. Patterns of N-acetyl-β-glucosaminidase isoenzymes in the epidermis and hepatopancreas and induction of N-acetyl-β-glucosaminidase activity by 20-hydroxyecdysone in the fiddler crab, *Uca pugilator*. Comp. Biochem. Physiol. Part C 124, 345–349.