ARTICLE IN PRESS
CBC-07429; No of Pages 4
Comparative Biochemistry and Physiology, Part C xxx (2008) xxx–xxx

Contents lists available at ScienceDirect
Comparative Biochemistry and Physiology, Part C
journal homepage: www.elsevier.com/locate/cbpc

Effect of an insect juvenile hormone analogue, Fenoxycarb® on development and oxygen uptake by larval lobsters *Homarus gammarus* (L.)

Katie E. Arnold [a,b], Colin Wells [b], John I. Spicer [a,*]

[a] Marine Biology and Ecology Research Centre, School of Biological Sciences, University of Plymouth, Plymouth, PL4 8AA, Devon, UK
[b] National Lobster Hatchery, Padstow, PL28 8BL, Cornwall, UK

A R T I C L E I N F O

Article history:
Received 21 May 2008
Received in revised form 12 September 2008
Accepted 13 September 2008
Available online xxxx

Keywords:
Endocrine disruption
Fenoxycarb®
Homarus gammarus
Hormone analogue
Insect juvenile hormone
Larval development
Oxygen uptake

A B S T R A C T

Little attention has been focused on the effect of anthropogenic compounds that disrupt the endocrine systems in crustaceans. Consequently, this study investigated the effects of the juvenile hormone analogue (JHA), Fenoxycarb® on selected physiological and developmental processes of the zoeal stages in the European lobster, *Homarus gammarus*. Chronic exposure to Fenoxycarb (50µg L^{-1}) resulted in a significant ($p < 0.05$) reduction in moult frequency and size at moult. Fenoxycarb exposure extended zoeal duration between zoea I to II ($p<0.05$) and resulted in total inhibition of the moult from zoea II to III. Significantly greater rates of O_2 uptake were observed in Fenoxycarb-exposed larvae in comparison with controls ($p<0.05$). All rates of O_2 uptake decreased significantly between 7 and 12d of exposure ($p<0.05$). At 12d, exposure to the solvent control no longer influenced rates of O_2 uptake, but it was not possible to attribute increased O_2 uptake to Fenoxycarb exposure directly, as treated individuals did not moult beyond zoea III. The low exposure concentrations of Fenoxycarb, comparable to those used in plant protection, resulted in endocrine disrupted responses in *H. gammarus* (albeit with little clear, demonstrable effect on metabolism) a finding that could have important ecological and commercial implications.

© 2008 Published by Elsevier Inc.

1. Introduction

The European lobster, *Homarus gammarus*, is a commercially important species (Wahle and Fogarty, 2006); therefore understanding of potential threats to lobster fisheries, particularly those that might affect larval recruitment, is paramount. Larval lobsters may be exposed to pollutants more as they inhabit the pelagic zone of shallow, coastal waters in regions that are heavily populated and subject to anthropogenic inputs from industry or agriculture (Timbrell, 2002). To reduce the impacts of pollutants such as organophosphates, more selective ‘third-generation’ pesticides have been developed (Grenier and Grenier, 1993) for use as biological control agents, due to their actions upon the recently elucidated insect neuroendocrine system (McKenney and Celestial, 1996). The most extensive group of synthetic insect growth regulators (IGRs) contains juvenile hormone analogues (JHA), acting by mimicry of juvenile hormones, which are integral for successful insect development (McKenney and Celestial, 1996). Specificity of JHAs results in lower toxicity to vertebrates (especially fish) but still retain toxicity against closely related species to the target organism, particularly other arthropods such as crustaceans (McKenney et al., 2004). JHA residues from pest control can enter the marine environment, either through direct application toward pests such as mosquitoes, or indirectly through agricultural/urban run-off (Key and Scott, 1994). There is some evidence of the disruption of crustacean development by JHAs, mainly as a result of their increasing the time taken to metamorphosis (Horst and Walker, 1999; McKenney, 2005), but nothing is known of their effect on metabolism. Therefore it is important to assess the potential impact of JHA residues in coastal waters on non-target commercially important marine species utilizing this area during early development (Key and Scott, 1994).

In the present study, the effect of JHAs on both growth and metabolism (using rates of O_2 uptake as a surrogate measure) of the zoeal (larval) stages of *H. gammarus* were investigated. Fenoxycarb, the insecticide used in this study, is a non-neurotoxic carbamate, belonging to one of the five different synthetic JHAs currently used in insect growth regulation (Van Lenteren, 1999) and shows strong JH-activity (McKenney et al., 2004).

2. Materials and methods

2.1. Animal material

Ovigerous, female, adult lobsters, *H. gammarus* supplied by local fishermen, were held in large aquaria at the National Lobster Hatchery (NLH) in Padstow, Cornwall. Each aquarium was constantly supplied with aerated, filtered re-circulating sea water (T = 19±1°C, S = 35) pumped directly from waters adjacent to the NLH. Water was pre-treated through a pressurised sand filter, passed through activated carbon, and UV irradiated. Adult lobsters were fed *ad libitum* with blue

* Corresponding author.
E-mail address: jispicer@plymouth.ac.uk (J.I. Spicer).

1532-0456/$ – see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.cbpc.2008.09.007

ARTICLE IN PRESS

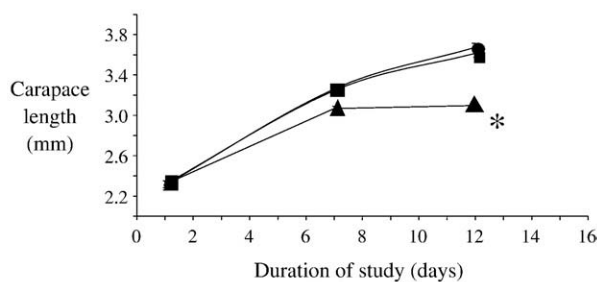

Fig. 1. Larval size after moult measured by length of the carapace (mm) of various aged *H. gammarus* larvae as influenced by continuous exposure to 50 μg L^{-1} Fenoxycarb. Values represent mean (n=40)±standard error; circles, control; squares, solvent control; triangles, Fenoxycarb. Asterisk denotes significant differences from control (p<0.05).

mussels, *Mytilus edulis*. When required, newly hatched, free-living larvae were removed from aquaria and used as described below.

2.2. Experimental procedure

Newly hatched larvae were carefully distributed between a number of aquaria (flasks vol. = 1L, n = 50 larvae per flask, T = 19±1°C), which contained one of the following aerated media: sea water ('untreated control'), sea water with solvent ('solvent control', see below for details); and Fenoxycarb solution ('treatment') (n = 3 for each treatment). Media changes were performed every 24h at which time moults and mortalities were removed. Larvae were fed after media changes using *Artemia spp.* nauplii (5 indiv mL^{-1} sea water) (Carlberg and Van Olst, 1976).

Individual larvae were removed at three different times (1d, 7d and 12d after exposure) and at random from each treatment. Larval stage was determined using Aiken (1973) and carapace length measured.

2.3. Preparation of test solutions

The Fenoxycarb treatment was prepared using Insegar® (Syngenta Crop Protection, Cambridge, UK), which contains 25% (w/w) of the active ingredient (a.i.) Fenoxycarb. Pre trial experiments found that Fenoxycarb was insoluble in water, therefore primary stock solutions were prepared by dissolving crushed Insegar® pellets (0.1g) into an acetone:water mixture (50/50) to a final concentration of 1mg mL^{-1} (0.25mg mL^{-1} a.i.). Exposure solutions were then constructed by diluting the stock solution with sea water. A preliminary experiment was carried out in order to determine a sublethal concentration of Fenoxycarb for use in the treatment described above, using a range of nominal concentrations (10, 20, 50, and 100μg L^{-1}) for 96h. From this test, and taking cognisance of previous studies on Fenoxycarb shown to elicit significant effects on growth and development in static renewal exposures (Cripe et al., 2003; McKenney et al., 2004), a nominal exposure concentration of 50μg Fenoxycarb L^{-1} was decided upon for the experiments. An acetone carrier solvent control was also constructed by adding acetone (Analar grade, vol. = 1mL) to sea water (1:1000 dilution).

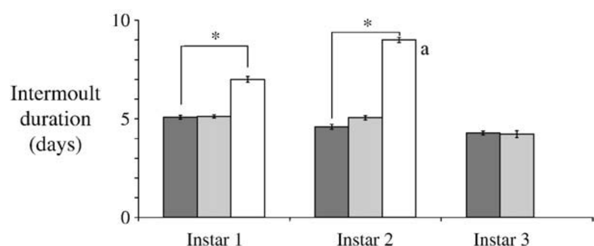

Fig. 2. Zoeal duration of *H. gammarus* throughout complete larval development (16 d) under continuous exposure to 50 μg L^{-1} Fenoxycarb. Values (n=40)±standard error; dark grey bar, control; light grey bar, solvent control; white bar, Fenoxycarb-exposed. Asterisk denotes significant differences from control (p< 0.05). [a] denotes failure to moult through to stage III the end of the experiment.

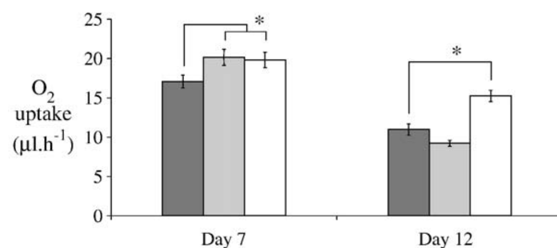

Fig. 3. Rates of O_2 uptake of various aged *H. gammarus* larvae as influenced by continuous exposure to 50 μg L^{-1} Fenoxycarb: bars represent the mean of 8 determinations for each± standard error; dark grey bar, control; light grey bar, solvent control; white bar, Fenoxycarb-exposed. Asterisk denotes significant differences from control (p< 0.05).

2.4. Measurement of O_2 uptake

Rates of O_2 uptake (as a measure of aerobic metabolism) of randomly selected individuals from each treatment (7d and 12d after exposure), were measured using a well-established, closed respirometry technique used previously to investigate the O_2 uptake of larvae of the Norway lobster, *Nephrops norvegicus* (Spicer and Eriksson, 2003). This protocol was followed precisely using exactly the same equipment (only at T = 19±1°C).

3. Results

There was a clear effect of developmental time and treatment on the size of larvae (2-way ANOVA, p<0.05 in each case). While there was no significant treatment effect after 1d, Fenoxycarb-exposed larvae were significantly smaller than either of the controls after 7d and to an even greater extent after 12d exposure (Fig. 1). There was also a significant effect of Fenoxycarb upon the duration of the first two zoeal stages (1 way ANOVA, p<0.05 in each case); 2d delay in moulting between zoea I and II and a 4d delay between zoea II and III (Fig. 2). By day 16 all larvae in control treatments (untreated and solvent) had metamorphosed into post-larvae. In contrast none of the Fenoxycarb larvae progressed beyond zoea II, hence the absence of data for zoea III in Fig. 2.

There was a significant effect of treatment and time on the O_2 uptake of larvae (2-way ANOVA, p<0.05 in each case, see Fig. 3). Although there was no significant difference between Fenoxycarb treated and solvent control individuals at 7d, both were significantly different (i.e. greater) than the untreated control. All of these values decreased significantly by 12d. However, there was no significant difference in O_2 uptake between the controls (untreated and solvent) but the controls were significantly different (i.e. lower) than the Fenoxycarb treatment (ANOVA, p >0.05). However, it should be noted that the comparison of 7d and 12d individuals is confounded by the fact that while control individuals were all zoea III, Fenoxycarb treated individuals were still zoea II (and did not moult subsequently). Thus if we replace the time at which the measurements were made with moult stage in our 2-way ANOVA, there is still a significant effect of treatment and developmental stage.

4. Discussion

Chronic exposure of larval lobsters to Fenoxycarb resulted in delayed moulting, and metamorphosis. The second zoeal was the most sensitive stage to toxicity from low concentrations of Fenoxycarb, with inhibition of moult into the third zoeal throughout the remaining duration of the experiment. Such JHA-related modifications in development, often at exposure concentrations similar to those used in this study, have been reported for a variety of crustaceans, including larval stages (Key and Scott, 1994; Hosmer et al., 1998; Nates and McKenney, 2000; Cripe et al., 2003; McKenney et al., 2004; Tuberty and McKenney, 2005).

Please cite this article as: Arnold, K.E., et al., Effect of an insect juvenile hormone analogue, Fenoxycarb® on development and oxygen uptake by larval lobsters *Homarus gammarus* (L.), Comp. Biochem. Physiol. Part C (2008), doi:10.1016/j.cbpc.2008.09.007

ARTICLE IN PRESS

During a time of morphological change, metabolic activity and energy utilization of an organism can be significantly altered (Capuzzo and Lancaster, 1979); therefore the higher rates of O_2 uptake in the 7d old larvae may be attributed to an increase in physiological processes in early larval stages. Processes regulated by hormones in crustaceans, such as energetic or metabolic activities, are believed to be sensitive to hormone disruption (McKenney, 1999). Rates of O_2 uptake in larvae exposed to Fenoxycarb were significantly increased, indicating a similar mode of action of insect JHAs on the endocrine system in crustaceans. Elevated respiratory rates were also observed in the larval shrimp *Palaemonetes pugio*, when exposed to the JHA methoprene (McKenney and Celestial, 1993). However, in the present study it was difficult to disentangle whether the increase in O_2 uptake was due to the duration of the exposure to Fenoxycarb or the developmental stage.

As the exposed larvae (12d) were still in the second zoeal of development, Fenoxycarb exposure resulted in immaturity and a prolonged state of morphogenesis. Similar relationships with O_2 have been shown to exist in larval crustaceans exposed to several organic xenobiotics, including petroleum hydrocarbons and various pesticides (Capuzzo et al., 1984).

Exposure to Fenoxycarb, throughout entire larval development in *H. gammarus*, caused a considerable delay to moult up to zoea II, with 100% incomplete metamorphosis to post-larval stage. Significant reductions in carapace length at each moult stage were also observed in exposed larvae, indicating that JHAs can display damaging effects as early as 7d into larval development. Previous studies have shown that sublethal exposure to Fenoxycarb caused impairment in growth in the mud crab *Rhithropanopeus harrisii* (Nates and McKenney, 2000). McKenney and Celestial (1993) found that reduced growth rates in larval shrimp corresponded with higher respiratory rates in exposed larvae, which supports results in this study. At day 12, the significantly reduced growth rate in larval *H. gammarus* is a result of the disruption to the moult cycle. Elevated respiration rates that were apparent at day 12 are also indicative of increased metabolic demands which allow for less assimilated energy available for growth (McKenney and Celestial, 1993). Juvenile hormones (JH) regulate developmental mechanisms such as growth and moulting (Chang, 1997), therefore exposure to a JHA may alter these JHs prior to completion of metamorphosis (Leonardi et al., 1996).

Insect growth regulators are used in nature to inhibit insect metamorphosis from larvae to adult working by a progressive lowering of the JH level through the larval stages (Downer and Laufer, 1983). As both insects and crustaceans possess the same moulting hormone 20-OH-ecdysone (Skinner, 1985), the evident incompletion of metamorphosis in Fenoxycarb-exposed *H. gammarus* larvae could indicate similar action to that seen in insects. Inhibition of successful completion to metamorphosis has also been observed in *P. pugio* (McKenney and Celestial, 1993) and *Homarus americanus* (Walker et al., 2005) when exposed to low levels of methoprene. If the period of larval development in *H. gammarus* is prolonged or prohibited completely in the pelagic zone, their population sustainability may become adversely affected, due to the higher risk of predation and exposure to further pollutants (Cripe et al., 2003).

There are difficulties associated with the toxicity of Fenoxycarb, due to the more insidious nature of its mode of action, and problems related with analytical detection (Grenier and Grenier, 1993), especially as effects are significant at low, continuous exposure concentrations (Retnakaran et al., 1985). The long-term persistence and efficiency of Fenoxycarb may trigger bioaccumulation further down the food chain, with effects on non-target biota potentially altering recruitment and impacting future populations (Grenier and Grenier, 1993). To our knowledge, there are no restrictions for the use of Fenoxycarb in the UK (PAN Pesticides Database, 2007). Therefore environmentally realistic concentrations that are effective against developing larvae of various insect pests (Hosmer et al., 1998) are comparable to the low values used in this study that cause chronic effects to larval lobsters. Studies like this will hopefully aid in the reassessment of FAO guidelines, and this research strongly denotes the importance of further evaluation into the potential impacts of JHA pesticides upon this ecologically and commercially important group of non-target marine invertebrates.

Acknowledgements

We thank Dom Boothroyd, Carly Daniels, and Rory Prior of the National Lobster Hatchery, Padstow for their assistance throughout these experiments.

References

Aiken, D.E., 1973. Proecdysis, setal development, and molt prediction in the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 30, 1337–1344.

Carlberg, J.M., Van Olst, J.C., 1976. Brine shrimp (*Artemia salina*) consumption by the larval stages of the American lobster (*Homarus americanus*) in relation to food density and water temperature. Proc. Annu. Meet.—World Maric. Soc. 7, 379–389.

Capuzzo, J.M., Lancaster, B.A., 1979. Some physiological and biochemical considerations of larval development in the American lobster, *Homarus americanus*. J. Exp. Mar. Biol. Ecol. 40, 53–62.

Capuzzo, J.M., Lancaster, B.A., Sasaki, G.C., 1984. The effects of petroleum hydrocarbons on lipid metabolism and energetics of larval development and metamorphosis in the American lobster (*Homarus americanus* Milne Edwards). Mar. Environ. Res. 14, 201–228.

Chang, E.S., 1997. Chemistry of crustacean hormones that regulate growth and reproduction. In: Fingerman, R., Nagabhushanam, R., Thompson, M. (Eds.), Recent Advances in Marine Biotechnology, Endocrinology and Reproduction, vol. 1. Science Publishers, USA, pp. 163–178.

Cripe, G.M., McKenney Jr., C.L., Hoglund, M.D., Harris, P.S., 2003. Effects of Fenoxycarb exposure on complete larval development of the xanthid crab, *Rhithropanopeus harrisii*. Environ. Pollut. 125, 295–299.

Downer, R.G.H., Laufer (Eds.), 1983. Endocrinology of Insects. Alan R. Liss, Inc., New York.

Grenier, S., Grenier, A.N., 1993. Fenoxycarb, a fairly new insect growth regulator: a review of its effects on insects. Ann. Appl. Biol. 22, 369–403.

Horst, M.N., Walker, A.N., 1999. Effects of the pesticide methoprene on morphogenesis and shell formation in the blue crab *Callinectes sapidus*. J. Crustac. Biol. 19, 699–707.

Hosmer, A.J., Warren, L.W., Ward, T.J., 1998. Chronic toxicity of pulse-dosed Fenoxycarb to *Daphnia magna* exposed to environmentally realistic concentrations. Environ. Toxicol. Chem. 17, 1860–1866.

Key, P.B., Scott, G.I., 1994. The chronic toxicity of Fenoxycarb to larvae of the grass shrimp, *Palaemonetes pugio*. J. Environ. Sci. Health B29, 873–894.

Leonardi, M.G., Cappellozza, S., Ianne, P., Cappellozza, L., Parenti, P., Giordana, B., 1996. Effects of the topical application of an insect growth regulator (Fenoxycarb) on some physiological parameters in the fifth instar larvae of the silkworm *Bombyx mori*. Comp. Biochem. Physiol. B 113, 361–365.

McKenney Jr., C.L., 1999. Hormonal processes in decapod crustacean larvae as biomarkers of endocrine disrupting chemicals in the marine environment. In: Henshel, D.S., Black, M.C., Harrass, M.C. (Eds.), Environmental Toxicology and Risk Assessment: Standardization of Biomarkers for Endocrine Disruption and Environmental Assessment. ASTM STP 1364, vol. 8. American Society for Testing and Materials, West Conshohocken, Pennsylvania, pp. 119–135.

McKenney Jr., C.L., 2005. The influence of insect juvenile hormone agonists on metamorphosis and reproduction in estuarine crustaceans. Integr. Comp. Biol. 45, 97–105.

McKenney Jr., C.L., Celestial, D.M., 1993. Variations in larval growth and metabolism of an estuarine shrimp *Palaemonetes pugio* during toxicosis by an insect growth regulator. Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol. 105, 239–245.

McKenney Jr., C.L., Celestial, D.M., 1996. Modified survival, growth and reproduction in an estuarine mysid (*Mysidopsis bahia*) exposed to a juvenile hormone analogue through a complete life cycle. Aquat. Toxicol. 35, 11–20.

McKenney Jr., C.L., Cripe, G.M., Foss, S.S., Tuberty, S.R., Hoglund, M., 2004. Comparative embryonic and larval developmental responses of estuarine shrimp (*Palaemonetes pugio*) to the juvenile hormone agonist Fenoxycarb. Arch. Environ. Contam. Toxicol. 47, 463–470.

Nates, S.F., McKenney Jr., C.L., 2000. Growth, lipid class and fatty acid composition in juvenile mud crabs (*Rhithropanopeus harrisii*) following larval exposure to Fenoxycarb®, insect juvenile hormone analog. Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol. 127, 317–325.

PAN (Pesticide Action Network) Pesticide Database, [online], 2007. International registration information for Fenoxycarb—summary of regulatory status. http://www.pesticideinfo.org/Detail_ChemReg.jsp?Rec_Id=PC33190.

Retnakaran, A., Granett, J., Ennis, T., 1985. Insect growth regulators. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology Biochemistry and Pharmacology, vol. 12. Pergamon Press, Oxford, pp. 529–601.

Skinner, D.M., 1985. Interacting factors in the control of the crustacean molt cycle. Amer. Zool. 25, 275–284.

Spicer, J.I., Eriksson, S.P., 2003. Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster *Nephrops norvegicus* (L.)? J. Exp. Mar. Biol. Ecol. 295, 219–243.

Please cite this article as: Arnold, K.E., et al., Effect of an insect juvenile hormone analogue, Fenoxycarb® on development and oxygen uptake by larval lobsters *Homarus gammarus* (L.), Comp. Biochem. Physiol. Part C (2008), doi:10.1016/j.cbpc.2008.09.007

Timbrell, J., 2002. Introduction to Toxicology3rd Edition. CRC Press, USA.
Tuberty, S.R., McKenney Jr., C.L., 2005. Ecdysteroid responses of estuarine crustaceans through complete larval development to juvenile hormone agonist insecticides. Integr. Comp. Biol. 45, 106–117.
Van Lenteren, J.C., 1999. Fundamental knowledge about insect reproduction: essential to develop sustainable pest management. Invertebr. Reprod. Dev. 36, 1–15.
Walker, A.N., Bush, P., Puritz, J., Wilson, T., Chang, E.S., Miller, T., Holloway, K., Horst, M.N., 2005. Bioaccumulation and metabolic effects of the endocrine disruptor methoprene in the lobster, *Homarus americanus*. Integr. Comput. Biol. 45, 118–126.
Wahle, R., Fogarty, M., 2006. In: Phillips, Bruce (Ed.), Chapter 1 Growth & Development: Understanding and Modelling Growth Variability in Lobsters: Biology, Management, Aquaculture and Fisheries. Blackwell Publishing, Oxford.

Please cite this article as: Arnold, K.E., et al., Effect of an insect juvenile hormone analogue, Fenoxycarb® on development and oxygen uptake by larval lobsters *Homarus gammarus* (L.), Comp. Biochem. Physiol. Part C (2008), doi:10.1016/j.cbpc.2008.09.007