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REVIEW

Spiny lobster development: mechanisms inducing metamorphosis to the puerulus: a review

Paulette S. McWilliam · Bruce F. Phillips

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Abstract This review outlines current knowledge of mechanisms effecting metamorphosis in decapod crustaceans and insects. The comparative approach demonstrates some of the complexities that need resolving to find an answer to the question raised frequently by ecologists: "What triggers metamorphosis in spiny lobsters?" It is evident that crustacean moulting and metamorphosis are genetically controlled through endocrine systems that mediate gene expression. The molecular mechanisms underlying these developmental processes have been studied intensively in insects, particularly in the fruitfly, *Drosophila melanogaster* (Diptera), and some lepidopteran species. Comparatively, there is minimal information available for a few decapod crustacean species, but none for spiny lobsters (Palinuridae). Nothing was known of hormone signalling transduction pathways, via nuclear receptors (NRs) and gene activation during larval moults in palinurids—until a recent, ground-breaking study of early phyllosomal development of *Panulirus ornatus* by Wilson et al. (Rock Lobster Enhancement and Aquaculture Subprogram. FRDC Project 2000/263, Australian Govt, Fisheries Research and Development Corporation and Australian Institute of Marine Science, Nov 2005). Their study not only identified homologues of five hormone NRs of *D. melanogaster*, but also patterns of gene regulation showing strong similarities to those of gene expression found in insect larval development. Their results indicated that control of moulting and metamorphosis in palinurids closely parallels that in insects, suggesting that insects can serve as model systems for elucidating molecular mechanisms in larval decapods. In insects and crustaceans, the steroid hormone, ecdysone, (20E) initiates moulting. In insects, juvenile hormone (JH) mediates the type of larval moult that occurs, either anamorphic or metamorphic. The latter results when the level of JH in the haemolymph drops in the final larval instar. High levels of JH inhibit the metamorphic moult during insect larval development. The interaction of 20E and JH is not fully understood, and the operative molecular mechanisms are still being elucidated. No nuclear receptor for JH has been identified, and alternative JH signalling pathways await identification. In decapod crustaceans, methyl farnesoate (MF), a precursor of JH, replaces the latter in other functions mediated by JH in insects; but there is little evidence indicating that MF plays a similar 'antimetamorphic' role in decapod larval moults.

Keywords Metamorphosis · Phyllosoma · Puerulus · Receptors · Spiny lobsters

P. S. McWilliam (✉)
18 Moroak Street, Hawker, ACT 2614, Australia
e-mail: pmcwilliamact@gmail.com

B. F. Phillips
Muresk Institute, Curtin University of Technology, GPO Box U1987, Perth, WA 6085, Australia

Introduction

The life cycle of spiny (or rock) lobsters (Decapoda, Palinuridae) is complex and includes a long, oceanic larval phase, varying in length (5–24 months) among species. Spiny lobsters hatch on the continental shelf as a planktonic, zoeal larva called a phyllosoma (about 1–2 mm long) and are transported off shore in the plankton and develop through a series of moults, increasing in size. Nine to eleven phyllosomal stages have been defined, arbitrarily, in most species in wild populations. Each stage does not necessarily represent a single instar and there are usually more instars than stages in a complete larval series in culture (see detailed drawings of stages in Matsuda et al. 2006). After developing through the early and middle stages in offshore waters, late-stage phyllosomas are returned towards the continental shelf by the deeper circulation. The final stage phyllosoma metamorphoses into the puerulus (the postlarva). The puerulus is a transitional stage which bridges the planktonic and benthic phases of the life cycle. It is a short-lived (ca 3–4 weeks), non-feeding stage (ca 30 mm long) which then swims across the continental shelf toward the shore. When the puerulus reaches shallow water near the shore, it settles. Its exoskeleton becomes pigmented and calcified just prior to its moult to the benthic juvenile stage which resumes feeding.

It is almost a decade since we reviewed the contentious question of what induces metamorphosis to the puerulus in palinurids (McWilliam and Phillips 1997). That paper focussed on phyllosomal development of the western rock lobster, *Panulirus cygnus*, endemic to Western Australia, and extrapolation from results of laboratory rearing of other larval decapods as well as other species of spiny lobsters. The physiology of metamorphosis was reviewed from the aspect of larval nutrition, bioenergetics and food availability. The main region of occurrence of metamorphosis in *P. cygnus* was inferred from the distribution and abundance of final phyllosomas, together with the relative numbers of pueruli collected. It was found to be in the slope region offshore of the shelf break. We found no evidence to support earlier suggestions that the metamorphic moult in this or other palinurid species was "triggered" by a direct, abiotic or biotic, exogenous cue associated with the shelf break or any other feature. Instead, all the available data led us to the conclusion that metamorphosis in *P. cygnus* results from the culmination of sustained nutrition through the larval phase and we suggested that it occurs when the final larval instar has reached some critical and specific level of stored energy reserves to support the swimming activity of the nektonic, non-feeding, puerulus, and its subsequent moult to the first juvenile stage after settlement.

This new review was prompted partially by the content of four of five articles published in the recent issue of *The Lobster Newsletter* (December, 2005, http://www.odu.edu/~mbutler/newsletter) The editors of that journal had invited several contributors to comment on the following topics:

> "Where does metamorphosis to the puerulus stage mainly take place among the shallow-water palinurids, and what factors—internal and/or external—are implicated in its progression?"

These contributions are of considerable interest, and where they deal with the second (physiological) question, will be examined as part of this review. The first (ecological) question will be addressed in a separate paper because of space limitations. Here, the approach we have taken represents a "paradigm shift". Because of the results of a recent study of early phyllosomas of a tropical spiny lobster, *Panulirus ornatus*, at the molecular genetics level by Wilson et al. (2005a), it now seems apposite to address the physiological question from the viewpoint of advances in molecular biology, specifically the knowledge of the endocrinology and genetics of moulting and metamorphosis in both insects and crustaceans. This knowledge has been accumulating over the past 50 years, particularly for insects, and similarities in the mechanisms underlying moulting and metamorphosis in crustaceans are now becoming apparent (Abdu et al. 1998; Chan et al. 1998; Marco et al. 2001; Anger 2001; Mu and Leblanc 2004; McKenney 2005; Wilson et al. 2005a, b).

The purpose of this review is to bridge the apparent gaps in knowledge of recent progress in spiny lobster biology by providing a simple introduction to aspects of molecular mechanisms underlying moulting and metamorphosis in insects and decapod larvae in general, and applicable to the

phyllosomas of palinurids in particular. It is hoped that this approach will provide a comprehensive background of more recent information for enhancement of spiny lobster larval development in future aquaculture studies, and for future fisheries research into larval development of these interesting, as well as commercially valuable, species.

Terminology

Before proceeding, it is necessary to define several terms, either because of the ambiguities of meaning in their use in the general literature, or because some readers may be unfamiliar with them.

Dimer

A compound formed by the union of two radicals or two molecules of a simpler compound (i.e., di- + mer, *cf* polymer),

Ligand

A molecule that binds specifically to a receptor, e.g., a hormone molecule. For details and configurations, see Laudet and Gronemeyer (2002),

Metamorphosis

This occurs in spiny lobsters at the last larval moult when the final phyllosoma emerges as a puerulus which has a body plan resembling the adult and vastly different from the phyllosomal morph; it also undergoes a change in behaviour and metabolism, and the appendages used for locomotion change from thoracic to abdominal. The term is used in this complete sense here. In all palinurids, metamorphosis occurs **before** settlement of the postlarva (see Gore 1985; Felder et al. 1985). This is true also of the postlarva of most known decapods that do not show abbreviated development (Felder et al. 1985). It is also true of slipper lobsters (Scyllaridae), another family in the Achelata (formerly Palinura), whose postlarva is called a nisto.

Nuclear receptors (NRs)

These are DNA-binding proteins of the nuclear receptor superfamily, which, on binding to an intracellular ligand can bind a specific nuclear chromatin region and inhibit or enhance transcription of target genes (i.e., they are latent transcription factors). All nuclear receptors bind to DNA as dimers. One NR is the ecdysone receptor (EcR); another is ultraspiracle (USP); the latter is a member of the vertebrate retinoid X receptor family (Laudet 1997; Wilson et al. 2005a). EcR is one of the most studied nuclear receptors. In the fruit fly, *Drosophila melanogaster*, it acts as a heterodimer with the nuclear receptor USP. The EcR/USP heterodimer when bound to ligand, activates expression of several genes in this insect.

RT-PCR

Reverse transcription polymerase chain reaction. A very sensitive amplification technique which enables detection of a few copies of an mRNA molecule from a sample. mRNA is isolated from a tissue or organ and then reversely transcribed to produce cDNA molecules from which many copies are then made by the PCR technique (Thain and Hickman 2004).

Molecular processes in early larval development of *Panulirus ornatus*

Nuclear receptors, which are specific to metazoan animals, play a key role in the complex endocrine systems that have evolved in these animals because they function in many processes from embryonic development to metamorphosis, and from physiological homeostasis to control of metabolism. They are grouped into a large superfamily (containing at least 70 members) which includes NRs for steroid hormones, ecdysone, retinoid and thyroid hormones, and others, and share a common evolutionary history. This is evident from their conserved structure and also their high degree of sequence conservation found across animal phyla, from nematodes to humans. They are ligand-activated transcription factors having the ability to bind selectively to DNA, and have a special role in gene regulation since they provide a direct link between the ligand, which they bind, and the target gene, whose expression they regulate (Laudet 1997; Devine et al. 2002; Laudet and Gronemeyer 2002: Bertrand et al. 2004; Escriva et al. 2004).

The study by Wilson et al. (2005a) is the first investigation into the role of hormone nuclear receptors in palinurid larval development. Their objective was "to identify triggers for moulting to evaluate a shortening of the larval phase" with the intention of reducing larval rearing time in cultures by hormonal manipulation. This identification was achieved by isolating 'candidate' nuclear hormone receptors from early phyllosomas of *P. ornatus*. At the same time they aimed to determine the extent of similarities in molecular mechanisms controlling crustacean larval development compared with those in insects. They used *D. melanogaster* as a model, since its genome sequence had been completed (Adams et al. 2000). Complete (nuclear) genome sequences for several species of invertebrates and vertebrates have been determined in the last few years (Escriva et al. 2004) thus making possible the systematic study of the whole set of NRs of an organism; but so far no crustacean genome has been determined, although the complete mitochondrial DNA (mtDNA) sequence of several crustacean species of different groups, including that of *Panulirus japonicus*, has been determined (Yamauchi et al. 2002).

Up until the time of this study of the larvae of *P. ornatus*, only nine partial or complete nuclear receptors had been identified from several crustacean species; all but one were decapods. One or more of these different NRs were found in the clawed lobster, *Homarus americanus* (see also El Haj et al. 1997); two crab species, *Uca pugilator* and *Carcinus maenas*; two penaeid prawns, *Metapenaeus ensis* and *Litopenaeus vannamei*, and the brine shrimp, *Artemia salina* (see Wilson et al. 2005a, Table 1.1). Wilson et al. (2005a) successfully identified five hormone NRs from phyllosomas of *P. ornatus*, each being a homologue of one of five different NRs from *D. melanogaster* (Table 1). They also developed sensitive assays (using the quantitative RT-PCR technique) to measure the activity of four of these five NRs (USP was omitted from the analyses) through early larval development across larval moults from Stage I to Stage IV phyllosomas of this species.

They did find patterns of gene expression of these four candidate nuclear hormone receptors in these early phyllosomal stages. More significantly, there was a strong similarity to the patterns of gene regulation found in insect larval development, which suggests that patterns of hierarchical gene expression occur in *P. ornatus* in a manner similar to that observed in *D. melanogaster* and the tobacco hornworm, *Manduca sexta* (Lepidoptera) (Riddiford 1993; Thummel 1996: Ashburner 1990; White et al. 1997). This also suggested that mechanisms controlling moulting and metamorphosis in crustaceans resemble those found in insects.

Wilson et al. (2005a) also measured ecdysteroid titres in phyllosomas of *P. ornatus* and confirmed that they varied in the same way as reported for larvae of insects and other crustacean species, namely, a surge in the titre occurred late in the actual physical process of shedding the old cuticle. In all its aspects, this study by Wilson et al. (2005a) provides evidence that control of phyllosomal development closely parallels that of larval development of holometabolous, dipteran and lepidopteran insects, such as *D. melanogaster*, *M sexta* and the silkworm, *Bombyx mori* (Riddiford et al. 2003) for which the published knowledge bank far outweighs that for decapod crustaceans

Overall, it has been recognised for a long time that the physiological processes that initiate moulting and metamorphosis in decapods are, or in the latter case are likely to be, similar to those in insects (Jenkin 1970; Spindler 1991; Chang et al. 1993). One seldom-mentioned example of conservation of hormonal activation across these arthropod phyla is the neuropeptide, crustacean cardioactive peptide (CCAP), released from the pericardial organs of decapods. This hormone, originally isolated from the shore crab, *C. maenas*, and later, the freshwater crayfish, *Oronectes limosus*, is directly associated with the onset of ecdysis behaviour in decapod crustaceans (Stangier et al. 1988; Phlippen et al. 2000), and later also found to have the same function in holometabolous insects (Jackson et al. 2001; Mesce and Farbach 2002). The CCAP gene was identified in the moth, *M. sexta* by Loi et al. (2001). In locusts, CCAP was also found to act as a releasing factor for adipokinetic hormone (AKH) which is synthesised in the corpora cardiaca and is important in lipid metabolism (Nijhout 1994; Jackson et al. 2001).

The evidence also indicates that the system of hormonal control of the moult cycle in larval decapods is similar to that found in their adults and is operative from hatching (Anger 2001). The studies

Table 1 The five nuclear receptors from the fruitfly, *Drosophila melanogaster*, with which the five candidate hormone nuclear receptors found in *Panulirus ornatus* phyllosomas by Wilson et al. (2005a) were homologous, and their function in the fruitfly

Nuclear receptor	Function in *D. melanogaster*	References
Ecdysone receptor (EcR)	Master receptor of ecdysone response. Binds ecdysone as a heterodimer with USP and activates genes with ecdysone response	Thummel (1996); Chung et al. (1998); Riddiford et al. (2003)
Ultraspiracle (USP)	Functions as a heterodimer with EcR to activate early response genes.	Ashburner (1990); Xu et al. (2002)
Hormone receptor 3 (HR3)	Induced by EcR. Functions in metamorphosis of the final larval instar by repressing "early genes" induced by ecdysone, and inducing competence factor for metamorphosis.	Koelle et al. (1992); White et al. (1997); Riddiford et al. (2003); Sullivan and Thummel (2003)
Hormone receptor 4 (HR4)	Evidence indicates it has a similar role to HR3. There is little data on its function but it is believed to act as a repressor, regulating genes at puparium formation. Its expression continues after cessation of HR3 expression.	Sullivan and Thummel (2003)
Hormone receptor 78 (HR78)	This receptor is reported to have a role in the onset of metamorphosis; it apparently acts as a critical regulator of gene expression.	Thummel (1996); Fisk and Thummel (1998)

of Wilson et al. (2005a) showed that insects can serve as model systems for elucidating the molecular mechanisms inducing metamorphosis in larval decapods, and particularly in phyllosomas of spiny lobsters.

Endogenous processes implicated in metamorphosis

Metamorphosis, moulting and the moult cycle in decapods

It is important to deal first with what is currently known about moulting and metamorphosis in palinurids, and other decapods, especially with the progress made by Wilson et al. (2005a) towards understanding these processes in palinurid larval development.

As pointed out earlier by McWilliam and Phillips (1997), metamorphosis in palinurids can occur anywhere from beyond the shelf break to well out in the open ocean Metamorphosis can also occur in aquarium cultures. To date, the complete larval development and metamorphosis to the puerulus of ten shallow-water palinurid species has been achieved in the laboratory (Table 2). They range from tropical to cool temperate water species. Metamorphosis has been successfully achieved, although no in vitro study could completely simulate all the abiotic and biotic environmental variables normally encountered by their larval counterparts developing in the wild. Furthermore, these phyllosomas metamorphosed under varied culture systems, although conditions such as food, (supplied regularly, even if suboptimal in quantity and quality), temperature (set to optimum levels for each species) and photoperiod, were mostly kept stable throughout the different rearing studies.

Successful rearing in the laboratory again suggests that there are some **endogenous** factors, common to all these palinurid species that control metamorphosis and determine the onset of the metamorphic moult to the puerulus.

Palinurid metamorphosis is dramatic because the form that emerges at the final larval ecdysis (which may only take a few minutes) differs dramatically from the phyllosomal form (See Fig. 1). However, apart from increase in size, most anamorphic (i.e., larva/larva) moults in palinurids involve some degree of morphological differentiation, mainly the addition, and/or differentiation of paired appendages of the cephalothorax and abdomen, from those of the hatched condition (Fig. 1a–c) as well as growth and development of the insignificant abdomen of the earlier larval stages. In general, in wild populations, segmentation of the abdomen and its appendages is more advanced in the penultimate larval stage, and more so in the final stage, when all the gill primordia

Table 2 Spiny lobster species whose complete larval development and metamorphosis have been achieved in the laboratory

Species	No. of instars	Duration of phyllosomal phase (months)	Duration of puerulus stage in laboratory (days)	Author(s)
Jasus lalandii	15	10	11	Kittaka (1988)
J. edwardsii	15–23	10.5–13.4	19	Kittaka (1990)*; Kittaka et al. (2005); Illingworth et al. (1997); Ritar (2004)*
Sagmariasus verreauxi	16–17	6.1–11.6	25.5	Kittaka et al. (1997); Moss et al. (2000)
Palinurus elephas	6–9	2.0–4.2	11–15	Kittaka and Ikegami (1988); Kittaka (2000)*
Panulirus japonicus	20–31	7.5–12.6	9–26	Kittaka and Kimura (1989); Sekine et al. (2000)
P. longipes bispinosus	17	9.1–9.5		Matsuda and Yamakawa (2000)
P. penicillatus	22	8.3–9.4		Matsuda et al. (2006)
P. homarus subsp				Murakami (2006)*
P. argus	23	5–7		Goldstein et al (2006)
P. ornatus				M.G.Kalis Pty Ltd[#]

*Pers comm, not published

[#] Press release, Perth, Aug 2006

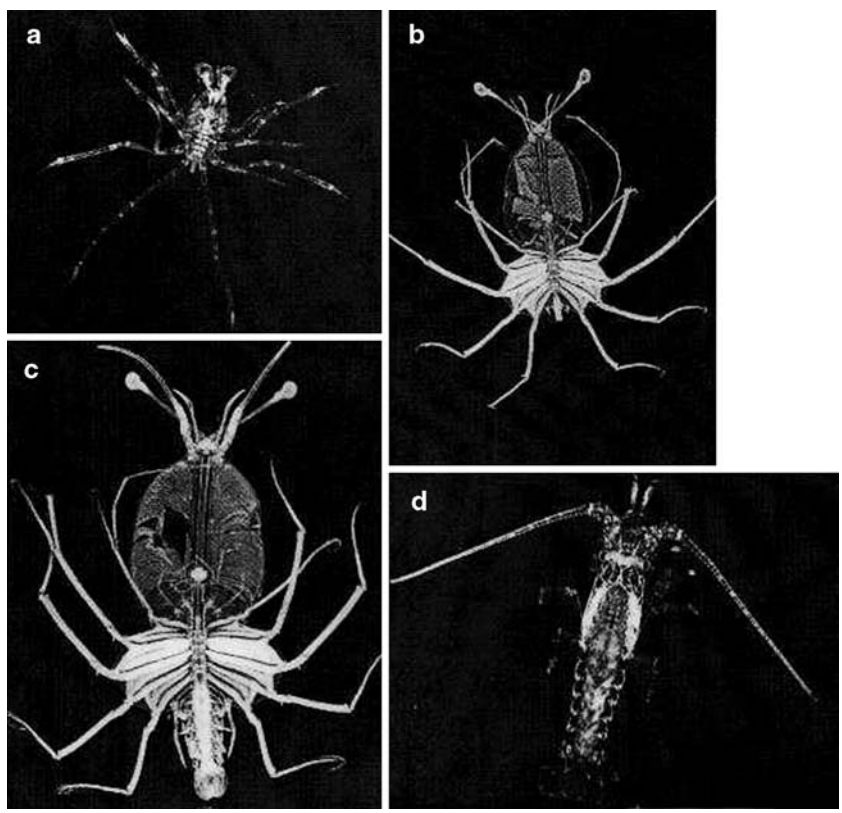

Fig. 1 Stages in early development of *Panulirus cygnus*: **(a)** newly-hatched Stage I phyllosoma; **(b)** late-stage phyllosoma, **(c)** final phyllosoma, (stage IX), **(d)** puerulus. (Photographs not to scale. Note: **(b)** and **(c)** are preserved specimens, and show sampling damage – ruptured cephalic discs and missing pereiopodal endopods.)

are visible Apart from this stepwise growth and development of sensory, feeding and locomotory appendages, the dorso-ventrally flattened body of the phyllosoma and its internal organisation is retained until the metamorphic moult. Moreover, it is often overlooked that the acquisition of the more adult-like shape and morphology of this transitional puerulus (a single-moult stage, shown in Fig. 1d,) resembling a transparent, miniature adult, does not appear until the metamorphic moult.

Laboratory observations of the final phyllosomal instars of *Jasus lalandii* and *Sagmariasus verreauxi* indicated that they stopped feeding a few days before metamorphosis, there was greater development of the pleopods and uropods, before their swimming activity declined and finally stopped; the hepatopancreas atrophied, and the whole body became turgid and opaque and the antennae flaccid, just prior to the metamorphic moult (Kittaka 2000); also, in *J. edwardsii* and other species, the abdomen of the final instar thickened a few days before this moult (Kittaka et al. 2005).

Moulting and the moult cycle

Like all crustaceans, and other invertebrates with a confining exoskeleton, phyllosomas must periodically shed their exoskeleton, the cuticle, in order to develop and grow beyond a certain level. They are also programmed intrinsically, to undergo the final larval moult which produces a very different phenotype. So it follows that what triggers the metamorphic moult, must also involve what triggers moulting, and hormonal activation of gene expression (or repression).

The complex process of crustacean moulting, which is regulated by hormones, basically involves the creation of a new cuticle, which is secreted by the underlying epidermal cell layer, followed by the physical process of shedding the old cuticle, i.e., **ecdysis;** this is followed rapidly by uptake of water and salts and then hardening of the new cuticle. It involves physiological as well as morphological processes, and it has been estimated that probably up to half the life of decapods is spent preparing for, or recovering from, ecdysis (Aiken 1980). The actual exuviation stage in ecdysis is the shortest part of the moult cycle but the most crucial step in the life cycle of crustaceans and other arthropods because death ensues inevitably if exuviation fails to be completed (Phlippen et al. 2000).

The decapod moult cycle has been officially divided into five sequential stages, A–E, for which there are alternative, more formalised terms for the generalised 'premoult', 'postmoult' and 'intermoult' stages used currently (See Aiken 1980). Staging is based on changes observed in the cuticle and the underlying epidermis and stages C and D include several detailed substages (Anger 2001). Stages A and B are together termed **metecdysis**, during which the new cuticle hardens and the animal recovers from the process of ecdysis, the endocuticle is formed and any degenerated muscle is repaired. In stage C, **anecdysis** or intermoult, there are no further cuticle changes, the animal resumes feeding and reserves for the next ecdysis are accumulated. Stage D, or **proecdysis**, starts with **apolysis**, the separation of the cuticle from the epidermis. This is followed by secretion of the outer layers of the new cuticle and the final preparations for actual **ecdysis**, or stage E.

It is still unclear what endogenous factor makes individual crustacean larvae 'competent ' to metamorphose, but, as shown by the Wilson et al. (2005a) study, the activation of hormonal systems, or their receptors, are among the possibilities, and particularly so for phyllosomas. Since the metamorphic moult is the climactic larval moult in palinurids, it must follow that the same hormones which regulate anamorphic moults throughout larval development must also be involved. An earlier review by Spindler (1991) of the roles of morphogenetic hormones in the metamorphosis of arthropods, indicated that three groups of hormones are involved in the regulation of metamorphosis in crustaceans; ecdysteroids, (neuro)peptides and juvenile hormones (JHs) (mostly terpenoids).

Endocrinology

It is now becoming evident, that crustacean moulting and metamorphosis, like that of insects, is genetically controlled through complex endocrine systems which regulate gene expression (Liu and Laufer 1996; Anger 2001; Bertrand et al. 2004; Wilson et al. 2005a).

Crustacean moulting is known to be under the control of two antagonistic hormones. It is stimulated by the steroid moulting hormone, 20-hydroxyecdysone

(20E), of which ecdysone is the precursor, (Chang et al. 1993), and suppressed by the moult-inhibiting hormone (MIH), a neuropeptide. Ecdysone is produced in the paired Y-organs of crustaceans and secreted into the haemolymph where it is converted enzymatically to the active form, 20E (Chang 1993). The Y-organs, of ectodermal origin, are located bilaterally in the cephalothorax. MIH is produced in the medulla terminalis of the X-organ and stored in the sinus gland; both the X-organ and sinus gland (often referred to as the 'X-organ-sinus-gland complex') are located in each eyestalk. The sinus gland is a neurohaemal storage organ, about 90% excretory (and apparently only about 10% secretory) in function (see details in Anger 2001, p. 63). It consists of a complex of the enlarged ends of nerve fibres coming from the X-organ and from numerous neurosecretory cells in the brain, in the ganglia of the optic lobe, and in the thoracic ganglia. The sinus gland is therefore mainly a collection vesicle and a distributing centre for various neurosecretory hormones, as well as MIH (Snodgrass 1956; Anger 2001).

Ecdysteroid molecules are known to be critical in controlling moulting and metamorphosis in insects and crustaceans. While 20E is the primary secretory ecdysteroid product of the crustacean Y-organ, and has been investigated mainly in the clawed lobster, *Homarus americanus*, (Charmantier and Charmantier-Daures 1988; Charmantier et al. 1991), production of some other ecdysteroids, namely 3-dehydroxyecdysone and 25-dehydroxyecdysone, also have been found in some crabs (Chang 1995). Y-organs secrete 20E and are functional from the Stage I decapod larva (Anger 2001) and throughout the juvenile and adult phases. There is also some evidence that 20E is not the only ecdysone involved in the ecdysteroid-regulated pathway of gene expression in larval development of *D. melanogaster* (Sullivan and Thummel 2003).

Insect moulting and metamorphosis are regulated by production of prothoracicotropic hormone (PTTH), secreted by the brain, and this stimulates the secretion of ecdysteroids by the prothoracic glands (Chang 1993). PTTH directs the timing of the moult as its release is governed by intrinsic factors, such as size and weight, and extrinsic factors such as photoperoid and temperature (Nijhout 2003; Riddiford et al. 2003).

Crustacean MIH regulates moulting by inhibiting the synthesis and/or release of ecdysteroids by the Y-organ of larval (except nauplii), juvenile and adult decapods (Chang et al. 1993; Anger 2001). It is not known what releases the Y-organ from MIH control, but there is some evidence suggesting that subsequent feedback from high titres of ecdysone in the haemolymph may reduce MIH activity (Watson and Spaziani 1985). Recent evidence for a negative feedback loop between the Y-organ and the X-organ, suggesting a role for 20E in regulating titres of MIH in juvenile *H. americanus*, was reported by El Haj et al. (1997). The MIH gene has been identified in a crab (Chan et al. 1998) and in the sand shrimp, *M. ensis* (Gu and Chan 1998). However, much more needs to be learned about the mechanisms underlying neurosecretory regulation of crustacean moulting and metamorphosis, and more neuropeptide hormones continue to be discovered in invertebrates (Hummon et al. 2006).

Ecdysteroids have been found in all arthropod taxa investigated for hormonal activity, but unlike vertebrates, arthropods are unable to synthesise sterols de novo (Spindler 1991) and crustaceans cannot synthesise cholesterol, the precursor of crustacean ecdysone (Teshima and Kanazawa 1971; McConaugha 1985; Watson and Spaziani 1985). Moreover, marine crustaceans are unable to synthesize several types of amino acids, and certain polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kanazawa 1994). Therefore dietary sources of cholesterol, as well as these other organics are essential for development and metamorphosis of planktotrophic larvae such as phyllosomas.

Less is known about crustacean JH. Methyl farnesoate (MF), the precursor of JH in insects, and structurally similar to a JH of insects (JH-III) (Chang 1993; Wainwright et al. 1996), was found to be the crustacean equivalent of the insect JH (Laufer et al. 1986; Borst et al. 1987). MF appears to be the only JH-like compound found in crustaceans so far. It is produced by paired, ductless endocrine glands, the mandibular organs (MOs), located near the base of the mandibles in decapod larvae as well as adults (Charmantier et al. 1997; Charmantier and Charmantier-Daures 1998). The production and release of MF and its precursor, farnesoic acid, was shown in vitro also in the MOs of several shrimp and crab species (Anger 2001). Overall, the message is that MF regulates many of the same processes that are

regulated by JH in insects (Laufer and Biggers 2001).

The action of ecdysone described by Wilson et al. (2005a) deserves quoting fully: "Ecdysone acts by binding to a receptor protein, the ecdysone receptor [EcR]. The ecdysone receptor is one of a family of proteins found throughout the animal kingdom which are activated by binding to a steroid hormone, and then act directly to regulate gene expression. In insects, the ecdysone receptor is the master switch in this system, as it responds with exquisite sensitivity to the circulating levels of ecdysone, being activated when ecdysone levels start to rise, but repressed once ecdysone reaches a maximal level. When activated, the ecdysone receptor triggers a cascade of gene activity, leading to the eventual moult and possible metamorphic transformation".

Prior to each moult in insects, ecdysteroid (mainly 20E) titres rise in the haemolymph and then return to basal levels just before the moult; if the levels do not fall, ecdysis does not occur (Zitnan et al. 1999; Riddiford et al. 2003). Furthermore, it is thought that the decline in the circulating ecdysteroids acts in conjunction with other signals that either specify the insect's readiness to moult, or provide circadian cues so that the insect moults during the appropriate phase of the light–dark cycle or in amenable temperatures (Truman 1996; Mesce and Fahrbach 2002). For instance, *D. melanogaster* moults at sunset thus avoiding desiccation through the heat of day before its new cuticle hardens. The circadian clock control of rhythmic behaviour in holometabolous insects, and the underlying genetic and biochemical mechanisms implicated in clock control of behaviour, were reviewed by Jackson et al. (2001).

Similar endogenous signals have been reported in cultured decapod larvae. Results of rearing larvae of *H. americanus* at 20°C in controlled photoperiod cycles, indicated a clear relationship between the timing of the dark (scotophase) period and the timing of their metamorphic moult (Waddy et al. 1990). Phyllosomas of *P. japonicus* cultured in natural light–dark cycles moulted synchronously within 1 h before and after sunrise. Under artificial light–dark cycle treatments the phyllosomas moulted around the start of lighting, irrespective of the photoperiod cycles used, and the start of lighting had a greater effect on the timing of larva/larva moults than did the end of lighting. Under a natural lighting system, metamorphosis to the puerulus occurred within 0.4 h before, and 1.2 h after, sunset, and the timing of metamorphosis was also changed artificially by regulating the end of lighting (Matsuda et al. 2003). Their experiments indicated that the timing of moulting and metamorphosis in phyllosomas of this species are regulated by endogenous processes, and were hypothesised to maximise survival. These patterns presumably evolved so the animals avoid the dangers of predation during their most vulnerable period, which is before hardening of the new cuticle and recovery from anamorphic or metamorphic moulting processes.

In early phyllosomal stages of the scyllarid lobster, *Thenus orientalis*, cultured under a natural photoperiod, moulting occurred synchronously around sunrise; but moulting was irregular in phyllosomas reared under conditions of continuous light or continuous dark periods. However, when these phyllosomas reared under normal photoperiods metamorphosed to the nisto, synchronous moulting changed from dawn to after dusk (Mikami and Greenwood 1997). These authors also cite cases where metamorphosis after dusk has been observed in aquarium-reared phyllosomas of another species of scyllarid and several palinurid species; whilst nocturnal metamorphosis has also been reported in other scyllarid species.

The crustacean moulting system is regulated by ecdysteroids and the neuropeptide, MIH, and this system also operates in spiny lobsters, as shown by Marco et al. (2001). They investigated these processes in adults of the South African spiny lobster, *Jasus lalandii*, and characterised the major circulating ecdysteroid in this species as 20E. They also found that the titre of ecdysteroids in the haemolymph varied greatly during the moult cycle, being highest in premoult and lowest in postmoult stages. Their in vitro experiments to characterise MIH activity in the sinus gland and its influence on Y-organ secretion levels of 20E, indicated that these titres were only inhibited by extracts from lobsters in the inter- and pre-moult stages, whilst Y-organs from post-moult animals were not. Ecdysteroid synthesis by Y-organs of intermoult J. lalandii was inhibited by extracts of sinus glands in a dose-dependent way—the highest dose produced the greatest inhibition. Their results from quantification of ecdysteroid levels during the moult cycle of this spiny lobster showed similar

fluctuations to those found in several other decapods, namely, a prawn, *Penaeus vannemai*, the crayfish, *O. limosus*, and the blue crab, *Callinectes sapidus* (reviewed by Marco et al. 2001). However, the X-organ secretes other neurohormones besides MIH so the latter neuropeptide may not necessarily be the sole inhibitor of ecdysone secretion in decapods

While it is known that ecdysones initiate the cascade of physiological and behavioural events that precede the moult, the JH of insects mediates the character of the moult; it determines whether it is larval or pupal. A high titre of JH is needed for insect anamorphic moults, the metamorphic moult occurs only when the JH titre drops during the final larval instar when the ecdysteroid titre is low also (Chang 1993; Riddiford et al. 2003; Dubrovsky 2005). Moreover, in certain species of insects, metamorphosis is associated with the attainment of a critical weight, whereas in most holometabolous insect species investigated, it is associated with attainment of a critical size (Nijhout 2003; Riddiford et al. 2003).

The control of PTTH secretion is complex and the stimulus for its secretion is known only for several species of the order Hemiptera, (hemimetabolous insects—which lack a pupal stage) such as the milkweed bug, *Oncopelius fasciatus*, in which it is controlled by stretch receptors in the larval abdomen that are activated when the animal reaches a particular critical size. Under normal conditions of growth, the abdominal stretch receptor is not activated until the larva has accumulated a critical amount of body mass and is thus determined by the quantity and quality of nutrition (Nijhout 2003).

A more complex mechanism operates in holometabolous insects such as *M. sexta* where secretion of PTTH and ecdysteroids in the final larval instar only are under inhibition by JH. If the corpora allata (which secrete JH) are removed early in this instar, the larva secretes PTTH and ecdysone prematurely and metamorphoses into a miniature adult. Conversely, if additional JH is injected into a final larval instar, PTTH secretion is delayed in a dose-dependent manner and metamorphosis begins at a much larger body size than normal (Nijhout 2003). The cessation of JH secretion is closely associated with the attainment of a critical weight, which in turn is determined by the weight of the larva at the **beginning** of the last larval instar. Circulating JH is broken down by JH esterase and the activity of this enzyme increases gradually throughout this final instar (Nijhout 2003). It is also notable that the level of JH esterase is strongly influenced by nutrition, and its activity drops to zero almost immediately if a larva is starved. Once dis-inhibited by the disappearance of JH, the timing of secretion of PTTH is controlled by a photoperiodic clock (see Nijhout 2003, and references therein).

The initiation of metamorphosis in the final insect instar means the switching-off of many larval-specific genes and the unmasking of previously unexpressed pupal cuticle genes, so the epicuticle becomes committed to pupation and can no longer express larval-specific genes. The underlying molecular key to this switch appears to be the Broad Complex (BR-C), a transcription factor which is regulated by JH (Riddiford et al. 2003) and specifies pupal development (Erezyilmaz et al. 2006). Quoting Wilson et al. (2005a), the effect of high titres of JH appears to be mediated in insects such as *D. melanogaster* "by selective repression of a set of regulatory genes that, when expressed, trigger the activity of a series of genes required for metamorphosis". Hence, the 'trigger' for metamorphosis in these insects may be the drop in the JH titre in the haemolymph in the final larval instar.

Some evidence suggests that, during the larval phase, JH may act by binding to the EcR/USP heterodimer and recruiting a co-repressor, thus preventing the activation of genes required for metamorphosis, but not those required for moulting (Maki et al. 2004; Dubrovsky 2005). Since the nuclear receptor USP was found to bind JHs, its role as a mediator of JH action was proposed earlier by Jones and Sharp (1997). Xu et al. (2002) also proposed that USP acts as a receptor for JH in *D, melanogaster*. However, the molecular basis of how insect JH intervenes to produce this effect of restraining insect metamorphosis is unknown. Chang (1993) reported that evidence for the existence of NRs for insect JH was characterised by Palli et al. (1990) and Palli et al. (1991); Riddiford (1993) followed with a review of a putative JH receptor found in insect larval tissues as "a 29-kDa nuclear protein that specifically binds JH with high affinity and represents a new class of intranuclear hormone receptor since it has no known DNA-binding domain and has a discrete subnuclear localization different from that of the EcR." However

its function remains unsolved, and to date no JH nuclear receptor has been defined in insects according to Riddiford et al. (2003) and Dubrovsky (2005), although the latter remarked that, of the two current candidates for the JH receptor, USP and the protein, MET (Methoprene-tolerant), found exclusively in the nucleus (Pursley et al. 2000), the former is more likely as there is some genetic evidence ruling against the latter (Dubrovsky 2005)

A more recent study suggests that MET interacts with BR-C in JH regulation of insect development (Wilson et al. 2006); while Erezyilmaz et al. (2006) found that the regulatory Broad gene (*br*) behaves differently in a hemimetabolous insect. This gene is expressed through embryonic development and all nymphal stages, disappearing at the moult to the adult; they also found that JH is necessary to maintain *br* expression throughout the nymphal stages.

Whether MF performs a similar mediating role to JH in crustacean larval development remains equivocal, and the possible molecular mechanism by which MF mediates the ecdysteroid-induced expression of larval decapod genes has not been defined (see also Laufer and Biggers 2001). Experiments using exogenous MF have been shown to affect decapod larval development and metamorphosis in different ways in different groups. For example, larvae of *H. americanus* treated with exogenous MF in seawater, resulted in only a small delay in metamorphosis and no morphological effects (Borst et al. 1987). In late larvae of the palaemonid prawn, *Macrobrachium rosenbergii*, fed with MF-enriched *Artemia* nauplii by Abdu et al. (1998), MF produced JH-like effects such as retarded larval development and an occurrence of intermediate morphological forms, depending on MF-dosage levels. This led these authors to suggest that MF "could be the direct hormonal agent controlling metamorphosis in crustaceans through an indirect effect of eyestalk neuropeptides".

For crustaceans, Mu and Leblanc (2004) have proposed a mechanism involving the interference of molecular action of ecdysteroid signalling by experiments in which they exposed embryos of the cladoceran, *Daphnia magna*, to solutions of 'juvenoids' (compounds which mimic the action of JH) namely, the synthetics, pyriproxyfen and fenoxycarb, as well as MF. All three were found to disrupt the ecdysteroid-regulated systems of embryo development in this species. They provided evidence showing that, following exposure to juvenoids, there is a reduction in EcR gene expression, possibly by competitive binding of the receptor partner protein, USP, (meaning, presumably, the latter is binding to something else, rather than to the usual EcR). The results of experimentation with larval development of two estuarine species, the shrimp *Palaemonetes pugio*, and the crab, *Rhithropanopeus harrisi*, exposed to solutions of JH agonist insecticides by Tuberty and McKenney (2005) seem to provide some support for this theory.

A neuropeptide hormone identified from the sinus glands of adults of the crab, *Cancer pagurus*, was found to repress synthesis of MF by the MOs, and therefore labelled mandibular organ inhibiting hormone (MOIH) by Wainwright et al. (1996). This neuropeptide hormone (occurring as two structurally similar forms, MOIH-1 and -2 and both found to be very similar to MIH in structure) represents two further members of the MIH group within the crustacean hyperglycaemic hormone (CHH) neuropeptide family (Wainwright et al. 1996). But these MOIHs and MIH appeared to be functionally distinct in their activity. These authors also noted that the MOIHs identified in their crab study showed no sequence similarities to the insect hormones that inhibit synthesis of JHs by the corpora allata, thus revealing a significant difference in neurohormonal control of synthesis of the JHs, MF and JH in crustaceans and insects, respectively. Two isoforms of MOIHs of the CHH neuropeptide family, having both functions, were also isolated from the sinus glands of the spider crab *Libinia emarginata* and bioassayed by Liu and Laufer (1996) who suggested that these hormones may be unique to crustaceans.

It is now notable that homologues of various CHH neuropeptides, still mainly of unknown function, have been found in insects, including *D. melanogaster*, as well as other arthropods (Gäde 2004: Liu et al. 2006). One example is the *Drosophila* Ion Transport Peptide (ITP) gene, a homologue of the ion-transport peptide gene which controls absorption of salts and water in the ileum of the hind-gut in locusts (Zhao et al. 2005). Further recent information on these hormones is given by Dai et al. (2007) who have identified in *D. melanogaster* and other insects, a conserved family of ITP/ITPL (ITPL = ITP-like) peptides, which have high amino acid sequence

homology with genes encoding crustacean CHH neuropeptides.

From the information now available it must be apparent that, despite intensive studies over several decades, the molecular mechanisms underlying JH regulation in insect metamorphosis remain enigmatic, as does the prospect that MF may be regulating metamorphosis of larval decapods—especially palinurids and scyllarids, in which this problem seems never to have been studied.

Exogenous factors implicated in metamorphosis

Together with temperature and photoperiod (mentioned above), food quality and quantity are of prime importance in growth and development of all decapod larvae, in culture and in nature (Kittaka 1994a; Anger 2001). Although none of these exogenous factors can be regarded as the 'trigger' for metamorphosis, the nutritional status of the final stage phyllosomas must have some bearing on metamorphic competency as noted in other decapod larvae, and other arthropods such as insects (see above).

Food and nutrition

As observed in rearing of other decapod larvae, phyllosomal nutrition also depends on dietary sources of food rich in PUFAs and of all dietary elements involved in completing larval development, lipids seem to be crucial for late-stage phyllosomas and vital in progression to metamorphosis to the puerulus (McConaugha 1985; Kittaka 1994b, 2000; Phleger et al. 2001; Nelson et al. 2006; Phillips et al. 2006). Lipids are the main components of cell membranes, and sterols of the moulting hormones; also, in the wild, phospholipid has been found to comprise the major energy storage source for the non-feeding, nektonic pueruli of *J. edwardsii* in New Zealand waters, with diacylglycerol (DAG) as a less important, secondary source. This seems unusual, as most marine animals use triacylglycerol (TAG) as a short-term energy source (Phleger et al. 2001). Protein catabolism was found to be unimportant in these New Zealand pueruli and carbohydrates such as glycogen and glucose less important as bioenergetic sources, although perhaps they may be used after exhaustion of lipid stores (Jeffs et al. 1999, 2001b). Histological analysis showed that phospholipids were concentrated in the bilateral fat bodies attached to the ends of the anterior and intermediate lobes of the hepatopancreas of *J. edwardsii* pueruli (Takahashi et al. 1994).

Phospholipid was also the main lipid class found in larvae of this species from Tasmanian and New Zealand waters, and of pueruli from Tasmanian waters only: while sterol, mainly cholessterol, was found to be the next most abundant lipid class, and TAG was not detected in wild pueruli, perhaps because of depletion of this energy source (Phleger et al. 2001). These authors went on to experiment with rearing of early phyllosomas of this species fed with *Artemia* encriched with the three essential PUFAs, arachidonic acid (AA), EPA and DHA, and also with "off-the-shelf" products which showed promise for future more successful larval culturing of the late stages (IX, X and XI in this species). Lipids and PUFAs such as these three fatty acids were also found to be important in early larval development (stages I–IV) of *P. cygnus* in culture, and lipid-enriched *Artemia* fed to these stages resulted in higher survival and growth (Liddy et al. 2004, 2005).

Success in culturing the larvae of spiny lobsters has always been thwarted by their lengthy larval phase and the fact that the food of wild larvae is unknown. It is only in the last few years that a start has been made to identify possible natural food items, particularly of late-stage phyllosomas, by obtaining signature lipid profiles of larvae of *J. edwardsii* (Phleger et al. 2001) for comparison with those of their potential prey items (Nichols et al. 2001). Recently, DNA-based methods were used to identify prey organisms in the gut contents of mid- to late-stage phyllosomas of palinurids and scyllarids (Suzuki et al. 2006).

Overall, the results of these studies indicate that phyllosomas were feeding mainly on gelatinous zooplankton, such as salps (Thaliacea) including *Thalia democratica* in some regions, also species of *Oikopleura* (Larvacea), scyphozoans and hydrozoans (Cnidaria), comb jellies (Ctenophora) and other soft-bodied zooplankters such as chaetognaths. To a lesser extent, possibly small crustaceans such as euphausiids comprise part of the diet of late phyllosomal stages of *P. cygnus* off southern Western Australia, and *J. edwardsii* off the North Island of New Zealand (Phillips et al. 2006). Their diets may also vary

seasonally and according to productivity of the region.

The ultimate goal of such studies of natural diets of phyllosomas is for improvement of formulated diets used in larval cultures in order to enhance survival, growth and successful development to and beyond the metamorphic moult. It is only through such aquacultural improvements, coupled with physiological and genetic investigations, that the molecular mechanisms of metamorphosis in spiny lobsters can be elucidated.

Discussion

The lobster newsletter articles

In the *The Lobster Newsletter* (December, 2005, http://www.odu.edu/~mbutler/newsletter) there were five articles proffering answers to the two questions detailed in our Introduction. Here, we are dealing only with their answers to the physiological question.

Jeffs (2005) provided a general view, suggesting that "there was very little hard evidence" serving to identify a possible exogenous "trigger" for metamorphosis. Statistical analyses of a small number of newly-metamorphosed pueruli of *J. edwardsii* collected off the North Island of New Zealand showed no correlation with any of the biotic or abiotic parameters (stored energy levels, water depth, distance offshore, phytoplankton biomass, sea surface temperature, salinity, or the distribution of late stage phyllosomas) chosen for comparison. However, there was a prior suggestion that metamorphosis of this species was associated with the inshore margins of the Waiarapa Eddy and its offshoots (Jeffs et al. 2001a). It is also doubtful whether the statistical analyses used in this study were valid because of the small numbers of newly-metamorphosed pueruli (33 out of the total 360 nektonic pueruli of any condition) caught. Moreover, it is doubtful whether correlations with water depth, sea surface temperature, or salinity would be useful parameters to select for statistical analyses indicating exogenous factors that may also be involved in inducing metamorphosis. Zooplankton biomass and composition, and times of sampling, may have been more relevant, but in any case these few newly-metamorphosed pueruli were collected over a wide stretch of offshore waters, (between 24 km and 216 km beyond the shelf break, mean of 94 km).

Jeffs (2005) was noncommittal about an answer to this question. He noted that metamorphosis appears to occur 'spontaneously' in cultured phyllosomas and therefore considered that it does not seem to depend on nutritional status, because cultured phyllosomas may often be 'nutritionally compromised' as so little is known about their diet and feeding requisites. Another factor overlooked here, is that, although cultured phyllosomas may not have been given the most nutritional and suitable diets, they have been fed regularly throughout their development, including those critical periods in the moult cycle when starvation could prove fatal, before metamorphosis is reached (see Gore 1985). Thus, poorer food quality and quantity, although provided regularly, may partially explain why, in earlier culture studies, only a few larvae have metamorphosed or, if so, most have failed to reach the first juvenile stage. Certainly it has been shown that nutritionally-enriched food has improved both the percentages of final instars metamorphosing and the numbers surviving as juveniles (Kittaka 2000).

The article by Yeung (2005) addressed the topic with respect to *Panulirus argus* stocks in the Florida Keys. She reported that its metamorphosis is believed to occur "offshore", but was thwarted in answering the question because catches of final-stage phyllosomas and pueruli of this species were too low to make any estimates. Yeung therefore focussed on larval transport pathways across the coastal eddies in a circulation model of the Florida Keys and offshoots of the Florida Current (see Yeung et al. 2001) and favoured an external stimulus as the 'trigger' for metamorphosis. Yeung (2005) suggested that "entrainment of the larvae in eddies of the coastal environment with different chemistry, biology and hydrodynamics from the surrounding ocean" would trigger their metamorphosis, and concluded that "with further field and laboratory investigation we might find that requisites for metamorphosis [of spiny lobsters] are variable among species, environments, or regions."

Dennis (2005) referred briefly to the alternative possibilities that metamorphosis in the tropical species, *P. ornatus*, was either a 'deterministic' process or not, meaning, apparently, either triggered by internal or external mechanisms, respectively.

Booth and Chiswell (2005), dealing with *J. edwardsii* phyllosomas and pueruli, from the same sampling program as Jeffs, off north-eastern New Zealand (see also Chiswell and Booth 1999; Jeffs et al. 2001a), were of directly opposite opinions to Jeffs (2005). They suggested that the external factor implicated in metamorphosis is when the final phyllosomas encounter more productive waters inshore of the Wairarapa Eddy—'right where most metamorphosis occurs,' (the criterion being most abundant final stage phyllosomas) and the 'trigger' is "the accumulation of sufficient stored energy reserves for the non-feeding puerulus, rather like the situation suggested by McWilliam and Phillips (1997) for *P. cygnus.*" In summary, Booth and Chiswell wrote: "What is important is that although the actual time of moult will be controlled by the phyllosoma's internal physiology, it will usually occur near shore, in the region of the shelf break, after the uptake of sufficient food."

Yoshimura (2005) had limited quantitative data on distribution and abundance of late- and final-stage phyllosomas and pueruli of *P. japonicus* in relation to the Kuroshio Current off southern Japan. He favoured an external cue "ín/around the Kuroshio such as sound, chemical, magnetic field, and so on—as reviewed by Jeffs et al. (2005) to initiate metamorphosis [sic] of the final phyllosomal instar." Despite his consideration of the successful results for cultured larval development to the puerulus of *P. japonicus*, under constant temperature, diet and photoperiod conditions, he commented that metamorphosis of this species in culture was because "some internal stimuli, such as time elapsed since molting or hatching, growth condition, or the amount of stored energy, may exist." But Yoshimura would not fully commit to an internal stimulus for phyllosomas to metamorphose, remarking that "since the rearing experiments have always used coastal water, it is difficult to entirely dismiss the possibility that some external stimulus such as a chemical, or salinity change, originating from coastal waters, is present".

In summary, one of the five articles (Jeffs 2005) indicated that the answer to the physiological question was probably a matter of molecular biology and genetics. Two other articles favoured an external, environmental stimulus for metamorphosis (Yeung 2005; Yoshimura 2005), despite Yoshimura's acknowledgement that metamorphosis can occur in culture situations, as well as in the wild. The two remaining articles (Booth and Chiswell 2005 and Dennis 2005) vacillated between the alternative views that metamorphosis was triggered by an internal or an external factor; Booth and Chiswell (2005) decided it was a mixture of both, and food was the external factor.

Response to these articles

We agree with Booth and Chiswell (2005) that a high-energy diet, and nutritional levels in the final stage larva (and perhaps the penultimate stage also) are implicated in successful palinurid metamorphosis (see McWilliam and Phillips, 1997, and references therein). But the advances in molecular biology within the last decade indicate that what probably induces metamorphosis in spiny lobsters is a much more complex, **endogenous** process than has been envisaged to date by most fisheries ecologists. So we agree with those contributors who mentioned the possibility that metamorphosis is 'programmed', and especially with Jeffs' (2005) final sentence referring to "recent advances in phyllosoma culture and biochemistry and genetic tools" having the potential to elucidate the problem.

We would go further and suggest that the onset of metamorphosis in all species of *Jasus* and *Panulirus* will probably be found to be genetically controlled, as is the onset of puberty in humans and mice through action of the gene, GPR54. This gene makes a receptor protein that probably is a key trigger of the hormonal cascade required for puberty to occur in these mammals (Seminara et al. 2003). The 'trigger' for palinurid metamorphosis may be a similar process, i.e., the "switching on" of a gene that makes a protein necessary for triggering a hormonal cascade that flows from a neural site to specific larval tissues and results eventually in the metamorphic moult to a fully-formed, functional, (but non-feeding) puerulus. Or maybe the converse occurs, the repression of a gene which inhibits the release of the critical hormonal cascade (Chang et al. 1993). Perhaps more than one gene is activated—or inactivated. Some form of epigenetic control of gene expression, causing chromatin alteration, may even be involved in the answer (see also Wolffe and Matzke 1999; Pennisi 2001: Beisel et al. 2002). Whatever the form of the putative, endogenous 'trigger', similarities in

its composition and mode of action seem likely to be found throughout larval development of all palinurids, and possibly throughout the Palinuroidea.

Meanwhile, there is still no definitive answer to the question 'what triggers metamorphosis in phyllosomas?' Expecting a simple answer would appear to be naïve at this early stage of investigation of molecular genetics in spiny lobster larvae, and judging by the complexities of the action, integration and 'cross-signalling' of these, and other crustacean hormones and genes not mentioned, or as yet, undefined; or perhaps of as yet undiscovered neurohormones.

In order to find answers to the question, further studies must first support or disprove the hypothesis that MF plays a similar role to JH in restraining metamorphosis during phyllosomal development in spiny lobsters, and identify the relevant hormonal signal, before the operative molecular mechanism(s) can be determined. As slipper lobsters are more closely related to spiny lobsters, than are prawns, clawed lobsters and crabs, such investigations could yield quicker results (because of their shorter larval period) by studying phyllosomas of *Thenus orientalis* cultured from egg to nisto. This coastal species (also a fisheries by-catch in Australian tropical waters) has a much shorter larval phase, <30 days in culture, and only four larval stages (I–IV) occur in the wild and in culture (Barnett et al. 1984; Mikami and Greenwood 1997).

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References

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

Adams MD, Celniker SE, Holt RA et al (196 co-authors) (2000) The genome sequence of D*rosophila melanogaster*. Science 287:2185–2195

Aiken DE (1980) Molting and growth. In: Cobb JS, Phillips BF (eds) The biology and management of lobsters. Physiology and behavior, vol 1. Academic Press, New York, pp 91–163

Anger K (2001) The biology of decapod crustacean larvae. AA Balkema Publishers, Lisse Abingdon

Ashburner M (1990) Puffs, genes and hormones revisited. Cell 61:1–3

Barnett BM, Hartwick RF, Milward NE (1984) Phyllosoma and misto stage of the Moreton Bay Bug, *Thenus orientalis* (Lund) (Crustacea: Decapoda: Scyllaridae), from shelf waters of the Great Barrier Reef. Aust J Mar Freshw Res 35:143–152

Beisel C, Imhof A, Greene J et al (2002) Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. Nature 419:857–862

Bertrand W, Brunet FG, Escriva H et al (2004) Evolutionary genomics of nuclear receptors: from twenty-five ancestral genes to derived endocrine systems. Mol Biol Evol 21:1923–1937

Booth J, Chiswell S (2005) Food is the factor. Lobster Newslett 18(2):15–18, http://www.odu.edu/∼mbutler/newsletter

Borst DW, Laufer H, Landau M et al (1987) Methyl farnesoate and its role in crustacean reproduction and development. Insect Biochem 7:1123–1127

Chan SM, Chen XG, Gu PI (1998) PCR cloning and expression of the molt-inhibiting hormone gene for the crab (*Charybdis ferriatus*). Gene 224:23–33

Chang ES (1993) Comparative endocrinology of molting and reproduction; insects and crustaceans. Ann Rev Entomol 38:161–180

Chang ES (1995) Current research on the hormonal control of crustacean molting. Lobster Newslett 8(1):8–9, http://www.odu.edu/∼mbutler/newsletter

Chang ES, Bruce MJ, Tamone SL (1993) Regulation of crustacean molting: a multi-hormonal system. Amer Zool 33:324–329

Charmantier G, Charmantier-Daures M (1988) Larval development and metamorphosis of the American lobster *Homarus americanus* (Crustacea, Decapoda) Effect of eyestalk ablation and juvenile hormone injection. Gen Comp Endocrin I70:319–333

Charmantier G, Charmantier-Daures M (1998) Endocrine and neuroendocrine regulations in embryos and larvae of crustaceans. Internat J Invert Reprod Develop 33:273–287

Charmantier G, Charmantier-Daures M, Aiken DE (1991) Metamorphosis in the lobster *Homarus* (Decapoda): a review. J Crust Biol 11:481–495

Charmantier G, Charmantier-Daures M, Van Herp F (1997) Hormonal regulation of growth and reproduction in crustaceans. In: Fingerman M, Nagabhushanam R, Thompson M-F (eds) Marine biotechnology, endocrinology and reproduction. Oxford and IBH Publishing, New Delhi

Chiswell SM, Booth JD (1999) Rock lobster *Jasus edwardsii* larval retention by the Wairarapa Eddy off New Zealand. Mar Ecol Progr Ser 183:227–240

Chung AC, Durica DS, Clifton SW et al (1998) Cloning of crustacean ecdysteroid receptor and retinoid-X receptor gene homologs and elevation of retinoid-X receptor mRNA by retinoic acid. Mol Cell Endocr 139:209–227

Dai L, Zitnan D, Adams ME (2007) Strategic expression of ion transport peptide gene products in central and peripheral neurons of insects. J Comp Neurol 500:353–357

Dennis DM (2005) Coral Sea *Panulirus ornatus*. Lobster Newsletter 18(2):4–15, http://www.odu.edu/~mbutler/newsletter

Devine C, Hinman VF, Degnan BM (2002) Evolution and developmental expression of nuclear receptor genes in the ascidian *Herdmania*. Internat J Develop Biol 46:687–692

Dubrovsky DB (2005) Hormonal cross talk in insect development. Trends Endocr Metab 16:6–11

El Haj AJ, Tamone SL, Peake M et al (1997) An ecdysteroid-responsive gene in a lobster—a potential crustacean member of the steroid hormone receptor superfamily. Gene 91:127–135

Erezyilmaz DF, Riddiford LM, Truman JW (2006) The pupal specifier *broad* directs progressive morphogenesis in a direct-developing insect. Proc Nat Acad Sci USA 103:6925–6930

Escriva H, Bertrand S, Laudet V (2004) The evolution of the nuclear receptor superfamily. In: Cambell PN (ed) Essays in biochemistry, vol 40, Published for The Biochem Soc (Great Britain) by Academic Press, London New York, p 11

Felder DL, Martin JW, Goy JW (1985) Patterns in early postlarval development of decapods. In: Wenner AM (ed) Larval growth. AA Balkema, Rotterdam

Fisk GJ, Thummel CS (1998) The DHr78 nuclear receptor is required for ecdysteroid signaling during the onset of *Drosophila* metamorphosis. Cell 93:543–555

Gäde G (2004) Regulation of intermediary metabolism and water balance of insects by neuropeptides. Ann Rev Entomol 49:93–113

Goldstein J, Matsuda H, Butler M (2006) Success! Caribbean spiny lobster, *Panulirus argus* is cultured from egg to juvenile for the first time. Lobster Newslett 19(1):3–4, http://www.odu.edu/~mbutler/newsletter

Gore RH (1985) Molting and growth in decapod larvae. In: Wenner AM (ed) Larval growth. AA Balkema, Rotterdam

Gu P-L, Chan S-M (1998) Cloning of a cDNA encoding a putative molt-inhibiting hormone from the eyestalk of the sand shrimp *Metapenaeus ensis*. Mol Mar Biol Biotech 71:214–220

Hummon AB, Amare A, Sweedler JV (2006) Discovering new invertebrate neuropeptides using mass spectrometry. Mass Spectro Rev 25:77–98

Illingworth J, Tong LJ, Moss GA et al (1997) Upwelling tank for culturing rock lobster (*Jasus edwardsii)* phyllosomas. Mar Freshw Res 48:935–940

Jackson FR, Schroeder AJ, Roberts MA et al (2001) Cellular and molecular mechanisms of circadian control in insects. J Insect Physiol 47:833–842

Jeffs AG (2005) Metamorphosis in spiny lobsters. Lobster Newslett 18(2):10–12, http://www.odu.edu/~mbutler/newsletter

Jeffs AG, Willmott ME, Wells RMG (1999) The use of energy stores in the puerulus of the spiny lobster *Jasus edwardsii* across the continental shelf of New Zealand. Comp Biochem Physiol Part A 123:351–357

Jeffs AG, Chiswell SM, Booth JD (2001a) Distribution and condition of pueruli of the spiny lobster *Jasus edwardsii* offshore from north-east New Zealand. Mar Freshw Res 52:1211–1216

Jeffs AG, Montgomery JC, Tindle CT (2005) How do spiny lobster post-larvae find the coast? N Z J Mar Freshw Res 39:605–617

Jeffs AG, Nichols PD, Bruce MP (2001b) Lipid reserves used by pueruli of the spiny lobster *Jasus edwardsii* in crossing the continental shelf of New Zealand. Comp Biochem Physiol Part A 129:305–311

Jenkin PM (1970) Control of growth and metamorphosis. Part II of Animal hormones: a comparative study. Pergamon Press, Oxford, UK

Jones G, Sharp PA (1997) Ultraspiracle: an invertebrate nuclear receptor for juvenile hormones. Proc Nat Acad Sci USA 94:13499–13503

Kanazawa A (1994) Nutrition and food. In: Phillips BF, Cobb JS, Kittaka J (eds) Spiny lobster management. Fishing News Books, Blackwell Scientific Publ, Oxford, UK

Kittaka J (1988) Culture of the palinurid *Jasus lalandii* from egg stage to puerulus. Nippon Suisan Gakkashi 54:87–93

Kittaka J (1994a) Larval rearing. In: Phillips BF, Cobb JS, Kittaka J (eds) Spiny lobster management. Fishing News Books, Blackwell Scientific Publ, Oxford, UK

Kittaka J (1994b) Culture of phyllosomas of spiny lobster and its application to studies of larval recruitment and aquaculture. Crustaceana 66:258–270

Kittaka J (2000) Culture of larval spiny lobsters. In: Phillips BF, Kittaka J (eds) Spiny lobster fisheries and culture. Fishing News Books, Blackwell Scientific Publ, Oxford, UK

Kittaka J, Ikegami E (1988) Culture of the palinurid *Palinurus elephas* from egg stage to puerulus. Nippon Suisan Gakkashi 54:1149–1154

Kittaka J, Kimura K (1989) Culture of the Japanese spiny lobster *Panulirus japonicus* from egg to juvenile. Nippon Suisan Gakkaishi 55:963–970

Kittaka J, Ono K, Booth JD (1997) Complete development of the green rock lobster *Jasus verreauxi* from egg to juvenile. Bull Mar Sci 61:57–71

Kittaka J, Ono K, Booth JD et al (2005) Development of the red rock lobster, *Jasus edwardsii*, from egg to juvenile. N Z J Mar Freshw Res 39:263–277

Koelle MR, Segraves WA, Hogness DS (1992) DHr3: a *Drosophila* steroid receptor homolog. Proc Nat Acad Sci USA 89:6167–6171

Laudet V(1997) Evolution of the nuclear receptor superfamily, early diversification from an ancestral orphan receptor. J Mol Endocr 19:207–226

Laudet V, Gronemeyer H (2002) The nuclear receptor factsbook. Academic Press, London

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

Laufer H, Borst DW, Baker FC et al (1986) The synthesis and regulation of methyl farnesoate, a new juvenile hormone for crustacean reproduction. In: Proceedings of the 4th international symposium of the International Society of Invertebrate Reproduction, Villeneuve-d'Ascq, France, 1–5 September 1986

Liddy GC, Kolkovski S, Nelson MM et al (2004) The lipid composition of early stage western rock lobster (*Panulirus cygnus)* phyllosoma; importance of polar lipid and essential fatty acids. J Shellfish Res 21:263–273

Liddy GC, Kolkovski S, Nelson MM et al (2005) The effect of PUFA enriched *Artemia* on growth, survival and lipid composition of western rock lobster, *Panulirus cygnus*, phyllosomas. Aquacult Nutrit 11:375–384

Liu L, Laufer H (1996) Isolation and characterization of sinus gland neuropeptides with both mandibular organ inhibiting and hyperglycemic effects from the spider crab *Libinia emarginata*. Archiv Insect Biochem Physiol 32:375–385

Liu F, Baggerman G, D'Hertog W et al (2006) *In silico* identification of new secretory peptide genes in *Drosophila melanogaster*. Mol Cell Protoeomics 5:510–522

Loi PK, Emmal SA, Park Y et al (2001) Identification, sequence and expression of a crustacean cardiaoactive peptide (CCAP) gene in the moth *Manduca sexta*. J Exp Biol 204:2803–2816

McConaugha JR (1985) Nutrition and larval growth. In: Wenner AM (ed) Larval growth, AA Balkema, Rotterdam

McKenney CL Jr (2005) The influence of insect juvenile hormone agonists on metamorphosis and reproduction in estuarine crustaceans. Integr Comp Biol 45:97–105

McWilliam PS, Phillips BF (1997) Metamorphosis of the final phyllosoma and secondary lecithotrophy in the puerulus of *Panulirus cygnus* George: a review. Mar Freshw Res 48:783–790

Maki A, Sawatsubashi S, Ito S et al (2004) Juvenile hormones antagonize ecdysone actions through co-repressor recruitment to EcR/USP heterodimers. Biochem Biophys Res Commun 320:262–267

Marco HG, Blais C, Soyez D, Gade G (2001) Characterisation of moult-inhibiting activities of sinus glands of the spiny lobster *Jasus lalandii*. Invert Reprod Develop 39:99–107

Matsuda H, Yamakawa T (2000) The complete development and morphological changes of larval *Panulirus longipes* (Decapoda, Palinuridae) under laboratory conditions. Fisheries Sci 66:278–293

Matsuda H, Takenouchi T, Goldstein JS (2006) The complete larval development of the pronghorn spiny lobster *Panulirus penicillatus* (Decapoda: Palinuridae) in culture. J Crust Biol 26:579–600

Matsuda H, Takenouchi T, Yamakawa T (2003) Diel timing of molting and metamorphosis of *Panulirus japonicus* phyllosoma larvae under laboratory conditions. Fisheries Sci 69:124–130

Mesce KA, Fahrbach SE (2002) Integration of endocrine signals that regulate insect ecdysis. Front Neuroendocr 23:179–199

Mikami S, Greenwood JG (1997) Influence of light regimes on phyllosomal growth and timing of moulting in *Thenus orientalis* (Lund) (Decapoda, Scyllaridae). Mar Freshw Res 48:777–782

Moss G, James P, Tong L (2000) *Jasus verreauxi* phyllosomas cultured. Lobster Newslett 13(1):9–10, http://www.odu.edu/∼mbutler/newsletter

Mu X, Leblanc GA (2004) Cross communication between signaling pathways:juvenoid hormones modulate ecdysteroid activity in a crustacean. J Exp Zool 301A: 793–801

Nelson MM, Bruce ME, Nichols PD et al (2006) Nutrition of wild and cultured lobsters. In: Phillips BF (ed) Lobsters: biology, management, aquaculture and fisheries. Blackwell Publishing, Oxford, UK

Nichols P, Mooney B, Jeffs A (2001) The lipid, fatty acid and sterol composition of potential prey items of the southern rock lobster *Jasus edwardsii*: an aid to identification of food-chain interactions. Report 2001-CMR/MP2, April 2001, CSIRO Marine Research, Hobart, TAS 7000, Australia

Nijhout HF (1994) Insect hormones. Princeton Univ Press, Princeton, New Jersey

Nijhout HF (2003) The control of body size in insects. Develop Biol 261:1–8

Palli SR, Osir EO, Eng WE et al (1990) Juvenile hormone receptors in insect larval epidermis: identification by photoaffinity labeling. Proc Nat Acad Sci, USA 87:796–800

Palli SR, Riddiford LM, Hiruma K (1991) Juvenile hormone and "retinoic acid" receptors in *Manduca* epidermis. Insect Biochem 21:7–15

Pennisi E (2001) Behind the scenes of gene expression. Science 293:1064–1067

Phillips BF, Jeffs AG, Melville-Smith R et al (2006) Changes in lipid and fatty acid composition of late larval and puerulus stages of the spiny lobsster (*Panulirus cygnus*) across the continental shelf of Western Australia. Comp Biochem Physiol Part B 143:219–228

Phleger CF, Nelson MM, Mooney BD et al (2001) Lipids and nutrition of the southern rock lobster, *Jasus edwardsii*, from hatch to puerulus. Mar Freshw Res 52:1475–1486

Phlippen MK, Webster SC, Chung JS et al (2000) Ecdysis of decapod crustaceans is associated with a dramatic release of crustacean cardioactive peptide hormone into the haemolymph. J Exp Biol 203:521–536

Pursley S, Ashok M, Wilson TG (2000) Intracellular localization and tissue specificity of the *Methoprene-tolerant* (*Met*) gene product in *Drosophila melanogaster*. Insect Biochem Mol Biol 30:839–845

Riddiford LM (1993) Hormone receptors and the regulation of insect metamorphosis. Receptor 3:204–209

Riddiford LM, Hiruma K, Zhou X et al (2003) Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manchuca sexta* and *Drosophila melanogaster*. Insect Biochem Mol Biol 33:1327–1338

Sekine S, Shima Y, Fushimi H, Nonaka M (2000) Larval period and molting in the Japanese spiny lobster *Panulirus japonicus* under laboratory conditions. Fisheries Sci 66:19–24

Seminara SB, Messager S, Chatzidaki EE et al (2003) The GPR54 gene as a regulator of puberty. N Engl J Med 349:1614–1627

Snodgrass RE (1956) Crustacean metamorphoses. Smithsonian Misc Coll 131(10):1–78

Spindler K-D (1991) Role of morphogenetic hormones in the metamorphosis of arthropods other than insects. In: Gupta AP (ed) Morphogenetic hormones of arthropods. Rutgers Univ Press, New Brunswick, New Jersey

Stangier J, Hilbich C, Dircksen H et al (1988) Distribution of a novel cardioactive neuropeptide (CCAP) in the nervous system of the shore crab, *Carcinus maenas*. Peptides 9:795–800

Sullivan AA, Thummel CS (2003) Temporal profiles of nuclear receptor gene expression reveal coordinate transcriptional responses during *Drosophila* development. Mol Endoc 17:2125–2137

Suzuki N, Murakami K, Takeyama K et al (2006) Molecular attempt to identify prey organisms of lobster phyllosoma larvae. Fisheries Sci 72:342–349

Takahashi Y, Nishida S, Kittaka J (1994) Histological characteristics of fat bodies in the puerulus of the rock lobster, *Jasus edwardsii* (Hutton, 1875) (Decapoda, Palinuridae). Crustaceana 66:318–325

Teshima S, Kanazawa A (1971) Biosynthesis of sterols in the lobster, *Panulirus japonicus*, the prawn, *Penaeus japonicus*, and the crab, *Portunis trituberculatus*. Comp Biochem Physiol 38B:597–602

Thain M, Hickman M (2004) The penguin dictionary of biology, 11th edn. Penguin Books Ltd., London, UK

Thummel CS (1996) Flies on steroids—*Drosophila* metamorphosis and the mechanisms of steroid hormone action. Trends Genet 12:306–310

Truman JW (1996) Ecdysis control sheds another layer. Science 271:40–41

Tuberty SR, McKenney CI Jr (2005) Ecdysteroid responses of estuarine crustaceans exposed through complete larval development to juvenile hormone agonist insecticides. Integr Comp Biol 45:106–117

Waddy SL, Aiken DE, Younglai WW (1990) Scotophase influences timing of the metamorphic molt in larval American lobsters (*Homarus americanus*). Amer Zool 30:A129 [Abstract only available]

Wainwright G, Webster SG, Wilkinson MC et al (1996) Structure and significance of mandibular organ-inhibiting hormone in the crab, *Cancer pagurus*, involvement in multihormonal regulation of growth and reproduction. J Biol Chem 271:12749–12754

Watson RD, Spaziani E (1985) Biosynthesis of ecdysteroids from cholesterol by crabY-organs, and eyestalk suppression of cholesterol uptake and secretory activity, *in vitro*. Gen Comp Endocr 59:140–148

White KP, Hurban P, Watanabe T (1997) Coordination of *Drosophila* metamorphosis by two ecdysone-induced nuclear receptors. Science 276:114–117

Wilson K, Swan J, Hall M (2005a) Reducing rock lobster larval rearing time through hormonal manipulation. Rock Lobster Enhancement and Aquaculture Subprogram. FRDC Project 2000/263, Australian Govt, Fisheries Research and Development Corporation and Australian Institute of Marine Science, Nov 2005

Wilson K, Hall M, Davey M et al (2005b) Biotechnology to improve reproductive performance and larval rearing in prawns and rock lobsters. In: Pandian TJ, Strusmann CA, Marian MP (eds) Fish genetics and aquaculture biotechnnology. Oxford and IBH Publishing Co Pty Ltd

Wilson TG, Yerushalmi Y, Donnell DM et al (2006) Interaction between hormonal signaling pathways in *Drosophila melanogaster* as revealed by genetic interaction between Methoprene-tolerant and Broad-Complex. Genetics 172:253–254

Wolffe AP, Matzke MA (1999) Epigenetics: regulation through repression. Science 293:481–485

Xu Y, Fang F, Chu YX et al (2002) Activation of transcription through the ligand-binding pocket of the orphan nuclear receptor ultraspiracle. Eur J Biochem 269:6026–6036

Yamauchi MM, Miya MU, Nishida M (2002) Complete mitochondrial DNA sequence of the Japanese spiny lobster, *Panulirus japonicus* (Crustacea:Decapoda). Gene 295:89–96

Yeung C (2005) The Florida spiny lobster. Lobster Newslett 18(2):12–13, http://www.odu,edu/∼mbutler/newsletter

Yeung C, Jones DL, Criales MM et al (2001) Influence of coastal eddies and counter-currents on the influx of spiny lobster, *Panulirus argus* postlarvae in Florida Bay. Mar Freshw Res 52:217–232

Yoshimura T (2005) The final-stage phyllosoma metamorphoses to the puerulus while caught up in the flow of the Kuroshio? Lobster Newslett 18(2):18–21, http://www.odu,edu/∼mbutler/newsletter

Zhao Y, Meredith J, Brock HW, Phillips JE (2005) Mutational analysis of the N-terminus in *Schistocerca gregaria* ion-transport peptide expressed in *Drosophila* Kcl cells. Arch Insect Biochem Physiol 58:27–38

Zitnan D, Ross LS, Zitnanova I (1999) Steroid induction of a peptide hormone gene leads to orchestration of a defined behavioral sequence. Neuron 23:523–535