The endocrine control of reproduction in Nereidae: a new multi-hormonal model with implications for their functional role in a changing environment

A. J. Lawrence[1],* and J. M. Soame[2]

[1]*Department of Life Sciences, University of the West Indies, Saint Augustine, Trinidad and Tobago*
[2]*Department of Biological Sciences, University of Hull, Hull HU6 7RX, UK*

Nereidae are vital to the functioning of estuarine ecosystems and are major components in the diets of over-wintering birds and commercial fish. They use environmental cues to synchronize reproduction. Photoperiod is the proximate cue, initiating vitellogenesis in a temperature-compensated process. The prevailing paradigm in Nereidae is of a single 'juvenile' hormone controlling growth and reproduction. However, a new multi-hormone model is presented here that integrates the environmental and endocrine control of reproduction. This is supported by evidence from *in vitro* bioassays. The juvenile hormone is shown to be heat stable and cross reactive between species. In addition, a second neuro-hormone, identified here as a gonadotrophic hormone, is shown to be present in mature females and is found to promote oocyte growth. Furthermore, dopamine and melatonin appear to switch off the juvenile hormone while serotonin and oxytocin promote oocyte growth. Global warming is likely to uncouple the phase relationship between temperature and photoperiod, with significant consequences for Nereidae that use photoperiod to cue reproduction during the winter in northern latitudes. Genotypic adaptation of the photoperiodic response may be possible, but significant impacts on fecundity, spawning success and recruitment are likely in response to short-term extreme events. Endocrine-disrupting chemicals may also impact on putative steroid hormone pathways in Nereidae with similar consequences. These impacts may have significant implications for the functional role of Nereidae and highlight the importance of comparative endocrinology studies in these and other invertebrates.

Keywords: Nereidae; reproduction; photoperiod; gonadotrophic hormones;
climate change; endocrine disrupters

1. INTRODUCTION

Emergent properties of ecosystems (i.e. their functional role) are now recognized as fundamental in relation to considerations of the value of a system with regard to poverty alleviation and human wellbeing (Millenium Ecosystem Assessment). This has led the Conference of the Parties (COP) to the Convention on Biological Diversity (CBD) to ask countries to apply the ecosystem approach as the major framework within National Biodiversity Strategy and Action Plans (NBSAPs) and as the primary mechanism to support the CBD and its mainstreaming into other national sectors.

The COP has also called for the integration or mainstreaming of climate change within NBSAPs. This initiative highlights the general consensus that anthropogenic impacts are leading to rapid climate change, with significant consequences for biodiversity and ecosystem processes. In particular, it is forecast that the resilience of many ecosystems is likely to be exceeded this century by a combination of climate change and pollution impacts, among other drivers (IPCC 2007).

Major ecosystem goods and services can be clearly recognized and appreciated. For example, marine systems provide food and other products, support tourism, act as carbon sinks or provide flood protection to coastal areas. However, while these functions can be recognized, the mechanisms and processes that maintain them are far more difficult to determine, monitor or model.

It has been suggested that the role of biodiversity on ecosystem processes depends on the functional characteristics of each species and the interactions among species present in the community (Loreau *et al.* 2001; Jonsson & Malmqvist 2003). While this would seem intuitive, the relationship between biodiversity and ecosystem goods and services depends on much more than this. It depends on, and therefore requires a fundamental understanding of, the underlying biological processes from molecular and cellular to physiology and reproduction of the organisms within the ecosystem, their environmental control and the linkage between these and population level responses. Critical within this pathway is the linkage between reproduction and fecundity and population/ecosystem structure. It is

* Author for correspondence (andrew.lawrence@sta.uwi.edu).

only with this that the relationship between the potential impact of biodiversity loss on ecosystem processes will be understood and some predictive capacity to foresee this impact become available.

In this context, the examination and understanding of endocrine systems is a fundamental prerequisite because the endocrine system acts as the transducer between the environment and the physiological or behavioural response of an organism. Consequently, it has been argued that comparative endocrinology has a key role to play in resolving mechanisms underlying responses to the environment (Wingfield 2008).

The aim of this paper is to present the scientific rationale for such a scenario and specifically to consider the potential impact of climate change and pollution on the reproduction of the Nereidae, marine polychaete worms, with regard to the implications for their functional role within some ecosystems. This includes a re-examination of the endocrine control of gametogenesis in these organisms.

2. THE FUNCTIONAL IMPORTANCE OF NEREIDAE

The Nereidae are common polychaetes living in coastal and estuarine habitats. Species such as *Nereis diversicolor* may live in these areas at densities up to 3700 m^2 (Scaps 2002). They may employ a number of feeding strategies including active predation, herbivory, suspension and deposit feeding (Riisgaard & Kamermans 2001; Volkenborn & Reise 2006).

It is now clear that the Nereidae, as well as other polychaete groups, play a fundamental role in the ecology and functioning of the systems they inhabit and as such may be considered keystone species. For example, as active predators, they regulate infaunal benthic populations (Commito 1982; Commito & Shrader 1985; Ambrose 1986). As suspension feeders within fjords, *N. diversicolor* populations can filter the whole water mass about three times per day, reducing the phytoplankton biomass by 50 per cent within less than 5 h (Riisgaard *et al.* 2004). Thus, Nereidae, especially *N. diversicolor*, may exert a pronounced controlling impact on the phytoplankton in sheltered coastal areas and provide a clear linkage between the benthic system and the overlying pelagic system.

The biogeochemical cycles of nutrients and contaminants within estuaries are greatly modified by the bioturbation of Nereidae (Davey & Watson 1995; Gunnarsson *et al.* 1999; Banta & Andersen 2003). Francois *et al.* (2002) classified *N. diversicolor* as a gallery-diffusor displaying a combination of bioadvective (fast uni-directional downward transport) and biodiffusive mechanisms (bi-directional mixing). As such, they have been shown to relocate a variety of plant and organic material into the sediment (Raffaelli 2000), including filamentous green algae, to a depth of 4 cm (Nordström *et al.* 2006). Bioturbation and herbivory by *N. diversicolor* also appear to affect *Spartina* distribution and lead to the loss of these pioneering species (Paramor & Hughes 2004, 2007).

The Nereidae and particularly the ragworm *N. diversicolor* also form the most important food source for many wading birds and benthic fish

(McLusky 1989; Masero *et al.* 1999; Moreira 1999; Lawrence & Soame 2004; Vinagre & Cabral 2008). For example, oystercatchers feed on several species including *N. diversicolor* (Boates & Goss-Custard 1992) and females show a distinct preference for this species (Ens *et al.* 1996). Oystercatchers also seem to switch between surface prey, such as *N. diversicolor*, to deep living prey, like *Macoma balthica*, in the spring and autumn (Zwarts *et al.* 1996). Black-headed gulls (*Larus ridibundus*) prey mainly on *N. diversicolor* in the summer (Moreira 1995) as do Dunlin in the Wadden Sea (Nehls & Tiedemann 1993). During the winter, *N. diversicolor*, *N. hombergii* and *S. armiger* constitute approximately 99 per cent of the prey for Bartailed Godwit (Scheiffarth 2001). Predation can be a key factor affecting the population dynamics of *N. diversicolor*, and one study suggests that levels of predation by birds and fish indicate that they can become a limiting resource (Rosa *et al.* 2008).

3. REPRODUCTION IN NEREIDAE

The reproductive biology of the Nereidae has been well studied over the past 60 years, most recently owing to their commercial value. Following a period of gametogenesis, strictly semelparous species such as the Nereidae spawn their gametes in a one-time terminal event. It is, essential, therefore, that gametogenesis in these species is coordinated across the whole population and that individuals release their gametes at the same time. Coordination is achieved through environmental and endocrine interactions to make sure all gametes develop at the same time, and pheromonal cues to ensure that individuals release their gametes in synchrony with one another (Bentley & Pacey 1992).

In the Nereidae, the age at which individuals enter reproduction, proceed to maturity and spawn is dependent on growth rate (Olive *et al.* 1986). In this respect, the environment is an important influence in determining the rate of somatic growth. In a food-rich environment, *Nereis virens* can mature and spawn in their first year, whereas this process normally takes 2 years (Bentley & Pacey 1992). Studies on other marine invertebrates also indicate that food supply is the most important controlling factor in determining maturation and spawning (Newell *et al.* 1982). However, a key element in the reproductive cycle of Nereidae is the irreversible transition from somatic growth to reproductive development several months prior to breeding (Olive 1995).

There are four principal stages in the gametogenic process: pre-vitellogenesis, vitellogenesis, corticogenesis and maturation (Dhainaut 1984) (table 1). During pre-vitellogenesis, oogonia divide mitotically to produce oocytes, which appear clustered in the coelomic fluid forming a dispersed ovary (Eckelberger 1986). The pre-vitellogenic phase effectively ends once the oocytes dissociate from the clusters to become individual cells floating in the coelom.

During vitellogenesis, vitellin (yolk protein) accumulates within the developing oocyte (Dhainaut 1984). The Nereidae follow a pattern of extraovarian vitellogenesis (Eckelberger 1986) with both glycoprotein-rich

Table 1. The different oocyte stages and their corresponding size ranges in the *N. virens, N. diversicolor* and *P. dumerilii*.

stage of oocyte development	*N. virens* (Clark & Ruston 1963)	*N. diversicolor* (Dhainaut 1984)	*P. dumerilii* (J. D. Hardege 2000, personal communication)
pre-vitellogenesis (μm)	less than 100	less than 70	less than 80
vitellogenesis (μm)	100–140	70–140	80–140
corticogenesis (μm)	140–180	140–180	140–180
maturity (μm)	more than 180	more than 180	more than 180

yolk and lipid droplets being laid down heterosynthetically. Vitellogenin, the vitellin precursor, is synthesized and released by eleocytes within the coelomic fluid (Fischer & Hoeger 1993). Corticogenesis or cortical alveoli formation follows vitellogenesis, and during this stage of development, carbohydrate material begins to accumulate in the ooplasm (Dhainaut 1984).

At the time of gamete maturation, many Nereidae species undergo a metamorphosis from the atokous to the epitokous form. This allows the animals to swim towards the surface of the sea at the time of spawning. *Nereis diversicolor* is an exception to this, with the female spawning in the atokous form within its burrow (Bartels-Hardege & Zeeck 1990). Spawning is precisely timed within populations of Nereidae. It is therefore considered that various environmental factors may act as zeitgebers to maintain the observed synchrony, and that this may be mediated through a possible control of hormone titre.

4. ENDOCRINE CONTROL OF REPRODUCTION

The consensus on the endocrine control of growth and reproduction in the Nereidae has been that of a single-hormone model. In this model, the supra-oesophageal ganglion secretes a juvenile hormone called nereidine. High concentration of nereidine promotes growth and regeneration of lost segments in young animals (Golding 1967). The hormone also permits the accumulation of oogonia into the coelom (pre-vitellogenesis) while inhibiting sexual maturation (Golding 1967; Franke & Pfannensteil 1984). Removal of the hormone leads to precocious sexual development, but oocytes form abnormally and degenerate. The hormone, therefore, appears to be required in low concentrations for normal oogenesis to occur.

As the animal ages, it has been suggested that there is a staged decline in the circulating hormone titre (Durchon & Porchet 1971; Porchet 1972). The animal's ability to regenerate lost segments is greatly reduced (Golding 1967), and this is coupled with a decrease in the growth rate of the animal. At the same time, the gametes begin to develop (Clark & Ruston 1963; Clark & Scully 1964; Andries 2001). The stage of oocyte development appears to be

correlated with the regenerative ability of the animal. For example, once the oocytes of *N. diversicolor* reach 140 μm, regeneration of amputated segments is not observed. Similarly, the removal of the supra-oesophageal ganglion at this stage does not result in the precocious development of the oocytes (Golding 1967).

Based on the single-hormone model, it has been suggested that the development of the gametes in Nereidae is stage-specific and, as such, there may be biochemical stages within gametogenesis that can only proceed in a specific, declining, endocrine milieu (Franke & Pfannensteil 1984). This was supported by Dhainaut (1984) who observed that brain removal halted vitellogenesis, with the advancement to corticogenesis in *Perinereis cultrifera*. With the discovery that vitellogenesis in Nereidae is heterosynthetic, it is now considered that decerebration causes the transition from heterotrophic yolk synthesis to autosynthetic corticogenesis (Porchet *et al.* 1989).

The single-hormone model therefore requires that the juvenile hormone of the Nereidae has many roles. In this model, it controls and integrates cellular functions associated with growth and regeneration; it controls somatic metamorphic events associated with the transition to the sexual condition; it also provides gametotrophic support for developing oocytes, inhibits the terminal maturation of oocytes and regulates the metabolic activities of the eleocytes (Olive 1997).

However, the presence of oocytes at different sizes and stages of development within an individual animal raises doubts over this theory (Olive & Garwood 1981; Golding 1983; Fischer 1984; Andries 2001). Furthermore, Golding (1983) reported little or no change in cerebral endocrine activity throughout gametogenesis in *N. diversicolor*. Oocytes only became homogeneous during the final five months when the juvenile hormone titre was found to decline (Golding 1983; Andries 2001). In addition, oestradiol-17β has recently been isolated from the coelomic fluid of *N. virens* where it has been shown to promote the secretion of vitellogenin by the eleocytes of mature females (Garcia-Alonso *et al.* 2006). These observations seem to contradict the idea of a single hormone controlling specific stages of development and suggest that the endocrine control of gametogenesis is not yet fully understood in the Nereidae.

Consequently, we have recently re-examined the endocrine control of gametogenesis in Nereidae, developing an *in vitro* bioassay using cultured oocytes from either *Platynereis dumerilii* or *Nereis succinea* incubated in Fisher's culture medium. The advantage of using these species is that both undergo rapid oogenesis and oocyte growth in the week prior to spawning, with the oocytes undergoing the full oogenic cycle in that period (J. D. Hardege 2000, personal communication). Oocytes were cultured with ganglia from juvenile or adult male or female ganglia to determine the activity of the juvenile hormone, the presence of any other endocrine factors, the heterospecificity and heat stability of the hormone and its interaction with other, known hormones.

Figure 1*a* illustrates the results obtained in the first assay carried out on *P. dumerilii* oocytes with an average oocyte diameter of 47 µm. Pair-wise comparisons showed that the growth rate of oocytes in the no-ganglia treatment was significantly higher than that observed in the juvenile ganglia treatment ($p < 0.01$). In addition, oocytes incubated with mature female ganglia increased in size significantly more than all other treatment groups ($p < 0.001$ all treatments).

Oocytes incubated with the juvenile ganglia showed the slowest rate of growth and were significantly smaller than those incubated with mature male ganglia ($p < 0.01$). This indicates that the growth of the oocytes in this treatment was inhibited by the juvenile hormone within the ganglia.

While the observed inhibition of growth by the juvenile ganglia supports the evidence from previous studies on the action of the juvenile hormone, the observation that mature female ganglia promoted growth significantly more than any other treatment is unique and is the first evidence to indicate that there is a second hormone, present within the supra-oesophageal ganglia of mature female *P. dumerilii*, which specifically promotes egg growth in a manner similar to that reported in some iteroparous species (Olive & Lawrence 1990; Lawrence & Olive 1995). As such, this hormone may be considered a gonadotrophic hormone.

In a second assay, using oocytes with a mean diameter of 50 µm, this pattern of differences in growth rate, under the varying endocrine influences, was found to be consistent over a 5-day period (figure 1*b*). Oocytes incubated with the mature female ganglia showed the greatest increase in size while those incubated with the juvenile ganglia showed little growth. The oocytes in the no-ganglia treatment increased steadily over the experimental period, but never to the extent of the female ganglia. The mature male treatment appeared to increase in size with the no-ganglia treatment. There were low levels of oocyte degeneration throughout the experiment. Consequently, the data support those of the previous assay and indicate that at least two hormones are involved in the control of oogenesis in *P. dumerilii*.

In a second set of tests, the bioassay was used to begin the characterization of the juvenile hormone through testing for heat stability as well as the heterospecificity of the hormone. *Platynereis dumerilii* oocytes with an average diameter of 133 µm were incubated in various treatments over a period of 3 days (figure 2*a*).

Results from this assay contrasted with those from the first assays. Pair-wise comparisons showed that oocytes incubated with no ganglia increased significantly more in diameter than any other treatment group ($p < 0.001$). Oocytes incubated with mature female ganglia also increased significantly more than oocytes incubated with no ganglia, juvenile ganglia, boiled juvenile ganglia and juvenile *N. virens* ganglia ($p < 0.001$). Oocytes from the juvenile ganglia treatment showed inhibited growth as did those with the boiled juvenile ganglia and juvenile *N. virens* ganglia, and there was no significant difference between these treatments (juvenile ganglia and boiled juvenile ganglia; juvenile ganglia and juvenile *N. virens* ganglia;

juvenile ganglia and the initial reading). There was no oocyte degeneration in the various treatments.

The data indicated, therefore, that the juvenile hormone is both heat stable and heterospecific between *N. virens* and *P. dumerilii*. The switch in pattern between the no-ganglia and mature female treatments indicates that the action of female ganglia is dependent on the initial oocyte size, with oocyte diameters in this experiment being larger than those used in the previous assays.

The heterospecificity of the hormone was further tested through the *in vitro* culture of *N. succinea* oocytes with an average diameter of 70 µm, cultured either with no ganglia, juvenile *N. succinea* ganglia, juvenile *P. dumerilii* or mature female *P. dumerilii* ganglia. Only a low level of oocyte degeneration was observed across all treatments.

Oocytes cultured with mature female *P. dumerilii* ganglia increased in diameter dramatically compared with the other treatments (figure 2*b*). Pair-wise comparisons showed this difference to be highly significant ($p < 0.001$). Oocytes from the no-ganglia treatment also increased significantly in size compared with the juvenile ganglia treatments ($p < 0.001$). Oocyte growth in the *P. dumerilii* and the *N. succinea* juvenile ganglia treatments showed little increase from the initial size, and there was no significant difference between these.

These results further support the evidence that the juvenile hormone is heterospecific between *P. dumerilii*, *N. virens* and *N. succinea*. Consequently, it appears that the structure and action of the hormone are highly conserved between species in the Nereidae. Furthermore, oocytes incubated with mature female ganglia grew beyond that observed in the no-ganglia treatment, supporting the evidence that the female ganglia may be the source of a growth-promoting, gonadotrophic hormone.

The effect of the known hormones, dopamine, melatonin, oxytocin and serotonin, on the activity of the juvenile hormone was also examined. In this assay, groups of 10 juvenile *P. dumerilii* were maintained *in vivo* in solutions of each of the hormones at a concentration of 10 µg ml^{-1} for 3 days prior to the collection and preparation of the ganglia. The ganglia were then tested on oocytes from *N. succinea* with an average diameter of 115 µm. Oocyte degeneration was low in all the experimental treatments.

Oocytes incubated with mature female ganglia showed the largest increase in size (figure 3). Pair-wise comparisons showed the size of these oocytes to be significantly larger than those in all other treatment groups ($p < 0.001$), except the juvenile ganglia incubated with oxytocin ($p > 0.10$). Oocytes incubated with juvenile ganglia were significantly smaller than those from all other groups ($p < 0.001$) except the initial group ($p > 0.67$). There was no difference in size between oocytes from the no-ganglia treatment and the juvenile ganglia with dopamine, or the juvenile ganglia with melatonin. The no-ganglia oocytes were, however, statistically different from all other treatment groups. The hormone treatment groups were all significantly different from one another, except serotonin and oxytocin ($p > 0.05$).
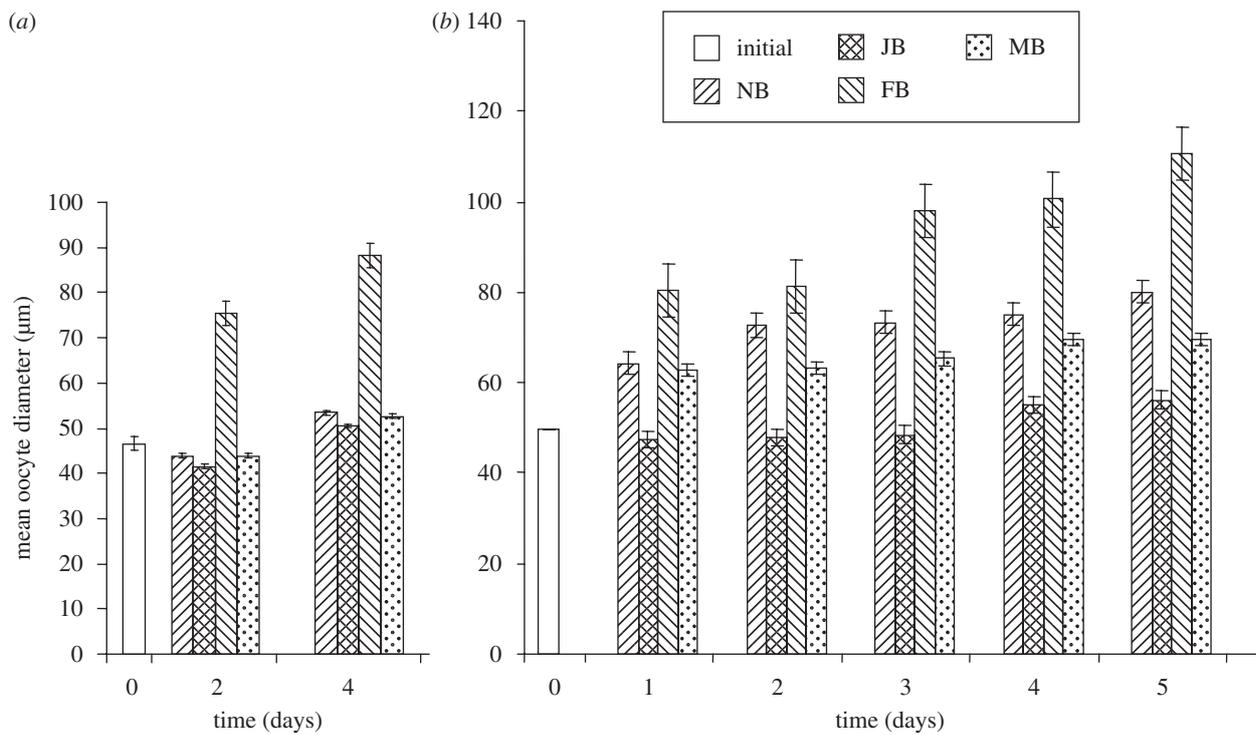
Figure 1. The average increase in diameter of *P. dumerilii* oocytes incubated *in vitro* with no ganglia (NB), juvenile *P. dumerilii* ganglia (JB), mature female *P. dumerilii* ganglia (FB) and mature male *P. dumerilii* ganglia (MB) and compared with an initial oocyte measurement. Bars denote standard error of the means. (a) Assay performed over 4 days. There were highly significant differences between treatments using a Kruskal–Wallis test, with Mann–Whitney U pair-wise comparisons ($n = 500$, $\chi^2 = 440.65$, d.f. $= 4$, $p < 0.001$). (b) Assay performed over 5 days. Two-way ANOVA showed that both treatment and time had a significant effect on the data ($p < 0.001$). A significant interaction between the two was also observed ($p < 0.001$). Games–Howell post hoc analysis found significant differences between each of the treatment group except the juvenile ganglia with the initial reading ($p > 0.74$).
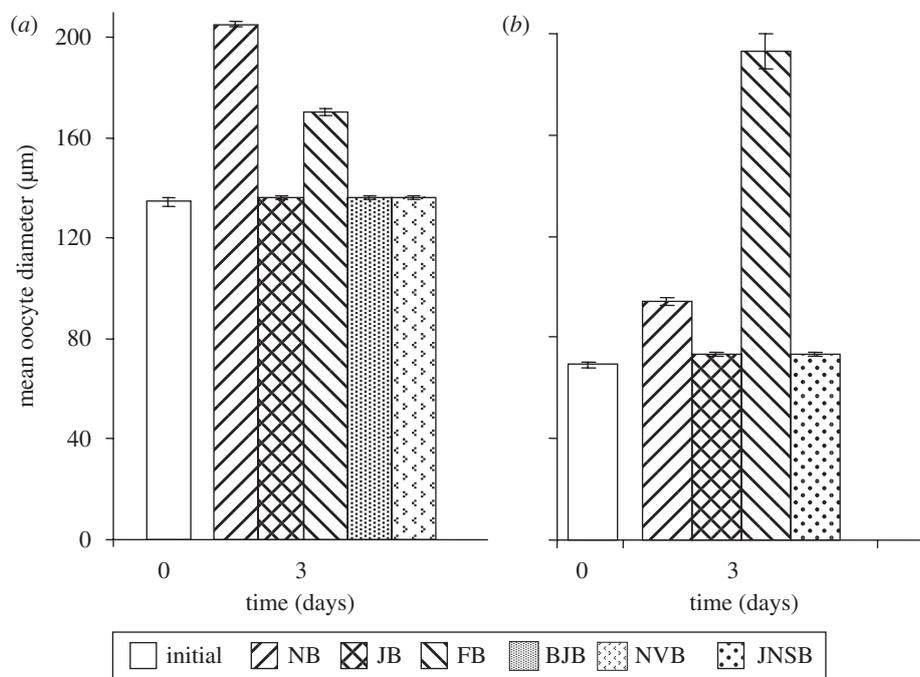

Figure 2. The average increase in diameter of *P. dumerilii* and *N. succinea* oocytes incubated *in vitro* and compared with an initial oocyte measurement. Bars denote the standard error of the means. (a) *Platynereis dumerilii* oocytes incubated with no ganglia (NB), juvenile *P. dumerilii* ganglia (JB), mature female *P. dumerilii* ganglia (FB), boiled juvenile *P. dumerilii* ganglia (BJB) and juvenile *Nereis virens* ganglia (NVB). There was a highly significant difference between treatments using the Kruskal–Wallis test ($n = 500$, $\chi^2 = 1600.70$, d.f. $= 5$, $p < 0.001$). (b) *Nereis succinea* oocytes incubated with no ganglia (NB), juvenile *P. dumerilii* ganglia (JB), mature female *P. dumerilii* ganglia (FB) and juvenile *N. succinea* ganglia (JNSB). There were significant differences between the treatments using the Kruskal–Wallis test ($n = 250$, $\chi^2 = 325.47$, d.f. $= 4$, $p < 0.001$).
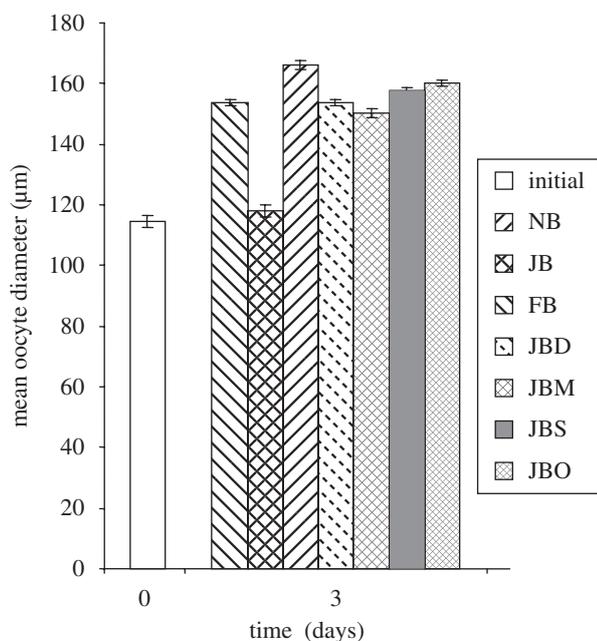
Figure 3. The average increase in diameter of *N. succinea* oocytes incubated *in vitro* with no ganglia (NB), juvenile *P. dumerilii* ganglia (JB), mature female *P. dumerilii* ganglia (FB), juvenile ganglia incubated for 3 days *in vivo* with dopamine ($10 \, \mu g \, ml^{-1}$) (JBD), melatonin ($10 \, \mu g \, ml^{-1}$) (JBM), serotonin ($10 \, \mu g \, ml^{-1}$) (JBS) and oxytocin ($10 \, \mu g \, ml^{-1}$) (JBO). Bars denote the standard error of the means. There was a highly significant difference between treatments using the Kruskal–Wallace test ($n = 250$, $\chi^2 = 544.46$, d.f. $= 7$, $p < 0.001$).

Overall, it would appear, therefore, that the known hormones do affect oocyte growth. Dopamine and melatonin appeared to switch off the action of the juvenile hormone, whereas serotonin and oxytocin appeared to have a positive effect on oocyte development. These hormones are known to be present in a wide variety of other invertebrates (Hardeland & Poeggeler 2003) and have been found to play a role in reproductive processes. Evidence for the existence of these hormones in the Nereidae is now coming to light. For example, oxytocin-like hormones have been identified in mature female *N. diversicolor* and *Perinereis vencourica* ganglia (Fewou & Dhainaut-Cortois 1995; Matsushima *et al.* 2002) and vasotocin (vasopressin/oxytocin)–neurophysin is expressed in the developing forebrain of *P. dumerilii* (Tessmar-Raible *et al.* 2007). Most recently, immunopositive staining for serotonin has been reported in *N. diversicolor* (Heuer & Loesel 2008). Furthermore, the fact that the supra-oesophageal ganglion of juvenile animals is responsive to these hormones *in vivo* indicates some inherent mechanistic competency within the neuroendocrine system to interact with these elements.

There is also a marked similarity in the reproduction of Nereidae with that of the nemertean *Lineus lacteus*. Gonad maturation in both is controlled by a gonad-inhibiting hormone, and the reproductive cycle is influenced by photoperiod and temperature. Melatonin, a key hormone in the photoperiodic control of reproduction in mammals and birds (Sirotkin & Schaeffer 1997), has been shown to occur in the cerebral ganglion of the nemertean, to vary during spawning and to affect gonad maturation in decerebrate animals (Arnoult & Vernet 1996). It was proposed, therefore, that light was detected by photoreceptors, which regulated melatonin production, which then regulated the synthesis of the gonad-inhibiting hormone in the cerebral ganglia (Arnoult & Vernet 1996).

Lafont (2000) has suggested that the structure of hormones is more highly conserved than their function. The fact that the ganglion of Nereidae is responsive to these hormones, and the resemblance to the process in the nemertean, might be taken as evidence for a common ancestry in the occurrence and role of these hormones.

5. THE ROLE OF PHOTOPERIOD AND TEMPERATURE ON REPRODUCTION IN NEREIDAE

The implication that dopamine and melatonin may be involved in switching off the juvenile hormone is interesting because of their known activity as chemical analogues of day and night, regulating photoperiodic responses in vertebrates (Doyle *et al.* 2002). In *P. dumerilii*, photoperiod has been shown to control the release of the hormone, and artificial moonlight can entrain a lunar cycle of spawning at the population level (Hauenschild 1960).

In addition, the possible role of opsins (light-sensitive membrane-bound receptors) in the process is being characterized. *Platynereis dumerilli* contain two types of photoreceptor cells (ciliary and rhabdomeric) that expresses two types of opsins: a rhabdopsin (r-opsin), which is homologous to vertebrate melanopsin, and a cilliary opsin (c-opsin) (Arendt *et al.* 2004). The ciliary photoreceptors occur within in the brain and may function in a photoperiodic photo-response. They appear, for example, to express a *Pdu-bmal* gene (an orthologue of *Drosophila* and vertebrate *bmal* genes encoding a key component of the circadian clock) with a circadian rhythmicity (Tessmar-Raible *et al.* 2007).

These observations highlight the fact that while hormones have been found to play an important role in the control of reproduction of the polychaetes, other environmental processes and cues are also important. Of these, studies by many authors have shown that temperature and photoperiod play an important role in the timing of specific stages of gametogenesis. These may act either directly or indirectly to reset or maintain internal clock mechanisms, and in some species to induce the actual spawning of the gametes (for review, see Bentley & Pacey 1992).

The role of environmental signals is particularly important in semelparous species for which the timing of reproduction must be tightly controlled, not only within the individual but also across the whole population. Spawning in these species is a terminal event. Consequently, the fitness of an individual is dependent upon releasing its gametes in synchrony with all members of a population who are to spawn in any given season (Bentley & Pacey 1992; Rees 1997; Lawrence & Soame 2004).

Both photoperiod and temperature show an annual cycle in northern temperate latitudes with a phased relationship between them. There is a slight delay between the two cycles, with the photoperiodic cycle followed by the temperature cycle (Olive 1995; Prandle & Lane 1995). However, of the two, photoperiod is the more predictable at any given latitude. Unlike temperature, photoperiod is not subject to variation. Temperature can vary significantly over short and long-term time scales. Consequently, of the two, photoperiod is a much better predictor of time of year (Lawrence & Soame 2004).

In temperate zones, seasonal changes in photoperiod and temperature influence biological cycles such as phytoplankton production. It is argued, therefore, that environmental cues are fundamentally important in ensuring that larvae are released or develop during periods of abundant food supply (Lawrence 1996; Rees 1997). Consequently, animals should respond to environmental cues to synchronize reproduction during the breeding season. Photoperiod offers the most reliable cue, and synchronization to this ensures that aspects of an individual's physiology are specifically linked to time of year.

(a) *Oogenesis and vitellogenesis*

As noted earlier, the reproductive process in the Nereidae begins with an irreversible transition from somatic growth to reproductive development several months prior to breeding (Olive 1995). *Nereis virens* reduce their feeding rates at the end of September, correlated with the change in photoperiod between mid-summer and mid-winter. Around this time, when photoperiod reaches LD 12 : 12, the animals appeared to switch from active body growth to the active production of oocytes (Last 1999).

The importance of photoperiod to the initiation of reproduction was further supported by Djuaendi (1995) who showed that animals exposed to the 'switch' to short daylengths in June rather than September became mature earlier. This has led to the conclusion that a photoperiod of LD 12 : 12 is crucial for the transition from somatic to reproductive development in *N. virens*. Consequently, Olive (1995) has argued that reproductive timing is independent of both feeding and temperature and that a photoperiod-controlled endogenous gated rhythm regulates the onset of sexual maturity.

However, other environmental cues, notably temperature, also play a role during oogenesis. For example, in populations of *N. virens* from N.E. England, low temperatures (7–12°C) encourage oocyte growth, whereas high temperatures inhibit it (Rees 1997). Similarly, short daylength (LD 8 : 16) promotes oocyte growth and long days (LD 16 : 8) inhibit it. This would indicate that there is a synergistic relationship between photoperiod and temperature with regard to oocyte growth during the winter months.

While it has been shown that female *N. virens* can be induced to produce new oocytes at any time of the year through either photoperiod or temperature changes, vitellogenesis can only be induced by switching the animals to short-day photoperiod (Rees & Olive 1999). Consequently, Rees (1997) and Rees & Olive (1999) conclude that temperature and photoperiod both influenced oocyte development in *N. virens*.

(b) *Final maturation*

Photoperiod also plays an important role in the final stages of maturation in *N. virens*. However, the mechanism appears to reverse the need for short daylengths required for vitellogenesis. It is thought that the concentration of juvenile hormone declines during vitellogenesis, which in *N. virens* occurs during the winter months. However, the final maturation of the oocytes is still inhibited, indicating that the juvenile hormone levels need to decline further. Rees (1997) and Rees & Olive (1999) found that, at this stage of development, long daylengths caused the animals to reach maturation and fertilizability quicker than short days and, in the natural population, this process happens in spring as the daylengths are increasing. This mechanism is similar to that reported in *Harmothoe imbricata*, an iteroparous species for which photoperiod controls reproductive development during the winter (Clark 1988).

Therefore, longer daylengths in the spring appear to promote the final reduction in juvenile hormone levels. Rees (1997) suggested two possible explanations: either a switch in the response of the animal to photoperiod or two hormones released from the ganglia involved in the regulation of oocyte development. The first suggestion, though possible, seems unlikely while the second would correlate with evidence from other polychaetes.

(c) *Spawning*

It is suggested that environmental factors may act to synchronize gamete release between individuals and that hormones and pheromones may be involved in the transduction of this information (Olive *et al.* 1990; Bentley & Pacey 1992). Environmental cues appear to prepare the animals to spawn over a restricted time period, but pheromones may be released from individuals to trigger the actual process of spawning (Hardege *et al.* 1998).

In the self-fertilizing hermaphrodite *Neanthes limnicola*, photoperiod alone appears to be fundamental in coordinating the time of spawning. In the natural environment, *N. limnicola* spawns in the spring in response to seasonally changing photoperiod (Fong & Pearse 1992). For this species, a seasonal cycle of decreasing and increasing light regimes was needed for maximum fecundity. Furthermore, to maintain reproductive synchrony, they needed to experience increasing daylength as is experienced in the winter through to spring (Fong & Pearse 1992).

In many marine invertebrates including polychaetes, however, pheromones have been identified that can stimulate gamete release and courtship. This is clearly demonstrated, for example, by the nuptial dance displayed by many nereid species (Zeeck *et al.* 1990; Hardege *et al.* 1998; Ram *et al.* 1999).

Bartels-Hardege & Zeeck (1990) found that spawning in *N. diversicolor* occurs in the spring. A temperature above 6°C was required to induce gamete maturation and the individuals spawned approximately four weeks later as the environmental temperature reached 12°C. Not only was the absolute temperature (more than 6°C) important, but so also was the timing of the rise in temperature (early spring) (Bartels-Hardege & Zeeck 1990). Animals maintained at a constant high temperature spawned asynchronously, suggesting that the rise in temperature from the winter to the spring was important in supporting the synchronization of gamete maturation and spawning (Bartels-Hardege & Zeeck 1990).

Temperature is important therefore in synchronizing the maturation of the oocytes in *N. diversicolor*, but a further mechanism imposes synchronization on the spawning of gametes. *Nereis diversicolor* females release their oocytes inside their burrows in synchrony with the semilunar cycle. Oocytes are spawned either at the new or full moon. This suggests that rising temperatures only induce maturation and initiate reproduction. The actual synchronization of the population is a consequence of the lunar cycle (Bartels-Hardege & Zeeck 1990).

Many reports concerning the spawning of Nereids describe both a temperature and lunar control, and it has been found that in many species a minimum temperature needs to be reached for swarming to be induced (Bentley & Pacey 1992). Therefore, spawning in some Nereidae may be temperature dependent. However, it is argued by Olive (1995) that this cannot be regarded as the primary cause of reproductive synchrony but may be adaptive in response to short-term changes in temperature.

6. A NEW MULTI-HORMONE MODEL FOR THE CONTROL OF REPRODUCTION IN NEREIDAE

Based on the environmental and endocrine control of reproduction in Nereidae, presented here, a new multi-hormonal model is presented in figure 4, which links environmental cues to gamete development and spawning. In the model, the critical photophase is experienced around the time of the autumn equinox. At this point, animals that are going to reproduce experience a fall in juvenile hormone titre, possibly in response to increased levels of dopamine or melatonin. This allows the gametes to progress through vitellogenesis. The process of vitellogenesis appears to be further enhanced by the oestradiol-17β-induced production and secretion of vitellogenin by eleocytes (Garcia-Alonso *et al*. 2006). The production of oestradiol-17β or uptake of vitellogenin is also enhanced by a second neurohormone present in the ganglion of mature females. This hormone appears to have a gonadotrophic function similar to that described in a number of iteroparous species (Lawrence & Olive 1995).

This multi-hormone model appears to resolve the problems highlighted with the single-hormone paradigm, and we have presented evidence to support it here. The evidence for the second, gonadotrophic

hormone, is unequivocal. However, while dopamine, melatonin, serotonin and oxytocin reduce juvenile hormone activity, and may even promote oocyte growth, their presence and precise role within the ganglia of Nereidae require further confirmation. Furthermore, while oestradiol-17β has been shown to enhance the production of vitellogenin, and its synthesis is considered to occur in gut epithelium (Garcia-Alonso & Rebscher 2005), this is also yet to be confirmed within the Nereidae. The endogenous origin of vertebrate-like steroids in invertebrates has been questioned in many cases (Swevers *et al*. 1991) and may raise doubts about their endocrine roles (Lafont 2000). However, oestrogen receptors have now been reported in *P. dumerilii*, providing evidence of an oestrogen signalling mechanism in the Polychaeta (Keay & Thornton 2009).

7. IMPACTS OF CLIMATE CHANGE ON REPRODUCTION IN NEREIDAE

Global warming is likely to affect all biological processes in Nereids, including growth and timing of reproduction. Furthermore, those species that use day-length as the proximate cue to predict time of year may be particularly vulnerable (Olive *et al*. 1990; Norse 1993; Lawrence 1996). Based on current predictions, climate change will cause a significant shift in the phase relationship between temperature and photoperiod, which in turn may impact on aspects of reproduction. This might include significant changes in the speed or timing of gametogenesis and spawning, fecundity and, ultimately, larval survival.

Climate change impacts on Nereidae are difficult to predict. As already highlighted, the initiation of vitellogenesis is fixed to time of year by photoperiod. In addition, a key element of gametogenesis is the requirement for low photoperiod and low temperature regimes over the winter. The shorter the day and lower the temperature, the quicker the gametes develop. Furthermore, final maturation and spawning are coordinated by the increase in both photoperiod and temperature in the spring.

The IPCC predicts that temperatures are likely to be 50–100% above the global mean in mid- to high latitudes during the winter, precisely when Nereidae require both low photoperiod and low temperature. Furthermore, it is feasible that temperatures may remain above the absolute minimum currently recognized as important for the induction of spawning (Goerke 1984) or lead to asynchronous spawning within populations.

For individual populations of species such as *N. virens*, which use photoperiod as the proximate cue, it has been suggested that the change of location poleward is unlikely because their reproductive cycle is fixed to an annual cycle of light that is set by latitude. Evidence to support this has been presented by Fong and Pearse (1992) who showed that exposure to different photoperiodic regimes negatively affected the fecundity of *N. limnicola*.

Individual fitness is dependent upon successfully synchronizing gametogenesis and spawning with that of the population. This is particularly critical for
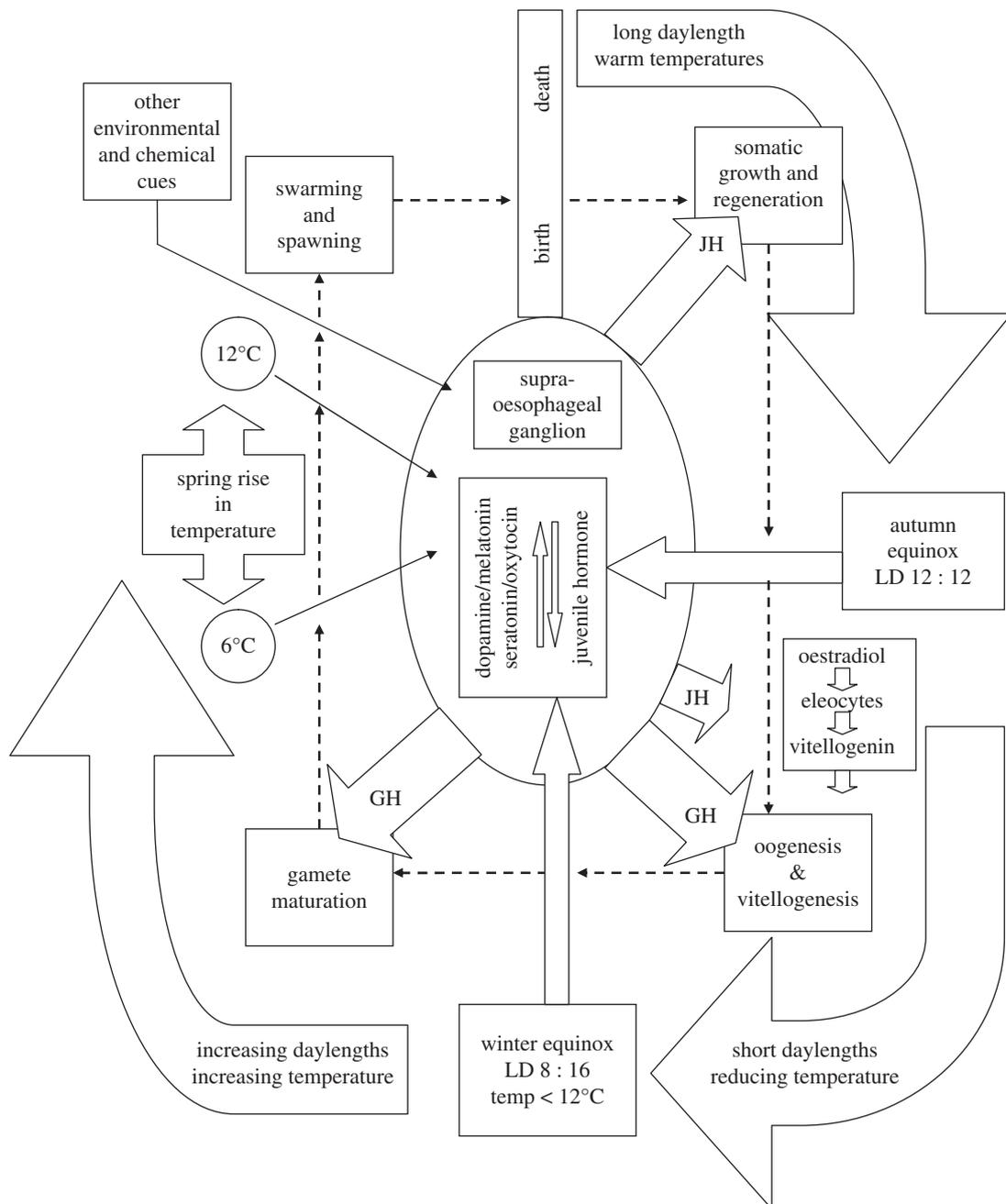
Figure 4. A multi-hormonal model showing the interaction between environmental and endocrine factors controlling the reproductive cycle of *Nereis*. JH, juvenile hormone; GH, gonadotrophic hormone.

semelparous species in which spawning is a one-time terminal event. Success relies on the interaction between temperature and photoperiod and the transduction of these signals via the endocrine system. Consequently, there should be strong selection for individuals within the population that conform to the cycle (Olive *et al.* 1990; Lawrence & Soame 2004). The ability of these animals to survive predicted climate change is therefore likely to depend on the speed of genotypic adaptation in the population compared with the speed of global warming (Olive *et al.* 1990; Lawrence 1996) and/or the degree of mixing between individual populations. *Nereis diversicolor* may be particularly vulnerable because of its lack of a planktonic larval stage and possible limited dispersal or population mixing.

For species with relatively short generation times, however, recent evidence indicates that genetic adaptation of the seasonal photoperiodic cue may be possible. For example, the pitcher-plant mosquito, *Wyeomyia smithii*, appears to have shown a shift in its genetically controlled photoperiodic onset of diapause over as little as 5 years in what appears to be an adaptive evolutionary response to recent global warming (Bradshaw & Holzapfel 2001). In contrast, great tit populations show genetic variation in the ability to adjust egg-laying date. However, genetic change has only been observed in the proportion of the population that is able to modify this timing and, despite this, the average lifetime reproductive success of the population as a whole is still declining. Consequently, the population appears unable to keep pace with environmental change and as such it has been argued that the ability to evolve in response to climate change does not ensure population survival (Nussey *et al.* 2005).

Furthermore, reports of adaptation in other groups do not require the temperature compensation component of the process reported in the control of reproduction in Nereidae. If a new, higher but stable, temperature was reached during winter months, then genotypic adaptation might be possible. However, with continuously shifting temperatures, the phase relationship between photoperiod and temperature is likely to remain uncoupled and continuously shifting. Therefore, global warming may have significant consequences for the Nereidae because time of year, set by photoperiod, will become uncoupled from, and out of phase with, that of temperature. This may result in reduced fecundity as spawning occurs too early in the year or fewer gametes are competent to be spawned.

Larval survival is likely to depend on the degree of match or mismatch between larval production and food availability or production cycle in the water column (Cushing & Dickson 1976). This is supported by Bhaud *et al.* (1995) who showed that short-term perturbations in temperature, often on a time scale of less than a month, may be the cause of variable reproductive success by either affecting breeding or larval success in marine environments. This mismatch between offspring production and food availability has also been reported in some bird species that rely on photoperiod to time reproduction (Visser *et al.* 1998, 2004).

This also highlights the point that global warming as a phenomenon is a relatively slow process. However, the occurrence of extreme events, including unseasonally warm winters in northern temperate latitudes, might cause a more significant problem in the short term. There is some evidence to support this. For example, observed ecological impacts to the North Atlantic Oscillation include changes in timing of reproduction, population dynamics, abundance and spatial distribution (Ottersen *et al.* 2001). Furthermore, losses of Shearwater in the Baltic, resulting from the impact of unseasonable climatic events, support this hypothesis. These studies have highlighted the fact that marine ecosystem structure and function are intimately linked to forcing from the atmosphere (Baduini *et al.* 2001; Napp & Hunt 2001).

8. IMPACTS OF POLLUTION ON REPRODUCTION

The Nereidae are often dominant in estuarine areas that are subject to a wide variety of human uses and impacts. Several studies have shown that Nereidae, notably *N. diversicolor*, may show tolerance to pollution in these systems (Bryan & Gibbs 1983; Mouneyrac *et al.* 2003) and that this tolerance has a genetic component (Grant *et al.* 1989; Burlinson & Lawrence 2007). However, pollution tolerance may be associated with a metabolic cost, and it has been suggested that tolerant individuals should be competitively inferior to sensitive worms in clean sediments (Grant *et al.* 1989; Posthuma & Van Straalen 1993).

This would suggest that any cost of tolerance might be reflected in the animal's energy stores, with possible implications for reproduction and fecundity. This has been reported in the case of *N. diversicolor* collected from relatively clean or polluted sites in France. Energy reserves were higher in worms from the clean site as were the number of oocytes per female, and the density of worms (Durou *et al.* 2007, 2008). These authors therefore suggest that these pollution biomarkers and population responses are related. Similar altered energy budgets have also been reported in *N. virens* (Carr & Neff 1984). Furthermore, Last (1999) reported that in the spring some maturing *N. virens* resumed feeding while others spawned, and concluded that those animals that resumed feeding did not have enough reserves to complete gamete maturation. This would suggest that energy availability is a significant factor affecting the gametogenic processes and support the link between energy resources and reproduction.

Matozzo *et al.* (2008) have suggested that the possible role of oestradiol-17β in vitellogenin production by eleocytes in *N. virens* clearly demonstrates the potential for xenoestrogens to impact on gametogenesis in Polychaeta. This is supported by Mouneyrac *et al.* (2006) who found that *N. diversicolor* from a polluted site showed reduced levels of the steroid hormones (progesterone, testosterone and oestradiol-17β), suggesting that these animals may have been exposed to endocrine-disrupting chemicals. Furthermore, a variety of known environmental-disrupting chemicals have now been shown to disrupt the transcriptional activity of oestradiol receptors in *P. dumerilii* in what appears to be the first example of an invertebrate ER that can be disrupted by xenobiotics (Keay & Thornton 2009). However, this contrasts with the study by Durou & Mouneyrac (2007) who correlated energy reserves and reproductive state to steroid hormone levels in *N. diversicolor* from polluted and control sites: while cycles of steroids were observed in both populations, no differences were observed between the sites.

There is also limited evidence to show the impact of pollution on the process of fertilization and embryogenesis. For example, Caldwell *et al.* (2002) found that aldehydes from diatoms could impact negatively on the fertilization success and embryogenesis of *N. virens* eggs. More recent evidence has also shown that the water-accommodated fraction of crude oil can impact on fertilization and have a teratogenic effect on early embryo stages (Lewis *et al.* 2008). Consequently, it can be argued that if significant pollution events occur during the short 'spawning window' for these species, then impacts on fertilization and embryogenesis could significantly impact on recruitment and population structure of Nereidae.

9. DISCUSSION

Among its many observations and scenarios, the 4th IPCC Synthesis Report makes three key predictions. First, the resilience of many ecosystems is likely to be exceeded this century by a combination of climate change, pollution and other anthropogenic drivers. Second, that up to 30 per cent of the species examined are likely to be at increased risk of extinction as a result of these impacts. Third, there are likely to be major changes in ecosystem structure, function and

ecological interaction with predominantly negative consequences on biodiversity and ecosystem goods and services (IPCC 2007).

This paper presents a scenario for such a situation. It is unlikely that the Nereidae will be among the groups at risk of extinction. However, the evidence presented here suggests that local populations may suffer significant impacts on their reproduction and fecundity through a combination of climatic and pollution events. These are likely to disrupt the endocrine transduction of environmental cues controlling the reproductive cycle of these species, leading to significant fluctuations in population structure. Ultimately, this is likely to lead to the degradation of the functional role of the Nereidae within estuarine environments, with implications for migratory birds and fish, as well as the structure and functioning of the estuarine environment.

Furthermore, while this paper has focused on the Nereidae, there are several other species of polychaete and mollusc that form significant components in estuarine systems. Several of these use interactions between photoperiod and/or temperature to cue reproduction, transduced via endocrine systems (Lawrence & Soame 2004). Again, such coordination by endocrine systems is likely to become compromised as a consequence of climate change and pollution, reinforcing the likelihood that populations across the estuarine ecosystem are likely to fluctuate, leading to significant declines in benthic productivity.

There are still many gaps in our understanding of the endocrine control of reproduction in Nereidae. We present evidence here of a multi-hormonal system, with unambiguous evidence for a second, gonadotrophic, neurohormone promoting oocyte growth. However, the presence and role of dopamine, melatonin, serotonin and oxytocin require further confirmation, as does those of steroid hormones including oestradiol-17β. In addition, evidence for the genetic variation and fitness consequences of climate change in marine invertebrates is still lacking, and no studies have considered the speed of genotypic adaptation in these species.

Lafont (2000) has argued that unless substantial effort is made to better understand invertebrate endocrinology, it will be difficult to determine the mechanisms involved in xenobiotic toxicity. The *in vitro* bioassay described here, as well as those reported previously, offers suitable tools with which these inter-relationships can be unraveled at least in the Nereidae and Polychaeta. Furthermore, Lawrence (1996) has previously argued that these assays could provide a suitable bioindicator with which to monitor future climate change or pollution impacts. This further supports the contention by Wingfield (2008) that the future of comparative endocrinology lies in coordinating investigations of endocrine disrupters while resolving the basic mechanisms underlying an animal's interaction with the environment.

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