Environmental influences on endocrine systems controlling reproduction in Polychaetes

*Matthew G. BENTLEY, Jeni BOYLE * & Allan A. PACEY***

Gatty Marine Laboratory, School of Biological and Medical Sciences
University of St Andrews, St Andrews KY16 8LB, Scotland, U.K.
*present address: Clyde River Purification Board, East Kilbride
Glasgow G75 0LA, Scotland, U.K.
**present address: Observatoire Océanologique, URA CNRS 671
B.P.28, 06230 Villefranche-sur-Mer, France

ABSTRACT

Photoperiod and/or temperature are known to influence gametogenesis and spawning in a number of polychaete species. Information is now available on the nature of endocrine systems controlling gametogenesis and spawning in representatives of several polychaete families. For relatively few species, however, is information available on the possible transduction of environmental signals by the endocrine system (BENTLEY & PACEY, 1992). We report here on the effects of photoperiod and temperature on the action of gonadotropin and spawning hormones in the polychaete *Nephtys hombergii*, and on the action of a gonadotropin hormone in the polynoid *Harmothoe imbricata*.

RÉSUMÉ

Influences des facteurs externes sur les systèmes endocrines contrôlant la reproduction chez les Polychètes

La photopériode et/ou la température agissent sur les processus de gamétogenèse et de ponte chez un grand nombre d'espèces de polychètes. Si les différents types de systèmes endocrines qui contrôlent la gamétogénèse et la ponte sont bien documentés chez de nombreuses espèces de polychètes, on a, par contre, très peu d'informations sur les interactions existant entre les facteurs du milieu et ces systèmes endocrines (BENTLEY & PACEY, 1992). Nous rapportons ici les effets de la photopériode et la température sur l'action de l'hormone gonadotropique et de l'hormone de ponte chez *Nephtys hombergii* ainsi que sur l'action de l'hormone gonadotropique, qui a récemment été décrite, chez *Harmothoe imbricata*.

INTRODUCTION

Nephtys hombergii Savigny, 1818 (Nephtyidae) and *Harmothoe imbricata* L., 1769 (Polynoidae) are both iteroparous polychaetes with an annual cycle of reproduction. *Nephtys hombergii* produces a single batch of gametes which are spawned in late April or May in populations around the coast of northern England or Scotland (OLIVE, 1978; BENTLEY *et al.*, 1984). *Harmothoe imbricata*, in contrast, produces two consecutive batches of gametes which are produced and spawned in quick succession in the spring (DALY, 1972, 1974; GARWOOD,

BENTLEY, M.G., BOYLE, J. & A.A. PACEY, 1994. — Environmental influences on endocrine systems controlling reproduction in Polychaetes. *In:* J.-C. DAUVIN, L. LAUBIER & D.J. REISH (Eds), Actes de la 4ème Conférence internationale des Polychètes. *Mém. Mus. natn. Hist. nat.*, **162** : 37-44. Paris ISBN 2-85653-214-4.

1981). Gametogenesis and spawning in *N. hombergii* are controlled by two hormones, a gonadotropin hormone and a spawning hormone (OLIVE, 1976; OLIVE & BENTLEY, 1980; BENTLEY & OLIVE, 1982; OLIVE & LAWRENCE, 1990) but to date, evidence for the influence of any environmental factors on reproduction has been speculative. A possible correlation was reported, for example, between insolation and daytime spawning of *Nephtys caeca* (BENTLEY *et al.*, 1984).

Studies on *Harmothoe imbricata* have revealed a sophisticated photoperiodic control of reproduction (GARWOOD & OLIVE, 1978, 1982; CLARK, 1988) but, only recently has a gonadotropin hormone been shown to operate in the control of oogenesis (OLIVE *et al.*, 1990). The first cohort of oocytes requires appropriate environmental input and endocrine support. The second oocyte cohort has been shown to develop autonomously, apparently without environmental control.

The results we describe in this paper show that in *Nephtys hombergii* environmental conditions under which animals are maintained may affect resorption and the onset of renewed gametogenesis. Resorption has been shown to be a common occurrence following spawning failure in this species (OLIVE *et al.*, 1981). We also show that there is endocrine support of development of the second cohort of oocytes in *Harmothoe imbricata* even though these do not require continued environmental support. In light of the fact that rapid growth of the first cohort of oocytes occurs under gonadotropin support apparently associated with increasing photoperiod in the spring (CLARK, 1988) then observations that continued gonadotropin secretion occurs, without further environmental stimulus, during the rapid vitellogenesis of the second oocyte cohort are as would be predicted.

MATERIALS AND METHODS

Collection and maintenance of specimens. — Specimens of *Nephtys hombergii* were collected by digging in sand at low water of spring tides from three sites: 'The Black Middens' in the estuary of the River Tyne, North-East England; Kirkcolm, Wigtownshire, Scotland and from Fairlie Sands, Ayrshire, Scotland. Individuals were maintained in the laboratory in groups of four in polyethylene containers containing 200ml sea water and sterilised sand to a depth of 1cm.

Specimens of *Harmothoe imbricata* were collected from beneath stones from the intertidal rocky shore at St. Andrews and Kingsbarns, Fife, Scotland. They were maintained individually in small glass jars containing 50ml of seawater. An empty *Patella vulgata* shell was included to provide shelter. Animals which were not used within one week of collection were fed weekly with pieces of *Nereis diversicolor* presented in forceps.

Light temperature regimes for laboratory maintenance of animals. — Animals were maintained in the laboratory in Gallenkamp cooled incubators with timed light cycling under a variety of environmental conditions as appropriate. The particular combinations of photoperiod and temperature were as follows:

Ambient conditions
Summer conditions of daylength and temperature 16L: 8D, 15°C
Winter conditions of daylength and temperature 8L: 16D, 5°C
Winter daylength / summer temperature 8L: 16D, 15°C
Continuous illumination, 7°C.

Sampling of coelomic / ovarian oocytes. — *Nephtys hombergii* were anaesthetized in 0.07% 3-aminobenzoic acid ethyl ester (Sigma Chemical Co.) in sea water. Samples of coelomic / ovarian oocytes were obtained by puncturing the body wall in the inter-parapodial region with a pulled micro-pipette which had been moistened with filtered sea water. Examination was carried out on a Leitz Diaplan compound microscope using a x40 objective lens.

Extraction of brain homogenates and their preparation for use in tissue culture. — Supra-oesophageal ganglia ("brains") were removed from *Nephtys hombergii* anaesthetized as described above by making a transverse incision in the dorsal body wall just posterior to the prostomium. Excised ganglia were kept on ice until homogenization. Homogenization was carried out using an MSE Soniprep 150 ultrasonic disintegrator fitted with a 9mm titanium steel probe. Crude extracts to be used for immediate bioassay or to be lyophilised for storage were homogenised in bidistilled water, the aqueous supernatant was then used for injection or lyophilised and stored at -20°C until used. Heat treated homogenate was prepared by suspending tubes containing crude homogenate in a water bath at 100°C for 1 min followed by centrifugation in an MSE micro-centaur; the supernatant was then used for subsequent addition to tissue culture wells.

Bioassay procedures for SH in *Nephtys*. — The bioassay of extracts of supra-oesophageal ganglia for Spawning Hormone (SH) activity was carried out as follows: gravid specimens of *Nephtys hombergii* were

anaesthetized as above and injected with extract to be assayed or with filtered sea water as a control. Injections of 10µl volume were made with a pulled, calibrated glass micropipette. The animals were then placed in an incubator at 12°C and allowed to recover from the anaesthetic. The spawning response was measured using standard counts of spawned gametes as described previously (OLIVE & BENTLEY, 1980).

Aseptic techniques. — *Culture method.* Animals were placed in streptomycin (2.5 mg/ml) and penicillin (5000IU/ml) for a period of 24 h before dissection of ovaries for tissue culture. Animals were anaesthetized as described above and ovaries were removed by excision of parapodia and lifting out of the ovaries with sterile watchmaker's forceps. The ovaries from several females were pooled for use in amino-acid incorporation experiments, where they were explanted into culture wells of Cel-Cult 24-well plastic tissue culture plates (Sterilin) each containing 2 ml of culture medium developed by BENTLEY & OLIVE (1982) for polychaete organ culture.

Radio-isotope procedures. — Ten ovaries were added to each tissue culture well. L-[4,5-H^3] leucine (Amersham International; specific activity 130-190 $Ci.mM^{-1}$) (≈10,000 counts. $well^{-1}$)). The plates were placed in sealed containers with moist tissue and kept in Gallenkamp cooled incubators at 15°C.

Harvesting of Cultures. — After culture periods of 96 h the ovaries and medium were removed from the wells and centrifuged briefly in an MSE micro-centaur to sediment the ovaries. The medium was discarded, the ovaries were resuspended in 1ml of 0.125 M Tris-HCl buffer at pH 8.5 and then homogenised in a Potter glass homogeniser. An aliquot of 0.5 ml (50%) of the homogenate was stored for subsequent protein analysis using the Bradford micro-protein determination method (BRADFORD, 1976). Three ml of ice-cold TCA was added to the remaining 0.5 ml of homogenate and this was left overnight at 4°C. The samples were centrifuged (at 2800g) in a MSE Mistral 3000 centrifuge for 30 min at 4°C. The supernatant was removed and 1ml of 0.15M NaOH was added to redissolve the proteins. The sample was divided into two, 3 ml Packard emulsifier scintillant 299 was added and the sample was then counted for 10 min on a Packard 2000 Tri-Carb liquid scintillation analyzer.

RESULTS

Observations on *Nephtys* gametogenesis. — Means and ranges of oocyte sizes in individuals which had been maintained under conditions of: a) winter photoperiod (8L: 16D) and temperature (5°C); c) summer photoperiod (16L:8D) and temperature (15°C); d) continuous illumination at 7°C; or b) recently collected from the field in September 1990 are represented in Fig. 1. It can be seen that group a) which had been maintained under winter conditions six of the 19 individuals had no vitellogenic eggs (diameter > 50 µm) and the mean oocyte diameter was <80 µm in eight of them. In contrast large vitellogenic eggs were found in virtually all worms in the other three groups. These observations were made on individuals taken from a field population which did not spawn in 1990 and which at the commencement of the experiment had unspawned vitellogenic oocytes in the coelom. It is quite clear that resorption of unspawned oocytes is accelerated in individuals maintained under conditions of winter temperature and photoperiod. Maintenance under low temperature alone (d) does not have the same effect.

Observations on *Nephtys* spawning hormone activity. — The results of assay of spawning-inducing activity of *Nephtys hombergii* supra-oesophageal ganglia taken from individuals which had been maintained under a range of photoperiod/ temperature regimes for a period of 219 days (from prior to the winter solstice) are shown in Fig. 2. Individuals injected with supra-oesophageal ganglion homogenate prepared from freshly collected animals show a response typical of *Nephtys hombergii*, both in terms of the numbers of gametes released and the percentage of individuals responding. The response is seen to be highly variable between individuals in that some individuals spawn out almost completely whereas others spawn only partially. Groups injected with supra-oesophageal ganglion homogenate prepared from individuals maintained under ambient conditions or under conditions of winter photoperiod and temperature showed a response similar to that in individuals described above. Groups injected with supra-oesophageal ganglion homogenate prepared from individuals maintained under summer conditions, or under conditions of winter photoperiod and summer temperature showed a very low level of response or no spawning at all.

Observations on gonadotropin hormone activity in *Harmothoe imbricata.* — Fig. 3 shows the results of amino acid incorporation into ovaries of *Harmothoe imbricata* following organ culture for 96 h in the absence or presence of brain homogenate. Incubation of ovaries of *Harmothoe imbricata* during the development of the first cohort of oocytes (8 March 1991) in the presence of brain homogenate and heat treated brain homogenate, and in medium (all containing H^3 leucine) shows enhanced uptake of the amino acid (ANOVA $F_{2,17} = 13.04$, $p=0.001$).

There is no difference observed on the degree of incorporation between ovaries cultured with fresh or heat-treated brain homogenate.

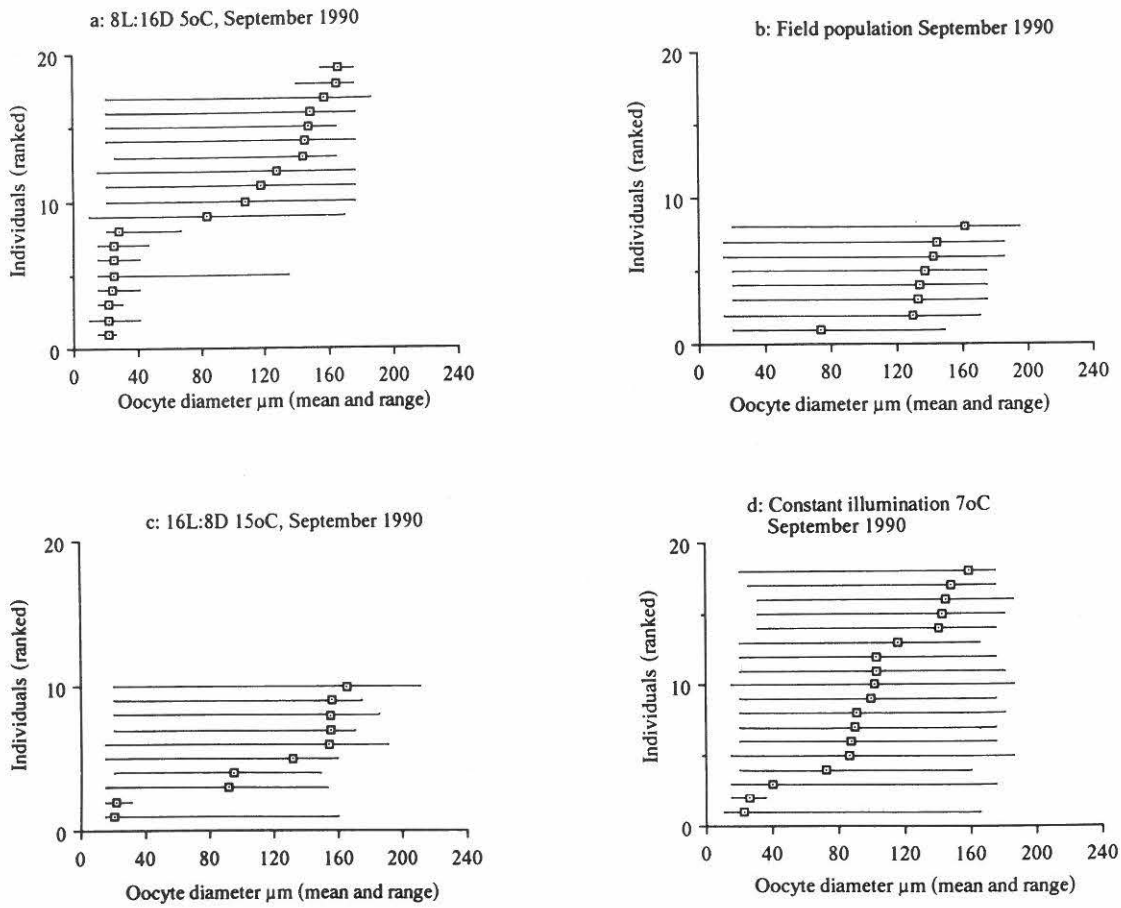

FIG. 1. — Oocyte diameters (means and ranges) in individuals of *Nephtys hombergii* which have been maintaind for a period of six months in the laboratory, and from the field in September 1990. The treatment groups were: a) maintained under a 8L:16D photoperiodic regime at 5°C, b) field collected animals, c) maintained under a 16L: 8D photoperiodic regime at 15°C, d) maintained under constant illumination at 7°C. It should be noted that, following spawning failure in the spring, individuals collected from the field in September still posess vitellogenic oocytes in the coelom.

Incubation of ovaries during the development of the second cohort of oocytes (29 April 1991) in the presence of brain homogenate prepared either from supra-oesophageal ganglia excised during development of the first or second oocyte cohorts also show enhanced uptake of the amino acid compared to the controls (ANOVA $F_{2,17}$ = 4.07, p=0.039) but there is no significant difference between the two brain homogenate treatments. This demonstrates quite clearly the effect of gonadotropin hormone on ovaries during the development of both the first and second oocyte cohorts.

DISCUSSION

The reproductive cycle of *Nephtys hombergii* is known to be controlled by two reproductive hormones (a gonadotropin and a spawning hormone) which are supposed to be secreted in a cyclical manner during the annual reproductive cycle (OLIVE, 1976, OLIVE & BENTLEY, 1980; OLIVE *et al.*, 1985). It has also been reported that

Nephtys hombergii is seen to undergo periodic spawning failure which may have an adaptive significance (OLIVE *et al.*, 1981). These spawning failures may occur in one of two ways: the production of a cohort of normal oocytes which are not released as a result of failure to secrete spawning hormone, or the premature resorption of gametes prior to the breeding season. It has been proposed that spawning failure and oocyte resorption may occur in response to 'poor condition' of the individual (measured by energy levels in the soma) (OLIVE *et al.*, 1985). The endocrine system is the means by which environmental (and somatic energy level) information is likely to be transduced. The results reported here on *Nephtys hombergii* suggest that photoperiod may influence the rate of oocyte resorption in unspawned oocytes and the onset of renewed gametogenesis. Short photoperiod appears to accelerate this process. Temperature does not appear to exert a marked effect. Photoperiod provides an accurate means of determining the timing of reproduction (BENTLEY & PACEY, 1992) whereas temperature cycles are less predictable and are variable from year to year.

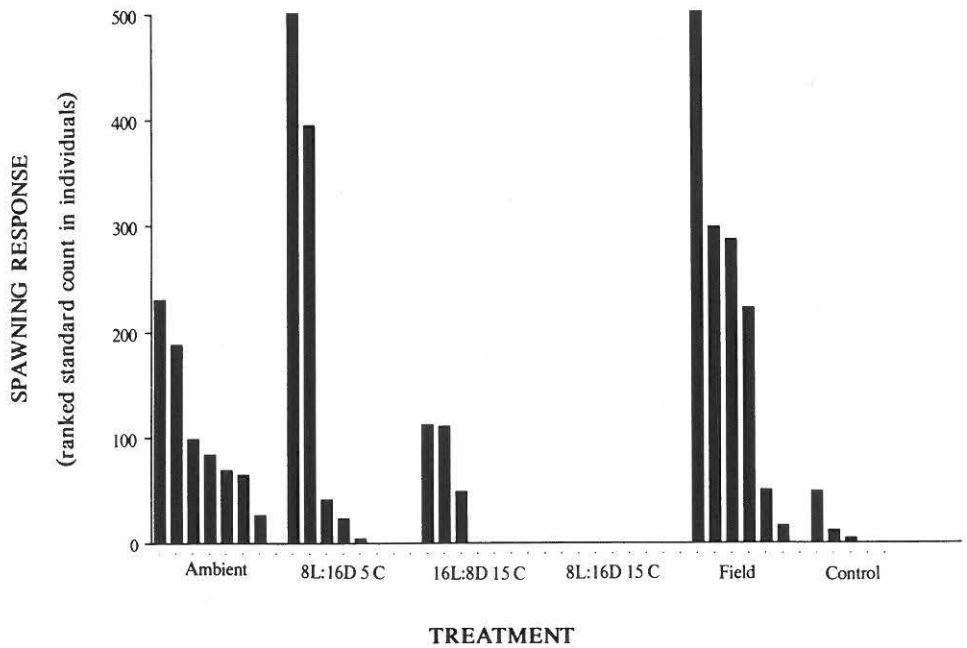

FIG. 2. — Spawning response in *Nephtys hombergii* injected with supra-oesophageal ganglion homogenate which had been obtained from individuals which had been maintained in the laboratory for a period of 219 days (from prior to the winter solstice) under: a) ambient photoperiod and temperature, b) a 8L:16D photoperiodic regime at 5°C, c) a 16L: 8D photoperiodic regime at 15°C, d) a 8L:16D photoperiodic regime at 15°C, or which had been obtained from individuals recently collected from the field. Control injected animals recieved a dose of 10µl of filtered seawater (solvent vehicle). Standard counts were those as used by OLIVE and BENTLEY (1980).

Observations on the production of spawning hormone in *Nephtys hombergii* show a rather different pattern. Prolonged exposure to short photoperiod alone is not sufficient to induce production of the hormone in the supraoesophageal ganglion. Low temperature, in contrast appears to induce spawning hormone production. Clearly production of spawning hormone is not critical in the timing of reproduction (only the timing of release plays a role in this respect), and temperature, therefore, may be an appropriate way of ensuring SH production. Observations on the field spawning of *Nephtys caeca* (BENTLEY *et al.*, 1984), and *Nephtys hombergii* may be similar, suggest that temperature may act as a 'trigger' for spawning. Temperature has been shown to play a role in controlling spawning of Nereidae (GOERKE, 1984) in which there are temperature "windows" for the swarming of species which are related to their geographical range. It is clear that the control of gametogenesis, hormone production and readiness to spawn in *N. hombergii* is a complex process in which temperature and photoperiod both appear to be involved.

There are few polychaete species in which both environmental and endocrine control of reproduction have been demonstrated. FRANKE (1983) has demonstrated the transduction of environmental information by the endocrine system of *Typosyllis prolifera* in an elegant series of experiments, but there are at present no other well

documented cases. The reproductive cycle of *Harmothoe imbricata* is controlled in a sophisticated manner by environmental factors, of which photoperiod is the most important (GARWOOD & OLIVE, 1978, 1982; GARWOOD ,

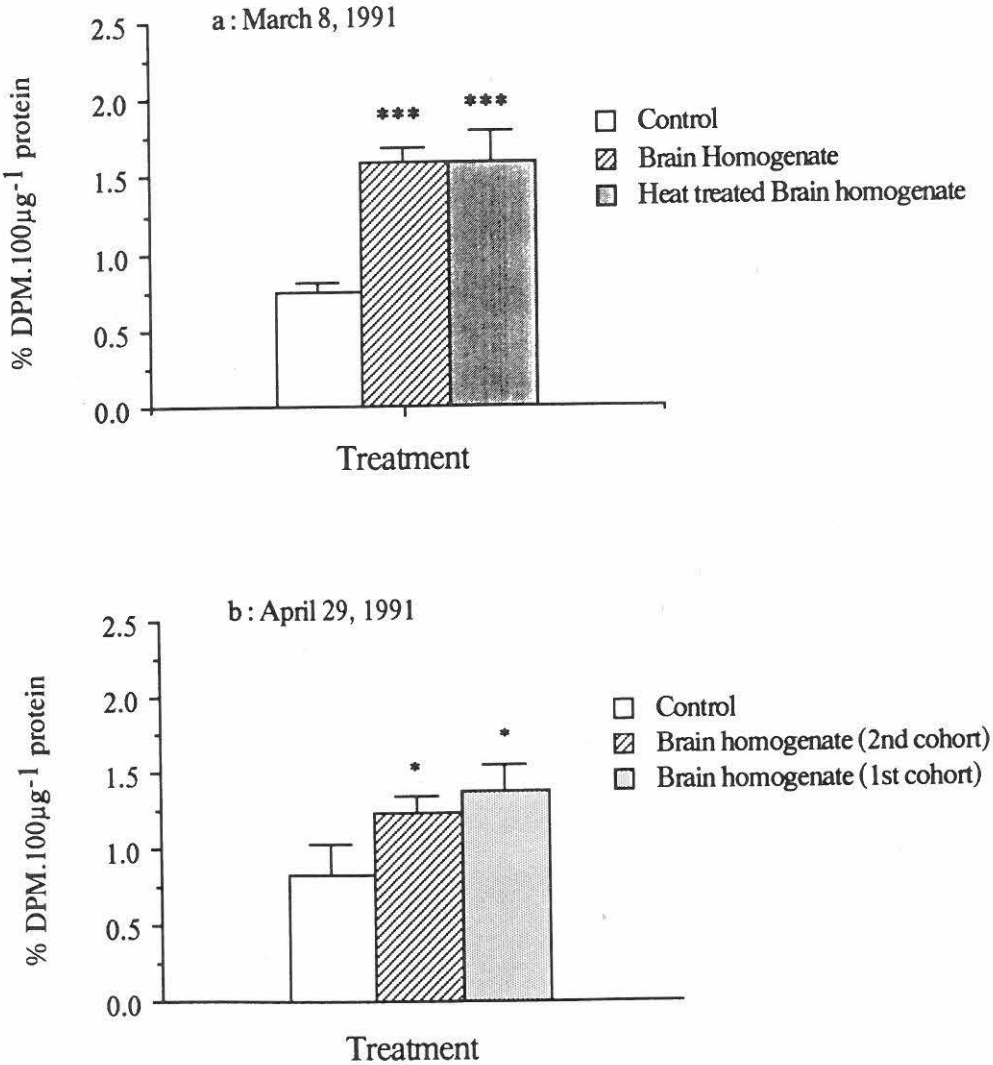

FIG. 3. — Incubation of ovaries of *Harmothoe imbricata* during: a) the development of the first cohort of oocytes (8 March 1991) in the presence of brain homogenate and heat treated brain homogenate, and in medium (all containing L-[4,5-H^3] leucine) and b) the development of the second cohort of oocytes (29 April 1991) in the presence of brain homogenate, prepared either from supra-oesophageal ganglia excised during development of the first or second oocyte cohorts, and in medium only.

1980; CLARK, 1988). Photoperiod acts to promote gametogenesis and to prevent abortion of the developing first cohort of oocytes (CLARK, 1988), and this information is relayed to the developing oocytes, at least in part, by a gonadotropin hormone (OLIVE *et al.*, 1990). The second cohort of oocytes, which develops independently of continued environmental input, we have demonstrated to be still under the gonadotropin hormone support. It may

be that once gonadotropin hormone secretion is switched on and the critical exposure to short days has been passed, secretion of the gonadotropin hormone cotinues throughout the development of the two oocyte cohorts.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Marianne Ghyoot for checking the French abstract. This work was supported by a Natural Environment Research Council grant (GR3/6841) to MGB.

REFERENCES

BENTLEY, M.G. & PACEY, A.A., 1992. — Physiological and environmental control of reproduction in polychaetes. *Oceanogr. Mar. Biol. Ann Rev.*, **30** : 443-481.

BENTLEY, M.G. & OLIVE, P.J.W., 1982. — An *in vitro* assay for gonadotrophic hormone in the Polychaete *Nephtys hombergii* Sav. (Nephtyidae). *Gen. Comp. Endocrinol.*, **47** : 467-474.

BENTLEY, M.G., OLIVE, P.J.W., GARWOOD, P.R. & WRIGHT, N.H., 1984. — The spawning and spawning mechanism of *Nephtys caeca* (Fabricius, 1780) and *Nephtys hombergi* Savigny, 1818 (Annelida: Polychaeta). *Sarsia*, **69** : 63-68.

BRADFORD, M.M., 1976. — A rapid and sensitive method for the quantification of microgram quantities of proteins using the principle of protein dye binding. *Anal. Biochem.*, **72** : 248-254.

CLARK, S., 1988. — A two phase photoperiodic response controlling the annual gametogenic cycle in *Harmothoe imbricata* (L.) (Polychaeta: Polynoidae). *Invertebr. Reprod. Dev.*, **14** : 245-266.

DALY, J.M., 1972. — The maturation and breeding biology of *Harmothoe imbricata* (L.) (Polychaeta: Polynoidae). *Mar. Biol.* **12** : 53-66.

DALY, J.M., 1974. — Gametogenesis in *Harmothoe imbricata* (Polychaeta: Polynoidae). *Mar. Biol.*, **25** : 35-40.

FRANKE, H.-D., 1983. — Endocrine mechanisms mediating light-temperature effects on male reproductive activity in *Typosyllis prolifera* (Polychaeta, Syllidae). *Wilhelm Roux' Arch. Dev. Biol.*, **192** : 95-102

GARWOOD, P.R., 1980. — The role of temperature and daylength in the control of the reproductive cycle of *Harmothoe imbricata* (L.) (Polychaeta: Polynoidae). *J. Exp. Mar. Biol. Ecol.*, **47** : 35-53.

GARWOOD, P.R., 1981. — Observations of the developing female germ cell in the polychaete *Harmothoe imbricata* (L.). *Int. J. Invertebr. Reprod.*, **3** : 333-345.

GARWOOD, P.R. & OLIVE, P.J.W., 1978. — Environmental control of reproduction in the polychaetes *Eulalia viridis* and *Harmothoe imbricata*. *In*: D.S. MCLUSKY & A.J. BERRY (eds), *Physiology and Behaviour of Marine Organisms*. Pergamon Press, Oxford, New York : 331-339.

GARWOOD, P.R. & OLIVE, P.J.W., 1982. — The influence of photoperiod on oocyte growth and its role in the control of the reproductive cycle of the polychaete *Harmothoe imbricata* (L.). *Int. J. Invertebr. Reprod.*, **5** : 161-165.

GOERKE, H.,1984. — Temperature dependence of swarming in North Sea Nereidae. *In*: A. FISCHER & H.-D. PFANNENSTIEL (eds), Polychaete Reproduction. *Fortschr. Zool.*, **29** : 39-44.

OLIVE, P.J.W. 1976. — Evidence for a previously undescribed spawning hormone in *Nephtys hombergii* (Annelida: Polychaeta). *Gen comp. Endocrinol.*, **28** : 454-460.

OLIVE, P.J.W., 1978. — Reproduction and annual gametogenic cycle in *Nephtys hombergii* and *N. caeca* (Polychaeta: Nephtyidae). *Mar. Biol.*, **46**: 83-90.

OLIVE, P.J.W. & BENTLEY, M.G., 1980. — Hormonal control of oogenesis, ovulation and spawning in the annual reproductive cycle of the polychaete *Nephtys hombergii* Sav. (Nephtyidae). *Int. J. Invertebr. reprod. Dev.*, **2** : 205-221.

OLIVE, P.J.W., BENTLEY, M.G., WRIGHT, N.H. & MORGAN, P.J., 1985. — Reproductive energetics, endocrinology and population dynamics of *Nephtys caeca* and *N. hombergi*. *Mar. Biol.*, **88**: 235-246.

OLIVE, P.J.W., CLARK, S. & LAWRENCE, A., 1990. — Global Warming and Seasonal Reproduction: Perception and Transduction of Environmental Information. *In*: M. HOSHI & O. YAMASHITA (eds), *Advances in Invertebrate Reproduction*. Volume **5**. Elsevier, Amsterdam, pp. 265-270.

OLIVE, P.J.W., GARWOOD, P.R. & BENTLEY, M.G., 1981. — Oosorption and reproductive failure in Polychaeta in relation to their reproductive strategy. *Bull. Soc. Zool. Fr.*, **106** : 263-268.

OLIVE, P.J.W. & LAWRENCE, A., 1990. — Gonadotrophic hormone in Nephtyidae (Polychaeta: Annelida): Stimulation of ovarian protein synthesis. *Invertebr. Reprod. Dev.*, **18**: 189-196.