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Photoperiodic regulation of parturition in the self-fertilizing viviparous polychaete *Neanthes limnicola* from central California

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Abstract. Seasonally-changing photoperiod controls the timing of parturition in the viviparous, self-fertilizing polychaete *Neanthes limnicola* (Johnson, 1901) from Watsonville Slough, a central California estuary. During 1987 to 1989, worms in the field gave birth mainly in the spring. Those born in late February from field-collected parents and maintained in the laboratory under in-phase photoperiodic conditions reproduced in 12 to 13 mo, under spring light-regimes. When maintained under light conditions 6 mo out of phase, they required only about 6 to 8 mo to reproduce, giving birth in the fall, but under spring light-regimes. Worms born in the laboratory in fall and then maintained in phase reproduced in the ambient spring, at 6 to 8 mo of age; those maintained out of phase took 12 to 13 mo, giving birth the following fall under spring light-regimes. Photoperiod treatments had no consistent effect on the number of young produced, and age and fecundity were only weakly correlated. Highest fecundities were in salinities of 15 to 20‰, with lower fecundities at higher salinities. Worms maintained in full-strength sea water (33‰ S) showed abnormal development and produced very few or no young. Salinity did not affect timing of parturition. Temperature differences of 3 to 7C° between treatments had no effect on timing of parturition or number of young produced, and marginal effects on life span. These results indicate that photoperiod regulates the timing of reproduction in *N. limnicola* in central California, while salinity mainly influences fecundity.

Introduction

The seasonal production of gametes and subsequent spawning by temperate marine invertebrates may be controlled by seasonal changes in a number of environmental conditions including sea temperature, salinity, and photoperiod (Giese and Pearse 1974). Early work summarized by Orton (1920) invoked changing sea temperature as the single most important environmental condition controlling reproduction in temperate marine animals ("Orton's rule"). Most studies of environmental control of reproduction in polychaetes have focussed on sea temperature as the main controlling factor. In *Nereis succinea*, increasing sea temperature correlates with increasing numbers of heteronereids in the field (Kinne 1954). Hardege et al. (1990) were able to induce heteronereid metamorphosis in this species by raising temperatures around the period of new moon. Artificial increase in temperature caused premature release of unviable gametes in *N. virens* (Bass and Brafield 1972). Spawning in *N. diversicolor* is delayed or suppressed below 10 °C (Olive 1984). Goerke (1984) contends that control of heteronereid swarming depends upon exceeding a minimum sea temperature which depends on geographical location and minimum tolerable temperature for the species.

However, Giese (1959) suggested that, as in terrestrial systems, photoperiod may be important in controlling reproductive events in marine animals. Recently, both seasonally changing and fixed daylengths have been shown to regulate gametogenesis in a number of temperate asteroids (Pearse and Eernisse 1982, Pearse et al. 1986a, Xu and Barker 1990), echinoids (Pearse et al. 1986b, Bay-Schmith and Pearse 1987, McClintock and Watts 1990), shrimps (Custer 1986) and lancelets (Fang et al. 1989). In addition. Olive and his colleagues (Garwood and Olive 1982, Olive and Pillai 1983, Olive 1984, Clark 1988) have demonstrated the importance of photoperiod in the control of oocyte maturation in the polychaetes *Kefersteinia cirrata* and *Harmothoe imbricata*. Moreover, Chu and Levin (1989) found significant synergistic effects of temperature and photoperiod on brooding in *Streblospio benedicti*.

Studies on photoperiodic effects on nereid reproduction are few. In *Nereis diversicolor*, daylength does not affect oocyte maturation rate (Garwood and Olive 1981).

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Worms go through a rapid phase of oocyte growth during the winter and early spring in combinations of either short (8 h light:16 h dark) or long (16 h light:8 h dark) daylengths in 5, 10, or 15 °C. In all cases, worms reared in the laboratory reach sexual maturity at the same time as those on shore. In *Neanthes succinea* from San Francisco Bay, neither frequency nor timing of heteronereid metamorphosis was significantly affected by photopheriod (Fong 1991). Worms showed the highest frequency of metamorphosis in the ambient spring-summer (May–June) independent of light regimes. However, several studies have shown lunar control of swarming rhythms in nereids (Hauenschild 1955, 1960, Hardege et al. 1990, Fong in preparation).

Neanthes limnicola (Johnson, 1901) is a viviparous, self-fertilizing hermaphroditic polychaete (Smith 1950), occupying sandy muds of brackish waters and a freshwater lake, along the Pacific coast of North America from British Columbia to central California. Smith studied the reproductive cycle of this semelparous species in the Salinas River estuary of central California. He concluded that the annual reproductive cycle in the population consists of a steady release of young from spring through summer, with a slack-off period from fall through winter, although reproductive worms could be found during almost any month. We now report on the effects of seasonally-changing photoperiod, salinity, and temperature on the timing of parturition and fecundity in *N. limnicola*.

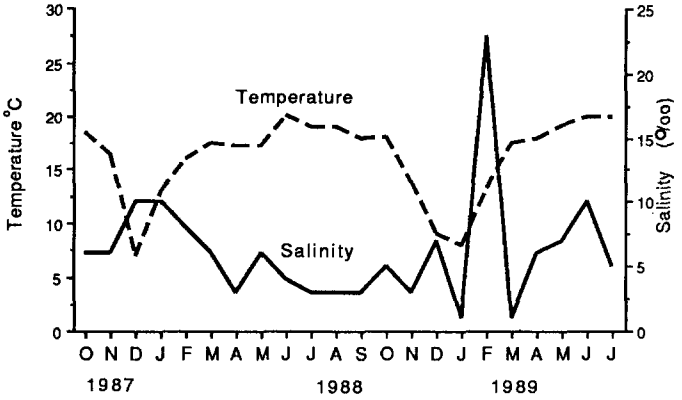

Fig. 1. Salinity and temperature profiles for Watsonville Slough, October 1987–July 1989, measured monthly

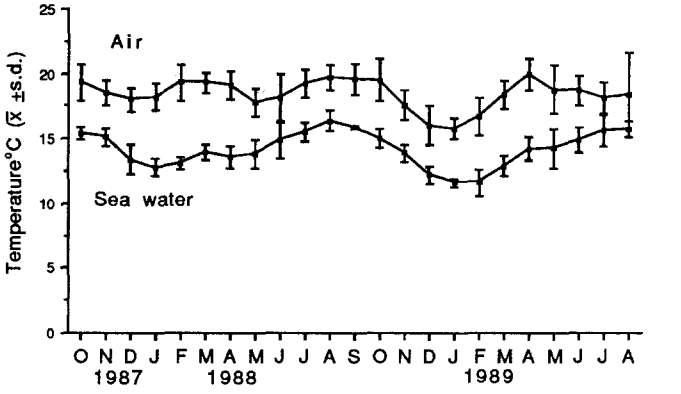

Fig. 2. Monthly temperature record of laboratory sea water and air at Long Marine Laboratory, October 1987–August 1989, measured daily

Materials and methods

Collection and culture of *Neanthes limnicola*

From October 1987 to May 1989, worms were collected monthly from poorly-sorted muds in Watsonville Slough, a tidal creek flowing into Monterey Bay, California (36°45'N; 121°45'W) about 15 km north of the Salinas River estuary. Besides *Neanthes limnicola*, the dominant macroscopic invertebrates in the shallow muds of the slough included the seasonally abundant estuarine amphipods *Anisogammarus confervicolus* and *Corophium spinicorne* and the isopod *Gnorimosphaeroma oregonese*. Worms with developing embryos were noted, and oocytes were collected from all worms by cutting off the posterior one-third of the body and smearing the coelomic fluid onto microscope slides for examination. For calculating an annual reproductive cycle, the first 50 oocytes encountered on each slide were measured. For worms with developing embryos, a diameter of 210 µm (size of a full-grown, fertilizable oocyte) was assigned to each of a total of 50 embryos. We considered this a reasonable estimate of "oocyte" size for such worms because they no longer have developing oocytes and all embryos are initially this same size. Some abnormally large oocytes (230 to 240 µm) were encountered in January 1988 and February-March 1989. These over-ripe oocytes (Smith 1950) were probably unviable, but were measured nonetheless. Slough water-temperature and salinity were measured monthly when the samples were taken (Fig. 1).

Worms were cultured singly, initially in small plastic petri dishes. After three weeks, each worm was transferred to an 80 × 100 mm pyrex culture dish supplied with sand or tygon tubing for it to occupy. Worms were kept isolated throughout their lives, and all offspring resulted from self-fertilization. To control daylength, cultures were maintained in one of two rooms equipped with fluorescent lights (General Electric F40D Daylight) and controlled by switches (Astronomic Time Switch, R. W. Cramer & Co., Type SY Model SOL). In one room, the lights were turned on and off at local sunrise and sunset, respectively (ambient or in phase). In the other room, the lights were set 6 mo out of phase with ambient photoperiod: the longest day of the year (14.8 h) was 22 December and the shortest (9.5 h) was 22 June.

Culture dishes maintained at sea water temperature were placed in sea tables and cooled with flowing sea water to a temperature within 2 C° of ambient ocean temperature. Air-temperature cultures were kept on shelves above the sea tables. Both sea water and air temperature were measured daily (Fig. 2). Desired salinities were attained by diluting sea water (33‰) with deionized water. No antibiotics were used. Worms were fed brine shrimp (*Artemia* sp.) once a week, and culture media were changed 1 to 2 d after feeding. Cultures were monitored daily for signs of reproduction. Cessation of feeding was generally a good indicator of parturient readiness. As birth approaches, adults lose their reddish color, take on a green hue, and become semi-transparent. Developing embryos are easily seen through the body wall at least one week before birth. At birth, young emerge through fissures in the body wall of the dying adult. Parturition date, number of young produced, and days spent in culture were recorded for each birth. Parturition date was assigned a numerical value according to the Julian calendar, with 1st January given the value 1, 1st February given the value 32, etc. From these values, mean parturition dates were generated for each photoperiodic condition (in phase or out of phase). As experiments progressed, it became apparent that most worms maintained at 33‰ S had abnormally green body color and were developing much slower than worms in either 5 or 15‰ S. Therefore, 33‰ S cultures were not included in analyses of reproductive timing, but were included in analyses of the number of young produced.

Table 1. Summary of experimental design. Juvenile *Neanthes limnicola* used in all laboratory experiments were born in laboratory in February 1987 (Experiment A), September 1987 (Experiment B), and from October 1987–January 1988 or February-March 1988 ("repeat experiments"). Juveniles which survived were cultured in the different salinity, temperature and photoperiod conditions shown. In the repeat experiments, juveniles which were born in out-of-phase light conditions were immediately shifted into in-phase conditions; those born in phase were immetiately shifted into out-of-phase conditions. (*n* surviving) are sample sizes for each three-factor experimental cell. Air: air temperature; SW: sea water temperature; IP: in-phase photoperiod; OP: out-of-phase photoperiod

Experiment	Dates and photoperiod juveniles born	*N* juveniles (surviving)	Environmental regimes in which juveniles were maintained			(*n* surviving)
			Salinity ‰	Temperature	Photoperiod	
(A) Spring-born juveniles	Feb. 1987 (in phase)	63	5	Air	IP	(4)
					OP	(4)
				SW	IP	(2)
					OP	(3)
			15	Air	IP	(8)
					OP	(10)
				SW	IP	(6)
					OP	(9)
			33	Air	IP	(5)
					OP	(3)
				SW	IP	(6)
					OP	(3)
(B) Fall-born juveniles	Sep. 1987 (out of phase)	23	20	Air	IP	(4)
					OP	(4)
			25	Air	IP	(4)
					OP	(3)
			30	Air	IP	(4)
					OP	(4)
(C) Repeat experiments	Oct. 1987–Jan. 1988 (out of phase)	20	5	Air	IP	(0)
				SW	IP	(7)
			15	Air	IP	(4)
				SW	IP	(9)
	Feb.–Mar. 1988 (in phase)	20	5	Air	OP	(2)
				SW	OP	(4)
			15	Air	OP	(8)
				SW	OP	(6)

Experiments with laboratory-born juveniles

All experiments (A, B, and C below) were begun with juveniles born in the laboratory from adults either freshly collected from the field or reared in the laboratory; thus, the worms experienced known photoperiodic conditions throughout their lives. Experimental culture conditions are summarized in Table 1.

Expt A: spring-born juveniles

Juveniles born during 20–28 February 1987 (from adults collected in the field) were split into two groups. One group was maintained under 6 mo out-of-phase daylengths. A control group was kept under in-phase daylengths. Cultures were maintained at laboratory sea water and air temperatures, and at salinities of 5, 15, or 33‰.

Expt B: fall-born juveniles

Juveniles born during 5–15 September 1987 were derived from parturitions that occurred in the out-of-phase cultures of Experiment A. They were maintained in both in-phase and 6 mo of-phase (control group) conditions, similar to those born in February, but the culture medium was composed of either 20, 25, or 30‰ S and all were maintained at laboratory air temperatures only.

Expt C: repeat experiments with 2nd generation; in phase shifted to out of phase; out of phase shifted to in phase

A third experiment utilized juveniles produced from both in-phase and out-of-phase conditions of Experiment A. Worms born under in-phase light regimes were shifted into out-of-phase conditions, worms born out of phase were shifted into in-phase light regimes, but no worms were kept under the light regimes of their birth. Culture salinities remained the same as those at birth; temperature conditions for 12 worms which were shifted into in-phase regimes were changed (10 from air to sea water and 2 from sea water to air), but these cultures showed no difference in any parameter from those ($N=28$) in which temperature conditions were not changed, and they were treated as in-phase shifted cultures.

Experiment with field-collected adults

To investigate the effect of sudden photoperiodic changes on reproductive timing and fecundity of field-collected, adult *Neanthes limnicola*, eight worms were collected on 27 October 1987, and their oocyte diameters determined by wet smears (20 oocytes/worm). Four worms were placed in phase, four out of phase, and all were maintained at 5‰ S and air temperature. Worms were fed brine shrimp once per week, and culture media were changed after each feeding. Worms were monitored daily for signs of reproduction.

Results

Field sampling

Oocytes were present in some individuals of *Neanthes limnicola* throughout the year, but rapid oocyte growth occurred mainly in mid-winter (Fig. 3). Embryos were present in adult coeloms mainly from late February through May. In February, March, April, and May 1988, all adults sampled had developing embryos and no oocytes. Since fertilized eggs take from 21 to 28 d to develop into juveniles that are ready to release (Smith 1950), parturition in Watsonville Slough probably takes place mainly in the spring and early summer. The newly released juveniles did not have oocytes (detectable in smears), but small oocytes 20 to 100 μm in diameter were present in some juveniles by late spring before all full-grown worms had released their young and died.

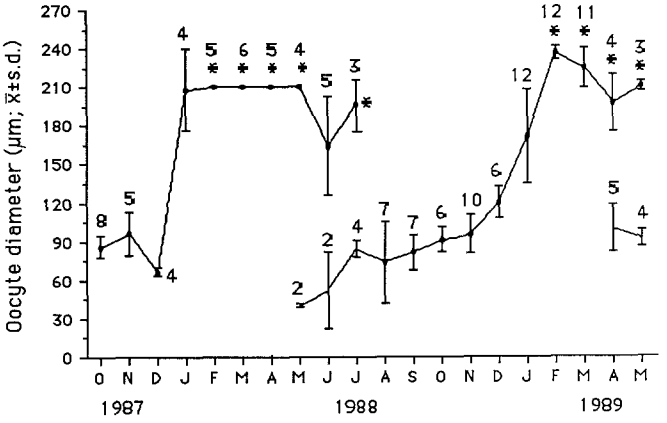

Fig. 3. *Neanthes limnicola.* Mean monthly oocyte diameter of field-collected worms, October 1987–May 1989. Asterisks indicate months when worms with developing coelomic embryos were observed; from February–May 1987, all worms had developing embryos or larvae equivalent in size to full-grown oocytes; numbers of worms sampled given above monthly datum points. Data are separated to account for overlapping cohorts from May–July 1988 and from April–May 1989; e.g. the May 1988 sample contained 4 specimens with embryos (equivalent to full-grown oocytes) and 2 specimens with small oocytes (40 to 50 μm)

Experiments with laboratory-born juveniles

Expt A: spring-born juveniles

Juvenile *Neanthes limnicola* which were born in spring 1987, and raised to adulthood gave birth mainly under spring light regimes, and photoperiod significantly affected the timing of parturition (Table 2). Parturition for the in-phase cultures was mainly in March–April 1988, while for out-of-phase cultures it was six months earlier, mainly in September–November 1987 (=spring light regime) (one-way ANOVA of mean parturition date based on Julian calendar, see first subsection of "Materials and methods", $F_{1,38}=98.36$, $P<0.0001$; Fig. 4A). There was some spread in the parturition dates; particularly notable were five in-phase worms that gave birth in September, >6 mo before the remainder but in phase with worms in the out-of-phase cultures. Photoperiod had a significant effect on the number of days worms spent in culture (one-way ANOVA, $F_{1,38}=35.43$, $P<0.0001$). Worms maintained in phase took much longer to reproduce ($\bar{x}_{\text{in phase}}=342.35$ d) than out-of-phase worms ($\bar{x}_{\text{out of phase}}=229.03$ d). Neither salinity nor temperature had a significant effect on parturition date (Table 2). Mean parturition dates for in-phase cultures in 5 and 15‰ S were 7 April and 16 May, respectively. Mean parturition dates for in-phase cultures at laboratory air and sea water temperature were 29 May and 29 March, respectively. Although temperature did not have an effect on parturition date, it did significantly affect days in culture (one-way ANOVA, $F_{1,38}=4.96$, $P<0.03$). Under in-phase conditions, worms at sea water temperature spent a significantly longer time in culture before giving birth than did worms at air temperature ($\bar{x}_{\text{water}}=472.3$ d, $\bar{x}_{\text{air}}=370.1$ d). However, this effect was not observed in out-of-phase cultures ($\bar{x}_{\text{water}}=277.6$ d, $\bar{x}_{\text{air}}=268.7$ d).

Salinity strongly affected the number of offspring produced (one-way ANOVA, $F_{2,51}=29.47$; $P<0.0001$). Sig-

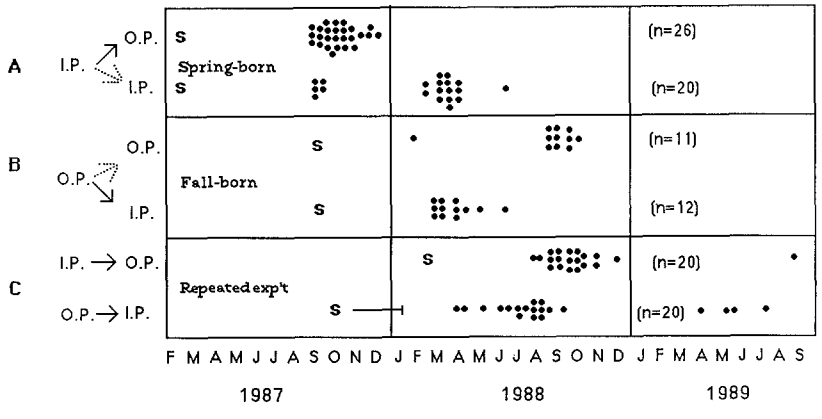

Fig. 4. *Neanthes limnicola.* Dates of juvenile release (parturition). Experiment A: parent worms born in spring 1987 and kept in phase (control) or immediately shifted to 6 mo out-of-phase light regime. Experiment B: parent worms born in fall 1987 and kept out of phase (control) or shifted to in-phase regime. Experiment C: parent worms born October 1987–January 1988 in out-of-phase regime and shifted to in-phase regime; parent worms born February–March 1988 in in-phase regime and shifted to out-of-phase regime. I.P.: in-phase light regime; O.P.: 6 mo out-of-phase light regime (e.g. worm born out of phase on calender day 1st September would be born in light regimes corresponding to March). Continuous arrows indicate parent worms reared in a light regime *different* from that under which they were born; dashed arrows indicate parent worms continued in the *same* light regime under which they were born (controls). S: starting dates of each experiment. Each point represents parturition by one worm

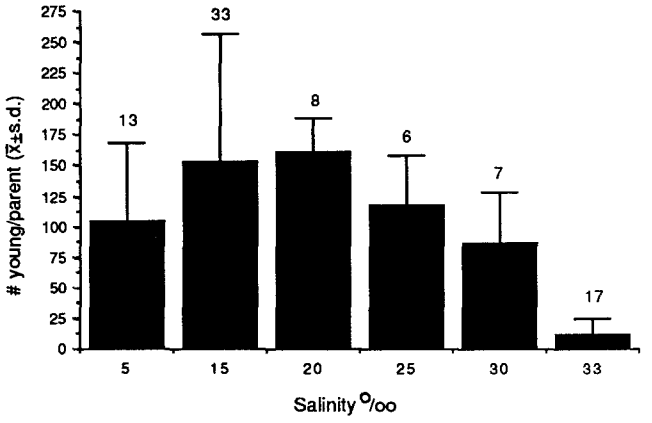

Fig. 5. *Neanthes limnicola.* Numbers of young produced at different culture-salinities. Numbers of adults giving birth appear above error bars. Results for 5, 15, and 33‰ are from Experiment A; those for 20, 25, and 30‰ are from Experiment B. The 17 worms giving birth in 33‰ S were not included in the analysis of reproductive timing (Fig. 4)

Table 2. *Neanthes limnicola.* Results of ANOVAs testing effects of photoperiod, salinity, temperature, and photoperiod × salinity interaction on parturition date, fecundity, and days in culture in each of three experiments (A, B, C). NS: not significant; nd: no data; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$

Variable and experiment	Photo-period	Salinity	Temper-ature	Photoperiod × salinity
Parturition date				
A	****[a]	NS	NS	NS
B	****[a]	NS	nd	NS
C	***[a]	NS	NS	NS
No. of young				
A	****[a]	****[a]	NS	**[b]
B	NS	**[1]	nd	NS
C	NS	NS	NS	NS
Days in culture				
A	****[a]	NS	*[a]	NS
B	****[a]	NS	nd	NS
C	***[a]	NS	NS	NS

[a] one-way ANOVA
[b] two-way ANOVA

nificantly more young were born in both 5 and 15‰ S cultures than in 33‰ S ($\bar{x}_{5‰}=104.2$; $\bar{x}_{15‰}=153.4$; $\bar{x}_{33‰}=12.1$, Scheffé multiple-range test, $P<0.05$ for both comparisons; Fig. 5). Worms in 33‰ S not only produced far fewer juveniles each, but 65% (11 of 17) of these births resulted in fewer than ten juveniles each, with four worms producing no juveniles at all. In the latter cases, adults had normal reproductive morphology, but produced no young. Photoperiod also had a significant effect on the numbers of offspring produced (one-way ANOVA, $F_{1,51}=18.22$, $P<0.0001$) with more young born under in-phase conditions ($\bar{x}_{\text{in phase}}=201.3$; $\bar{x}_{\text{out of phase}}=92.0$). Given this last result, mean differences in numbers of young born were analyzed separately for each photoperiodic condition. Under in-phase conditions, salinity significantly affected fecundity (one-way ANOVA, $F_{2,28}=23.11$, $P<0.0001$) and more young were produced in both 5 and 15‰ than in 33‰ ($\bar{x}_{5‰}=157.3$; $\bar{x}_{15‰}=220.2$; $\bar{x}_{33‰}=13.0$; Scheffé multiple-range test, $P<0.05$ for both comparisons). Similarly, under out-of-phase conditions, salinity affected fecundity (one-way ANOVA, $F_{2,29}=6.78$; $P=0.004$), but only 15‰ cultures produced significantly more young than those in 33‰ ($\bar{x}_{5‰}=58.7$; $\bar{x}_{15‰}=104.3$; $\bar{x}_{33‰}=10.5$; Scheffé multiple-range test, $P<0.05$).

Two-way and three-way ANOVAs showed no significant combined effects of photoperiod, salinity, and/or temperature on timing of parturition, although the interaction of temperature and photoperiod had nearly significant effects on both parturition date and days in culture ($P<0.07$ for both analyses). Salinity-photoperiod interaction had a significant effect on numbers of offspring produced (two-way ANOVA, $F_{2,51}=4.76$, $P<0.01$).

Expt B: fall-born juveniles

Juvenile *Neanthes limnicola* born in fall 1987 and raised to adulthood gave birth mainly under spring light-regimes, and photoperiod significantly affected the timing of parturition as it did in Experiment A (Table 2). Mean parturition date for worms maintained 6 mo out of phase was 1 September 1988 (= March light regime), but for in-phase worms it was 6 mo earlier, 7 April 1988 (one-way ANOVA, $F_{1,17}=55.41$, $P<0.0001$; Fig. 4B). Accordingly, photoperiod had a significant effect on days in culture (one-way ANOVA, $F_{1,17}=63.55$, $P<0.0001$). However, photoperiod had no significant effect on the number of young produced ($\bar{x}_{\text{in phase}}=115.5$; $\bar{x}_{\text{out of phase}}=135.6$), even though out-of-phase worms lived nearly twice as long in culture than those in phase. Salinity did not significantly affect the timing of parturition ($\bar{x}_{20‰}=19$ June, $\bar{x}_{25‰}=10$ May, $\bar{x}_{30‰}=14$ July), but it did affect the number of offspring produced (one-way ANOVA; $F_{2,15}=7.01$; $P<0.007$). Worms maintained in 20‰ S produced significantly more young than worms in either 25 or 30‰ S ($\bar{x}_{20‰}=161.3$; $\bar{x}_{25‰}=118.1$; $\bar{x}_{30‰}=86.8$; Scheffé multiple-range test; $P<0.05$ for both comparisons; Fig. 5). No significant interaction effects were observed.

Results from both Experiments A and B indicate that worms produce more young in salinities of 5 to 20‰, and most are born in 15 to 20‰. For worms that took one year or more to reproduce, the in-phase cultures of Experiment A (5 to 15‰ S) produced more young than the out-of-phase cultures of Experiment B (20 to 30‰) [$\bar{x}_{\text{in phase}}$ (A) = 238.2; $\bar{x}_{\text{out of phase}}$ (B) = 135.6; Mann-Whitney U-test, $U'=120$; $P<0.001$], probably as a result of the salinity effect.

Expt C: repeat experiments; in phase shifted to out of phase; out of phase shifted to in phase

Photoperiod significantly affected the timing of parturition in these experiments. Worms which were born under out-of-phase conditions between 14 October 1987 and 29 January 1988, then raised in phase, showed a mean parturition date of 13 July 1988, compared with the

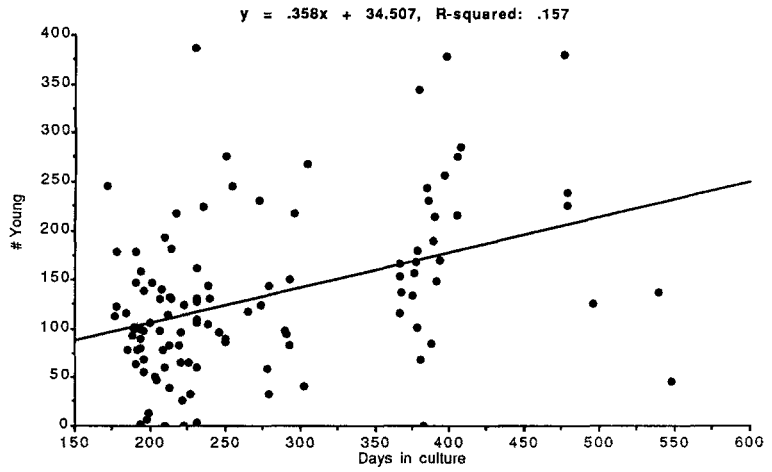

Fig. 6. *Neanthes limnicola.* Regression of numbers of young born on days in culture. Data are from pooled results of Experiments A (except 33‰ S cultures), B, and C ($N=109$)

mean parturition date of 10 October 1988 (=April light regime) for worms born in phase between 19 February and 29 March 1988 and then raised out of phase (one-way ANOVA, $F_{1,36}=17.47$, $P<0.0002$; Fig. 4C). Worms maintained out of phase also spent fewer days in culture than in-phase worms (one-way ANOVA, $F_{1,36}=3.28$, $P<0.07$; $\bar{x}_{\text{out of phase}}=228.6$ d, $\bar{x}_{\text{in phase}}=304.7$ d). Five individuals (4 shifted in phase and 1 shifted out of phase) lived longer than one year (478 to 548 d) in culture, but still gave birth in spring-summer light conditions. Although photoperiod had a significant effect on timing of births, it did not affect numbers of offspring ($\bar{x}_{\text{in phase}}=134.6$; $\bar{x}_{\text{out of phase}}=119.7$). There was also no difference in numbers of offspring produced by worms in 5 or 15‰ *S* ($\bar{x}_{5‰}=122.0$; $\bar{x}_{15‰}=129.2$;).

Temperature did not significantly affect the timing of births, but it did have a small effect on the number of days worms spent in culture (one-way ANOVA, $F_{1,36}=3.47$, $P<0.07$), with worms in sea-water temperature cultures taking longer ($\bar{x}_{\text{seawater}}=292.4$ d) to give birth than those in air-temperature cultures ($\bar{x}_{\text{air}}=220.8$ d).

Age versus number of young produced

Although the mean number of young produced by worms 6 to 8 mo-old was less than half the mean number produced by worms 12 to 13 mo-old in Experiment A (92 vs 201), there was no significant difference in mean number of young produced between 6 to 8 mo- and 12 to 13 mo-old worms in Experiment B (115.5 vs 135.6). When all the data are combined (except for worms held at 33‰ S), considerable scatter is seen between the number of young produced and the age of the worms, with only a weak positive correlation (Fig. 6). Six-month old worms produced a mean of about 100 young, while 1 yr-old worms produced a mean of only about 150. The highest number of young produced (387) was from a worm only 230 d-old. The worm that matured and released young earliest (171 d) released 245 young; the worm that lived the longest in culture (548 d) produced only 46 young when it finally matured.

Table 3. *Neanthes limnicola.* Oocyte diameters ($\bar{x}$ + range) and dates of juvenile release for worms maintained under in-phase light conditions ($N=4$), and 6 mo out-of-phase conditions ($N=4$). All worms collected from Watsonville Slough on 20 October 1987, and 20 oocytes/worm measured on 27 October 1987

Initial oocyte diam (μm)	Date (1988) of juvenile release of worms in:	
	in phase	out of phase
96.78 (90–105)	–	12 Jan.
70.50 (35–105)	–	14 Jan.
83.50 (65–105)	–	29 Jan.
79.75 (30–115)	–	30 Jan.
85.75 (40–155)	22 Mar.	–
93.25 (30–115)	1 May	–
86.12 (40–120)	6 May	–
93.50 (35–130)	7 June	–

Experiment with field-collected adults

Sudden changes in photoperiod significantly affected the timing of parturition in worms collected from the field on 27 October 1987, and then placed in both in-phase and out-of-phase conditions, regardless of size of oocytes when collected (Table 3). Mean parturition date for worms maintained in phase was 1 May 1988, but for out-of-phase worms it was 21 January 1988 (=July light regime) (Mann-Whitney *U*-test, $U=16$, $P<0.025$). Photoperiod did not affect the numbers of young produced ($\bar{x}_{\text{out of phase}}=249.0$, $\bar{x}_{\text{in phase}}=194.0$; Mann-Whitney *U*-test, $U=9$, $P<0.20$).

Discussion

Environmental factors are known to strongly influence a number of reproductive processes in polychaetes (reviewed by Schroeder and Hermans 1975, Olive 1984). In the present study, seasonally-changing photoperiod controlled the timing of parturition in *Neanthes limnicola* in laboratory experiments. Field data indicate that worms

release young mainly during the spring, and a new cohort grows to maturity and reproduces in one year. In experimental cultures, worms gave birth mainly (95%) in spring light regimes, independent of when (spring or fall) they themselves were born. Parturition usually occurred when worms were either 6 mo or 1 yr of age. Most worms that reproduced after only about 6 mo in culture experienced decreasing daylengths initially, followed by short days, and then increasing daylengths before finally giving birth. However, a few in both in-phase and out-of-phase conditions reproduced about 6 mo early after experiencing increasing, long, and decreasing daylengths. Worms taken directly from the field in October (with an 11 h light:13 h dark light-regime) and then immediately exposed to spring conditions of increasing daylength gave birth three months early under summer light regimes. In Experiment C, five individuals lived longer than a year (478 to 548 d) and still reproduced at appropriate times, indicating strong regulation by photoperiod.

Experiments shifting the annual photoperiodic cycle, as did ours, have shifted annual reproductive cycles in echinoids (Pearse et al. 1986b, McClintock and Watts 1990), asteroids (Pearse and Eernisse 1982, Pearse and Walker 1986, Pearse et al. 1986a, Xu and Barker 1990), and decapod shrimps (Custer 1986). In echinoids, fixed photoperiods <12 h stimulated gametogenesis, while those >12 h inhibited gametogenesis (Bay-Schmith and Pearse 1987). Lancelets also appear to respond to fixed daylengths, but long daylengths stimulate gametogenesis and gonad growth (Fang et al. 1989). In contrast, the asteroids (Pearse et al. 1986a) and shrimps (Custer 1986) studied to date maintain an annual reproductive cycle in phase with field populations when held on photoperiods of fixed daylengths either more or less than 12 h; they appear to have an internal calendar that responds only to changing daylengths. The animals in these studies are all iteroparous. *Neanthes limnicola*, like other nereids, is semelparous; it released a single batch of young after 6 mo to 2 yr in our cultures. Our data suggest that oocyte growth in *N. limnicola* may be stimulated by either decreasing or short daylengths, but that completion of oocyte growth and parturition of young occurs during periods of increasing daylength. Fixed daylength experiments incorporating seasonally changing photoperiods will be necessary to elucidate whether fixed daylengths, changing daylengths, or both, synchronize reproduction in this species.

The annual reproductive cycle of *Neanthes limnicola* fits the model of gated reproductive rhythms for nereids proposed by Olive (1984). In the model, the length of the reproductive cycle is entrained by an external zeitgeber which maintains synchrony within the population. Worms can initiate a rapid phase of gametogenesis only during a specific period of time ("gate-open period"). Once they have entered the "gate", worms are committed to spawn after rapid oogenesis is completed. Time for completion ("reaction time") is determined by various environmental factors such as sea temperature. If worms fail to enter the gate, they must wait another full cycle for the gate to reopen. In the case of *N. limnicola*, the exogenous zeitgeber is photoperiod, perhaps daylengths of 12 h or less, and the gate is open for rapid gametogenesis in fall and winter, with a reaction time of 90 to 150 d. In Experiments A and B (Fig. 4A, B), a few individuals apparently reached the gate early, and they gave birth within only 6 mo. In contrast, in Experiment C (Fig. 4C), five worms apparently missed the gate and had to wait ~ 12 additional months for the gate to reopen.

Parturition occurred mainly when worms were ~ 6 to 8, 12 to 13, or 16 to 18 mo-old, depending on photoperiod regime and the time they reached the gate-open period; however, length of life did not have a close relation to number of young produced (see Fig. 6). These results are unexpected; life-history theory predicts that longer life will allow animals to gain more resources that could be utilized for reproduction (Bell 1980). In these semelparous polychaetes, however, full reproductive potential apparently can be reached within half their normal life span, after which metabolic activity may be directed mainly toward maintenance until the beginning of the gate-open period. There appears to be little trade-off between life span and reproduction, but other trade-offs, or "dynamic linkages", may occur (Stearns 1989). In particular, our demonstration of photoperiodic control suggests that selection for timing of reproduction (parturition) may be critical . More data on the relationship of life-span to feeding, metabolic rates, growth, fecundity, and offspring size and survival are needed before such life-history features can be fully evaluated for this species.

Salinity significantly affected the number of young born. Worms produced more young in salinities from 5 to 25‰, with the largest broods being produced in 15 to 20‰; and fecundity was much reduced at higher salinities. Moreover, worms exposed to salinities of 33‰ often looked abnormal and produced very few or no young. It is not known how higher salinities affect the reproductive physiology of *Neanthes limnicola*. This species osmoregulates below about 12‰ S, above which it osmoconforms (Oglesby 1968). Since some successful births occurred at 33‰ S, fertilizations can take place. Early in the gametogenic cycle of nereids, a few oocytes go through a fast initial growth phase, but most develop slowly and go through a rapid phase of growth just before spawning. Full-strength sea water may interfere with this final rapid phase of oocyte growth; thus, perhaps only those oocytes which grew to large size early on were mature enough to be fertilized. The detrimental effects of full-strength sea water to embryonic development and fecundity in *N. limnicola* may explain why this species is restricted to brackish and fresh-water habitats along the Pacific coast of North America.

Temperature did not affect the timing of parturition in culture. The sharp increase in temperature in the field (Fig. 1) about the time when worms began to give birth suggests its importance in the later stages of oocyte growth. But, worms did not appear to be sensitive to laboratory temperatures whose means range only from about 12.0 to 16.0 °C in sea water and 16.0 to 19.0 °C in air (Fig. 2). Moreover, worms in culture gave birth during periods of both increasing and decreasing temperature. Temperature may play a role in synchronizing reproduction in the field, but when the worms are released

from this influence in the laboratory, photoperiodic cues are sufficient to focus timing of parturition. Similar results were obtained by Pearse and Walker (1986), who found photoperiodic control of gametogenesis in the North Atlantic sea star *Asterias vulgaris* maintained under the less extreme temperature regimes of central California.

Synchronization of reproductive cycles and spawning may increase the chances for successful fertilization in free-spawning species (Pearse 1990). However, *Neanthes limnicola* is a self-fertilizing hemaphrodite with no indication of any cross fertilization. Because benthic juveniles are released directly from the parents, seasonal reproduction cannot be an adaptation directed at survival of larvae. *N. limnicola* is a surface-deposit feeder, and reproduction during the spring, when food for emerging juveniles is probably more abundant, may select for spring parturition. In support of this suggestion, two deposit-feeding amphipods, *Anisogammarus confervicolus* and *Corophium spinicorne*, were also gravid or in precopula during the spring in Watsonville Slough (Fong unpublished observation), indicating that this is a favorable period for reproduction for other direct-developing taxa. Releasing large numbers of young during a few select months may also be a way of swamping predators. Moreover, increasing temperature during the spring may speed the growth of young so they can attain larger sizes and avoid predation. Alternatively, *N. limnicola* may be descended from a species in which reproductive responses to seasonally-changing photoperiod were at one time selected for, but with a viviparous mode of reproduction the evolutionary constraint for this behavior disappeared, resulting in a neutral trait. *N. limnicola* is closely related to and may be derived from *N. japonica,* which swarms and free-spawns (Izuka 1908, Imajima 1972). Analysis of the strength of photoperiodic regulation in *N. japonica* may assist in evaluating the possibility of such an evolutionary memory in *N. limnicola.*

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