**Old Dominion University**
## ODU Digital Commons

OEAS Faculty Publications        Ocean, Earth & Atmospheric Sciences

1993

# A Reproductive-Resting Stage in an Harpacticoid Copepod, and the Significance of Genetically Based Differences Among Populations

D. J. Lonsdale

P. Weissman

Fred C. Dobbs
*Old Dominion University*, fdobbs@odu.edu

# A REPRODUCTIVE-RESTING STAGE IN AN HARPACTICOID COPEPOD, AND THE SIGNIFICANCE OF GENETICALLY BASED DIFFERENCES AMONG POPULATIONS

*D. J. Lonsdale, P. Weissman and F. C. Dobbs*

## ABSTRACT

Dormancy is an important life-history strategy which allows copepods to increase their fitness by delaying growth and reproduction until harsh environmental conditions have ameliorated. For marine species, the primary strategies identified to date include the production of dormant eggs by shallow-water species, and copepodite overwintering in deep-water species. Herein, we describe a third strategy in which fertilized adult females enter a "reproductive-resting" stage during the late fall that allows them to overwinter and provide a first source of spring naupliar recruitment. This strategy has been observed in the estuarine copepod *Coullana canadensis,* but may also occur in other species. Laboratory studies indicate that daylength and temperature are the environmental cues that induce the developing female copepodite to switch between active reproduction and reproductive-resting stage. In Maine populations, daylengths equal to 14 h induce >90% of the females to reduce development rate and accumulate lipid before maturation and mating. The resulting females, however, do not develop ova regardless of food level. A similar reproductive-resting stage is triggered at daylengths <14 h in animals collected from Maryland. Transition from reproductive-resting stage to active ova production may be triggered in both populations by increased photoperiod and/or dramatically increased temperature. Cross breeding experiments indicate that the daylength triggered switch to reproductive-resting is under tight genetic control. Daylength likely serves as a critical cue for all populations in differentiating between the onset of harsh (i.e., winter) and favorable (i.e., spring) environmental conditions. At these times water temperatures are similar, but daylengths are different. Population differences in the daylength necessary to trigger the reproductive-resting strategy likely reflect latitudinal variation in the period over which environmental conditions are conducive to population growth.

Resting and diapause stages are common life-history attributes among planktonic copepods, particularly the Calanoida and Cyclopoida (e.g., *Labidocera aestiva, Acartia hudsonica* and *Mesocyclops edax*; Marcus, 1979; Sullivan and McManus, 1986; Wyngaard, 1988), and likely are adaptive responses to avoid harsh environmental conditions such as desiccation, low food availability, and high predation pressure (Hairston and Olds, 1987). A seasonal pattern in adult encystment has been reported in one species of marine and several species of freshwater Harpacticoida, but in general, little is known about diapause or resting strategies in this order of copepods and benthic species in general (Coull and Grant, 1981; Williams-Howze and Coull, 1992; reviewed in Williams-Howze, 1992).

*Coullana canadensis* [i.e., *Scottolana canadensis* (Willey); Por, 1984] is a brackish-water, harpacticoid copepod which occurs over a wide geographic range on the east coast of North America (Willey, 1923; Coull, 1972; Lonsdale and Levinton, 1985), and populations are interfertile from at least Maine to South Carolina (Lonsdale et al., 1988). *Coullana* also was collected on both the east and west coasts of Florida, but they are reproductively isolated from all other northern populations (Lonsdale et al., 1988). *Coullana* is usually seasonally restricted throughout its entire range, with highest naupliar densities occurring in early spring to early summer months, although egg production appears to be continuous throughout much of the summer (e.g., Maryland and Maine; Lonsdale, 1981b, this study). In Chesapeake Bay, for example, spring naupliar densities of *C. cana-*

*densis* range from 5,000 to 15,000 m$^{-3}$, but during times of unusually low summertime salinities (0 to 14‰), nauplii can persist through the summer and early fall (Heinle et al., 1977). Reduced salinity may favor population growth of *C. canadensis* (Lonsdale, 1981a) and reduce predator densities (Heinle et al., 1977). Invertebrate predation by ctenophores and adult *Acartia tonsa* may be a major factor in regulating naupliar densities during the summer (Lonsdale, 1981b). Copepodites and adults are likely prey for fish because they inhabit muddy substrata, a preferred feeding site for bottom-feeders such as juvenile spot (Coull and Dudley, 1985; Smith and Coull, 1987; S. Bell, pers. comm.).

Previous studies of *Coullana canadensis* indicated puzzling differences in the frequency of reproduction in newly matured and mated females. For example, the frequency of reproduction was considerably lower in Maine copepods compared to Maryland copepods (18.2% vs. 100%, respectively) when reared in the laboratory at 15°C under high food conditions (Lonsdale and Levinton, 1986). Also, lipid accumulation in the form of droplets was pronounced in the oviducts and other cavities (e.g., leg segments) of nonreproductive females (microscopic observations made using Oil Red O; methods from Gallager and Mann, 1981). In the field, however, naupliar recruitment occurs in Maine at 13° to 15°C (Lonsdale and Levinton, 1985). A similar lack of concordance in growth rates between laboratory-reared and field populations of Maryland *C. canadensis* at 15°C was also found by Lonsdale (1981b). This led us to hypothesize that the condition was induced by a pathogenic organism (Lonsdale and Levinton, 1986), or alternatively, that it was a critical part of the life-history strategy of the species which allowed it to succeed in diverse environments. Our histological investigations of females using light and transmission electron microscopy minimized the possibility of pathogens as the cause for the lack of reproduction. Thus, our objectives in this study were to test the alternative hypothesis that the life-history variation observed among *C. canadensis* populations was genetically based, and to elucidate its adaptive significance.

## MATERIALS AND METHODS

*Batch-culturing of Copepods.* — The life-cycle of *Coullana canadensis* includes planktonic naupliar molt-stages (6) and benthic copepodite (5) and adult (1) molt-stages. Females carry eggs in two external sacs, and can produce multiple clutches in a lifetime without remating (Lonsdale, 1981a). To obtain *C. canadensis* for laboratory experiments, 100 to 200 planktonic nauplii were obtained with a 63-µm mesh net from three sites on the east coast of North America (details in Table 1). In the laboratory, field-collected copepods were batch cultured at 20–21°C in 15‰ seawater and under a 14:10 h light : dark cycle (detailed in Lonsdale and Jonasdottir, 1990). Algal species for copepod rearing were grown in f/2 enrichment medium (Guillard, 1975; also see Lonsdale and Levinton, 1985). Algal cell densities were assessed with a hemacytometer. A mixture of two species, *Isochrysis galbana* (ISO; ~4–6-µm diameter) and *Thalassiosira pseudonana* (3H; ~4-µm), was added to cultures to produce a minimum of 2.5·10$^5$ cells·ml$^{-1}$ or ~2,887 µg C·liter$^{-1}$ (according to the Strathmann equations; Strathmann, 1967), a density within the range found in many estuaries (e.g., 500–3,388 µg·C·liter$^{-1}$ for Narragansett Bay; Durbin et al., 1983). For experiments, an algal suspension was prepared by adding cells (in the same amounts as for batch culturing) to 20-µm filtered, autoclaved seawater (15‰).

Environmental or maternal sources of physiological and morphological differences within or among populations were minimized by common-rearing of copepods for several generations in batch culture. Thus, any phenotypic differences were presumed to have a genetic basis. Hybrid crossing experiments were performed to verify the common-rearing results.

*Reproduction Experiments on Laboratory Populations.* — To test the hypothesis that the previously found difference in reproductive frequency among Maine (ME) and Maryland (MD) females (Lonsdale and Levinton, 1986) was genetically determined, gravid copepods (N = 18 from each locale) were taken from batch culture and isolated in separate 17-ml polystyrene wells containing 8 ml of algal suspension, and covered to minimize evaporation. These females served as the source of offspring for observations on the first (P$_1$) generation. Daily observations on females were made and newly hatched

Table 1.  Location and water temperature and salinity during collection (~1 m water depth) of *Coullana canadensis* nauplii

| Collection site | Date | Temperature (°C) | Salinity (‰) |
|---|---|---|---|
| Saco River Biddeford, ME | May 1988 | 17 | 10 |
| Saco River Biddeford, ME | June/July 1990 | 18–20 | 9–12 |
| Quantuck Creek Quogue, NY | May 1991 | 16 | 19 |
| Patuxent River Lusby, MD | April 1990 | 21 | 11 |

nauplii were equally sorted into two separate wells (up to 16 per well) for incubation at either 15 or 21°C and under a 14:10 h light : dark cycle (as in Lonsdale and Levinton, 1986). Replicated data (N = 2) were obtained for each copepod family by using nauplii hatched from a second clutch of eggs. To reduce temperature shock, the initial temperature of the algal suspension to which nauplii were added was 21°C. Culture wells were replenished with a concentrated algal suspension ($10^6$ cells·$ml^{-1}$) three times weekly, and copepods were transferred to clean wells with 100% fresh algal suspension weekly.

Observations on copepod survival and development were made daily. When $P_1$ females reared at 21°C reached ~Copepodite IV, two to six individuals per family were removed and placed in wells for both intra- and interpopulational crosses (control and hybrid crosses, respectively). Although mature males may form pairs with immature females, mating does not occur until the female molts to adult (Lonsdale et al., 1988). Offspring from these crosses were used to obtain reproductive statistics for the $f_1$ generation. Family replicates were not conducted. Newborns were not obtained from some $P_1$ family lines (N = 12 to 14) to study the $f_1$ generation because of skewed sex ratios (high proportion of males) or selected females did not reproduce. All females were monitored for mating status (i.e., pair formation), reproductive condition (i.e., ovum formation or presence of lipid droplets). Observations were terminated 10 days after no more pairs were observed. Usually at 15 or 20°C, reproductive females became gravid within 4 days following the terminal molt (Lonsdale and Levinton, 1986). This methodology was repeated for the $f_1$ generation.

A separate experiment was conducted following the above study to better define geographic gradients in this trait. Batch-cultured copepods collected from the south shore of Long Island, New York (NY; Table 1) were reared for one generation ($P_1$; N = 11 families), and under the same laboratory environmental conditions as described above.

Photoperiod was a major environmental difference between life-table studies of MD *Coullana canadensis* in which the reproductive frequency varied substantially at 15°C (Lonsdale, 1981b vs. Lonsdale and Levinton, 1986). In the former study, a 12:12 h light : dark cycle was used. Thus, to test the hypothesis that light cycle variation would alter the reproductive-resting response, batch-cultured copepods from all three sites were reared in a similar manner as already described, but under a 12:12 h light : dark cycle for one generation to obtain reproductive statistics.

For a clearer understanding of what role reproductive-resting females may play in the natural population dynamics of *Coullana canadensis*, experiments were conducted to determine if they could be induced to reproduce. Reproductive (having eggs in oviducts and/or carrying egg sacs) and reproductive-resting females (N = 27 for each reproductive condition) were obtained from 1988 batch cultures from ME held at 25° and 15°C, respectively. Females were individually placed in separate wells of a multi-depression dish held in an airtight, opaque plastic box. Distilled water in the bottom of the box served to reduce evaporation from the wells. Following acclimation at 20°C for 1 day, nine females of each reproductive condition were maintained for 14 days at 15°, 20°, or 25°C with the above mentioned algal suspension. The algal suspension was completely replaced daily. The light : dark cycle was 14:10 h.

*Data Analyses.*—The above experimental design was intended to allow detailed statistical analyses using standard ANOVA procedures. However, in analyzing the results of the experiments, it rapidly became apparent that the variances in the data were not normally distributed. For example, as discussed below, 100% of the MD females were reproductive at 21°C. As a result, we have had to use non-parametric statistics to analyze this data since a fundamental condition required to apply parametric statistics (i.e., ANOVA) was not met (Sokal and Rohlf, 1981).
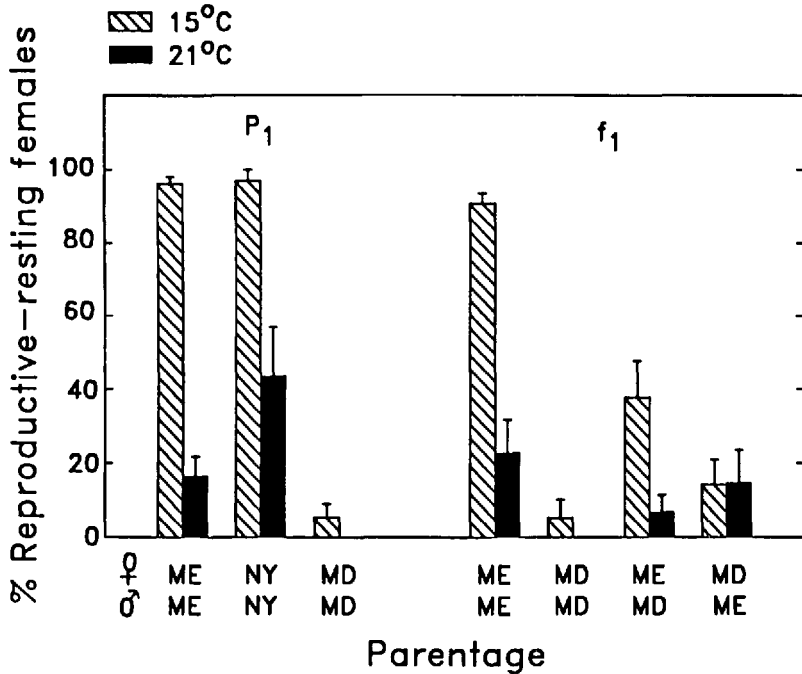
Figure 1.   Percentage of reproductive-resting females within families of *Coullana canadensis* obtained from intra- ($P_1$ and $f_1$ generations) and interpopulational ($f_1$ generation) crosses. Copepods were reared in the laboratory at two temperatures under a 14:10-h light : dark cycle. Mean ± 1 standard error are indicated (see Table 2 for number of families). The percentage of reproductive females can be determined by subtracting the percentage of reproductive females from 100%.

## RESULTS

*Locale Differences in Reproduction.* — The reproductive response of *Coullana canadensis* to temperature at daylengths typical of late summer (14:10 h light : dark cycle) varied dramatically between populations collected in Maryland (MD) and Maine (ME) (Fig. 1). The mean percentage of reproductive females within families was higher for MD copepods at both temperatures. These differences were largest at 15°C with more than 96% of the females reproductively active while NY and ME copepods were less than 5% active. At 21°C, all MD females became reproductive, whereas a percentage of females from the northern locales went into a reproductive-resting state (e.g., 16.5% and 43.7% for ME and NY, respectively, in the $P_1$ generation).

Non-parametric analysis indicates that we must reject the null hypothesis for both temperatures, i.e., that the frequencies of reproductive and reproductive-resting females reared under a 14:10 light : dark cycle (Table 2) were independent of parentage in the first generation ($P_1$) (Table 3a). At both water temperatures, the frequency of reproductive females was higher in MD *Coullana canadensis,* and no difference was found among ME and NY copepods. The $P_1$ results strongly indicate that the reproductive-resting response at 14:10 h photoperiod is a genetically controlled life-history trait. The identical responses among ME and MD copepods in the $P_1$ and $f_1$ generations provide additional support for the hypothesis that genetic differences among *C. canadensis* populations for this trait have evolved.

Table 2. Number of reproductive and reproductive-resting females of *Coullana canadensis* reared at two temperatures and a 14:10-h light:dark cycle. Females from the $P_1$ generation (2A) at 21°C were mated to batch culture males to obtain control and hybrid offspring for $f_1$ experiments at two temperatures (2B)

| | 2A | | | | | | 2B | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | $P_1$ generation | | | | | | $f_1$ generation | | | | | | | |
| | 15 | | | 21 | | | 15 | | | | 21 | | | |
| Temperature (°C) | | | | | | | | | | | | | | |
| Maternal source locale | ME | NY | MD | ME | NY | MD | ME | ME | MD | MD | ME | ME | MD | MD |
| Paternal source locale | ME | NY | MD | ME | NY | MD | ME | MD | MD | ME | ME | MD | MD | ME |
| No. families | 18 | 8 | 17 | 16 | 10 | 16 | 12 | 12 | 13 | 13 | 12 | 12 | 14 | 13 |
| No. reproductive females | 9 | 1 | 110 | 119 | 18 | 127 | 6 | 76 | 43 | 44 | 40 | 102 | 58 | 50 |
| No. reproductive-resting females | 164 | 21 | 3 | 35 | 10 | 0 | 53 | 52 | 4 | 9 | 18 | 13 | 0 | 14 |

Table 3. Results of a statistical test of the null hypotheses that the frequencies of reproductive and reproductive-resting females reared under a 14:10-h light:dark cycle were independent of parentage in the $P_1$ and $f_1$ generations (3A and 3B, respectively) (R × C tests of independence; Sokal and Rohlf, 1981). Superscripts on parentage denote non-significant differences at the 5% level within a test temperature. Crosses are indicated as maternal × paternal parentage

| Temperature (°C) | $P_1$ generation (3A) | | $f_1$ generation (3B) | |
|---|---|---|---|---|
| | 15* | 21† | 15‡ | 21§ |
| Parentage | ME × ME[a] | ME × ME[a] | ME × ME[a] | ME × ME[a] |
| | NY × NY[a] | NY × NY[a] | ME × MD[b] | ME × MD[b] |
| | MD × MD[b] | MD × MD[b] | MD × MD[c] | MD × MD[c] |
| | | | MD × ME[c] | MD × ME[a,b] |

* df = 2, $G$ = 305.3, $P$ < 0.001.
† df = 2, $G$ = 54.9, $P$ < 0.001.
‡ df = 3, $G$ = 101.4, $P$ < 0.001.
§ df = 3, $G$ = 32.1, $P$ < 0.001.

In the $f_1$ generation, statistical analysis of the crosses (Table 2) indicates that the null hypothesis that the frequencies of reproductive and reproductive-resting females were independent of parentage must be rejected at 15° and 21°C (Table 3b). At 15°C, hybrid crosses between MD and ME $P_1$ copepods dramatically increase the percentage of reproductive females compared to ME $f_1$s (Fig. 1).

Frequency distributions of the percentage of reproductive-resting females within families suggest that the reproductive-resting trait may be genetically controlled by only a small number of loci with a low level of additivity. If the trait were under polygenic control, a normal distribution in percentages would have been expected in the $f_1$ hybrids (Fig. 2).
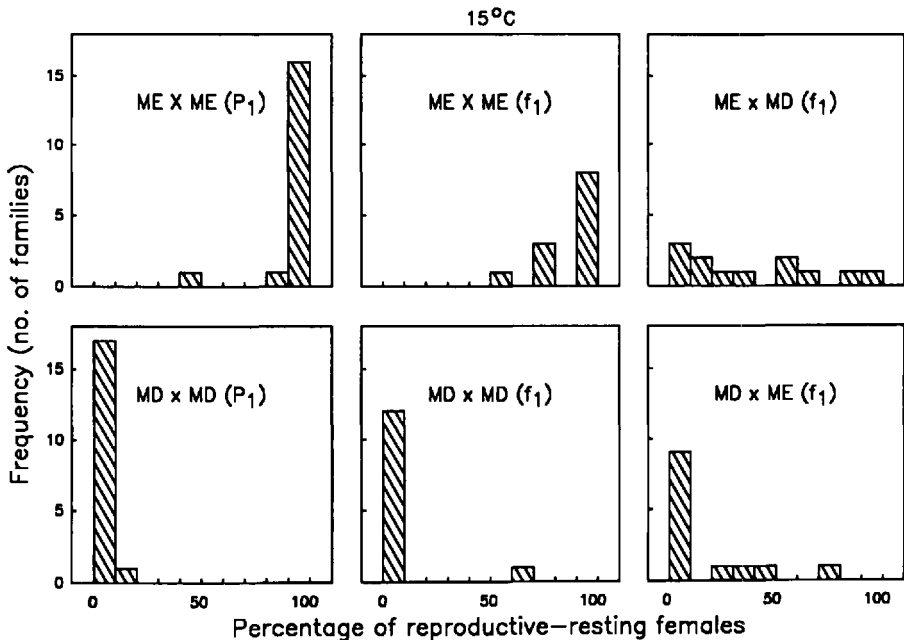


Figure 2. Frequency distribution of the percentage of reproductive-resting females of *Coullana canadensis* from intra- and interpopulational crosses, reared in the laboratory at 15°C. Crosses are indicated as maternal × paternal parentage.

Table 4. Number of reproductive and reproductive-resting females of *Coullana canadensis* reared at two temperatures and a 12:12-h light:dark cycle

| | Temperature (°C) | | | | | |
| | 15 | | | 21 | | |
| | ME | NY | MD | ME | NY | MD |
|---|---|---|---|---|---|---|
| Maternal source locale | ME | NY | MD | ME | NY | MD |
| Paternal source locale | ME | NY | MD | ME | NY | MD |
| No. families | 14 | 15 | 10 | 10 | 10 | 11 |
| No. reproductive females | 1 | 4 | 0 | 14 | 16 | 23 |
| No. reproductive-resting females | 34 | 57 | 13 | 11 | 14 | 4 |

*Light Cycle Variation.*—The reduction in photoperiod to a 12:12 h light:dark cycle dramatically altered the life-history response of MD copepods at 15°C (Table 4). All females entered the reproductive-resting stage, and exhibited visually detectable lipid accumulation. The null hypothesis that the frequencies of reproductive and reproductive-resting stages were independent of parentage could not be rejected at 15°C, but was rejected at 21°C (Table 5). New York copepods had a significantly lower frequency of reproductive females compared to MD copepods.

*Reproductive Recovery.*—These results supported our hypothesis that the trait in question was a reproductive-resting response. When exposed to the highest water temperature (i.e., 25°C), 67% of the reproductive-resting females began ova and egg sac production during the 14 day interval, but there was no apparent reproductive recovery for ME resting females maintained at 20° or 15°C (Fig. 3). A switch to the reproductive-resting strategy by active females was not apparent because most maintained egg production even at the lower temperatures.

## DISCUSSION

*Coullana canadensis* nauplii are numerically important in estuarine plankton during spring (Heinle et al., 1977). The first source of their yearly recruitment is most likely overwintering females, a hypothesis supported by the observation that the reproductive-resting stage can be overridden by increasing water temperature. Moreover, reproductive-resting females accumulate more wax esters, triglycerides, and sterols, compared to reproductive females that have more polar lipids (unpubl.). Storage of wax esters in particular may fuel females during the nutritionally

Table 5. Results of a statistical test of the null hypothesis that the frequencies of reproductive and reproductive-resting females reared under a 12:12-h light:dark cycle were independent of parentage (R × C tests of independence). Superscripts on parentage denote non-significant differences at the 5% level within a test temperature. Crosses are indicated as maternal × paternal parentage

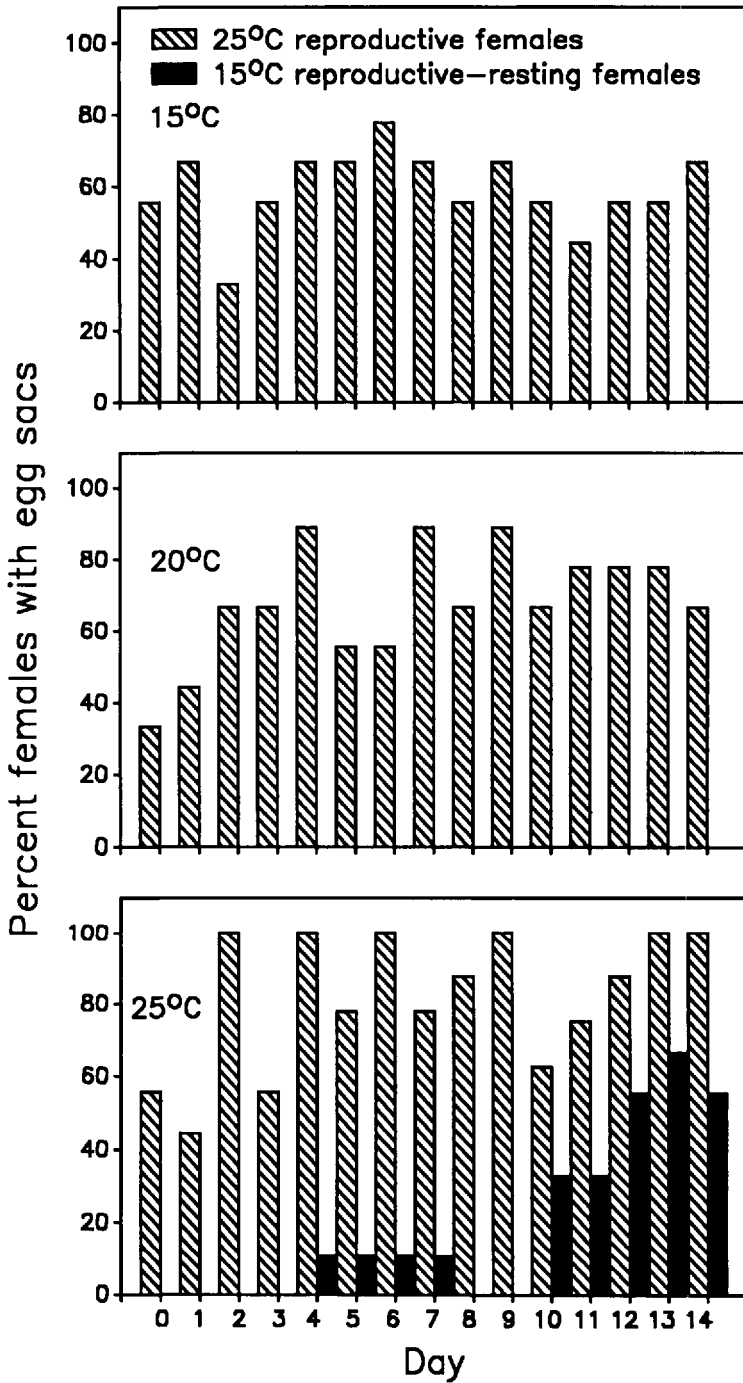| Temperature (°C) | df | G | P | Parentage |
|---|---|---|---|---|
| 15° | 2 | 2.0 | >0.1 | ME × ME[a]<br>NY × NY[a]<br>MD × MD[a] |
| 21° | 2 | 8.1 | <0.025 | ME × ME[a,b]<br>NY × NY[b]<br>MD × MD[a] |

Figure 3. Percentage of reproductive and reproductive-resting females of *Coullana canadensis* carrying egg sacs over 14 days at 15, 20, and 25°C.

dilute winter, and ultimately, support egg production in the early spring (e.g., as found in marine calanoid copepods; Ohman et al., 1989; also see Sargent and Henderson, 1986 for review). Thus, in addition to providing a refuge from harsh environmental conditions, female overwintering could be advantageous if egg production preceded the spring bloom, a food resource which hatched nauplii subsequently could utilize (e.g., as found for *Neocalanus* spp.; Frost et al., 1983). This advantage would be similar to that for nauplii hatched from dormant eggs in other shallow-water copepod species (see Donaghay, 1988 for discussion), and may be operative at least for MD *C. canadensis*. Mean chlorophyll *a* levels are highest around mid-April (ranging from mid-March to mid-May depending on location and year; Malone et al., 1988) when nauplii first recruit into the Chesapeake Bay plankton (Heinle et al., 1977; Lonsdale, 1981b). Apparently in ME, however, naupliar recruitment occurs 2 months after the late February to mid-March spring bloom (Townsend, 1984; as discussed in Lonsdale and Jonasdottir, 1990). In the northern locale, lipid accumulation may fuel female survival over the winter, but initial egg production may be more dependent on spring bloom resources. Teasing out the physical constraints to reproduction (e.g., temperature), and how they may dictate the relative importance of maternal and environmental food resources at various latitudes will shed light on the adaptive significance of this overwintering strategy.

The potential life span of reproductive-resting females of *Coullana canadensis* should allow overwintering as a recruitment strategy. Reproductive-resting females from MD maintained at 15°C under a 12:12 h light : dark cycle survived for over 90 days in the laboratory, and some subsequently reproduced when moved to 20–25°C. Whether or not mature males enter a similar reproductive-resting stage or store energy reserves for overwintering remains to be determined, but we have occasionally observed in the laboratory males with "lipid-like" droplets. Spring naupliar recruitment, however, could occur without males present because reproductive-resting females are already mated, and produce viable clutches without remating (as also found in a cyclopoid copepod; Naess and Nilssen, 1991).

Resting or diapause eggs are an important recruitment mechanism in other shallow-water copepod species. This may, however, not be the case for *Coullana canadensis* as no nauplii hatched from sediment taken from two sites in Stony Brook Harbor during January and March 1990, although other species did hatch (e.g., *Acartia* spp.). We incubated sediment under nine different conditions, 5, 12, or 20°C and 5, 12, or 25‰, for 12 days. At 20°C, *C. canadensis* eggs take only 3–4 d to hatch (Lonsdale and Levinton, 1985). The lack of evidence for resting eggs points to the importance of overwintering adult copepods as the source of spring naupliar recruitment for *C. canadensis*.

Re-analyzed data from published work (Lonsdale and Levinton, 1986) suggest that by CI, the reproductive-resting trait has been cued, and copepodite developmental rates are altered (Fig. 4). For ME females which eventually entered the reproductive-resting stage, mean stage-specific development time at 15°C was significantly slower for copepodites, but not for nauplii compared to those which became reproductive (Table 6). The timing of the developmental "switch" by CI may be adaptive for several reasons. First, the slower development rate of cued copepodites may allow energy diversion away from growth and into lipid accumulation essential for overwintering. A similar change in copepodite growth and lipid accumulation was also found in a diapausing freshwater harpacticoid, *Canthocampus staphylinus* (Sarvala 1979, cited by Williams-Howze and Coull, 1992). Second, the very shallow light compensation depths of most estuarine and coastal waters during the spring and summer may preclude photoperiod as an effective environmental cue for benthic animals. This latter hypothesis is supported by
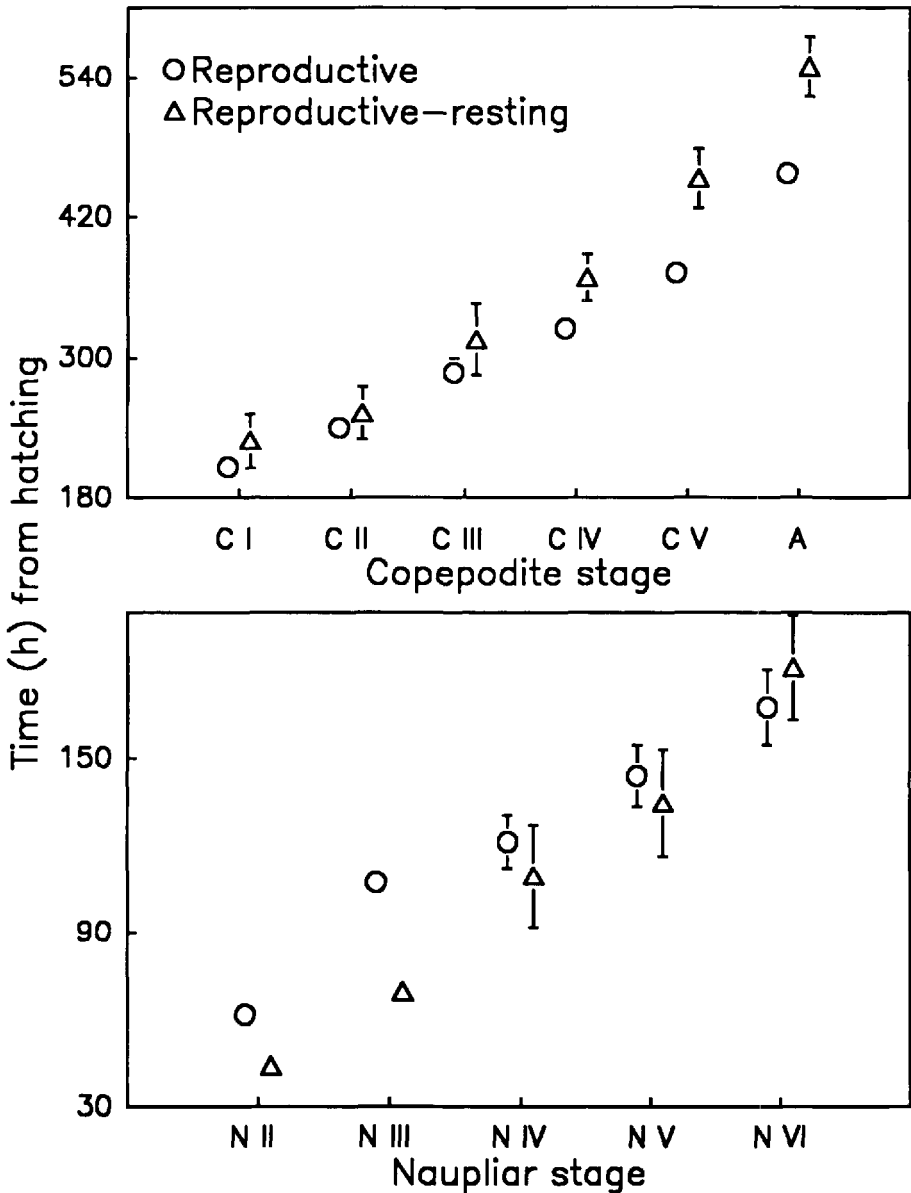
Figure 4. Time (h) from hatching of reproductive and reproductive-resting females of *Coullana canadensis* collected from ME and reared at 15°C. Mean ± 1 standard error are indicated. Data are re-analyzed from Lonsdale and Levinton (1986), and expressed separately for naupliar and copepodite stages. Sample size for both reproductive types was low; N = 3 for reproductive-resting copepods and N = 2 for reproductive copepods. Females selected to compute reproductive-resting developmental rates were those which 1) had lipid inclusions, and 2) never showed signs of egg production. Missing standard errors are smaller than the height of the symbol.

findings that photoperiod manipulation could not induce summer encystment in another shallow-water, marine harpacticoid *Heteropsyllus nunni* (Williams-Howze and Coull, 1992). This species, like most harpacticoids, does not possess a planktonic stage.

The adaptive significance of the light : dark cycle as a proximate cue for the

Table 6.   Results of a statistical test of the null hypothesis that development rates of copepods (stages d⁻¹) are independent of life-history (i.e., reproductive versus reproductive-resting strategy) (paired $t$-test; Sokal and Rohlf, 1981)

| Life-stage | N | $t_s$ | P |
|---|---|---|---|
| Naupliar | 5 | 1.519 | >0.2 |
| Copepodite/Adult | 6 | 3.438 | <0.02 |

induction of the reproductive-resting response is suggested from monthly records of daylength (Fig. 5). Daylength may be important to copepods in differentiating between the onset of favorable (spring) and harsh (winter) environmental conditions when water temperatures are similar, ~13°C and 15°C (open water), respectively, for MD, and ~13°C and 18(?)°C for ME populations, respectively (Heinle et al., 1977; Lonsdale, 1981b). We are uncertain about the timing of the seasonal decline in ME *Coullana canadensis,* but found large numbers of copepodites and adults in mid-July 1990 in Saco Bay. Although the reproductive response of ME *C. canadensis* under daylengths >14 h at 15°C was not tested, we predict that developing copepodites and reproductive-resting females would become reproductive as found for MD copepodites under longer daylengths (>12 h). These findings would explain the lack of concordance between ME naupliar recruitment found in the field and our laboratory results at 15°C. The result showing that reproduction in MD *C. canadensis* at 15°C is tightly linked to light regime explains a similar lack of concordance previously found by Lonsdale (1981b). It may also be possible that reproductive-resting females break this stage after a given period of time without changes in environmental cues (e.g., as found in *Mesocyclops edax* diapausing individuals; Wyngaard, 1988), although after 90 days, reproductive-resting females maintained at 15°C did not begin reproduction.

Population differences in the absolute period of daylength related to the induction of the reproductive-resting response may reflect a shorter period when environmental conditions are conducive for growth and reproduction in ME copepods compared to MD. Latitudinal variation in the length of the growing season may be the selective force driving genetically based differences in growth rate among fish populations (Conover and Present, 1990; Conover, 1991), and diapause egg production in *Labidocera aestiva* populations (Marcus, 1984). *Coullana canadensis* nauplii first appear in appreciable numbers in April in Chesapeake Bay and May in the Saco River when daylength periods exceed 12 h and 14 h, respectively (Fig. 5; Heinle et al., 1977; Lonsdale, 1981b; Lonsdale and Levinton, 1985). It is also likely that the demise of growing conditions occurs earlier in ME, and is suggested by the observation that at 21°C and a 14:10 h light:dark cycle, a percentage of ME females entered the reproductive-resting state, whereas all MD females became reproductive. A similar trend regarding temperature and photoperiod influences on diapause egg production was found in populations of *L. aestiva* collected from Massachusetts to North Carolina (Marcus, 1984). Moreover, copepods collected from Florida, which exhibit a year-round pattern of abundance, had lost the genetic capacity to produce diapause eggs. Our result suggesting more genetic similarity of NY and ME copepods compared to MD is similar to that found for *Fundulus heteroclitus* in which mtDNA analyses revealed a northern and southern genetic assemblage, and that the transition occurred between northern and southern New Jersey. In this example, temperature adaptation and past geological events may explain this pattern (Gonzalez-Villasenor and Powers, 1990).
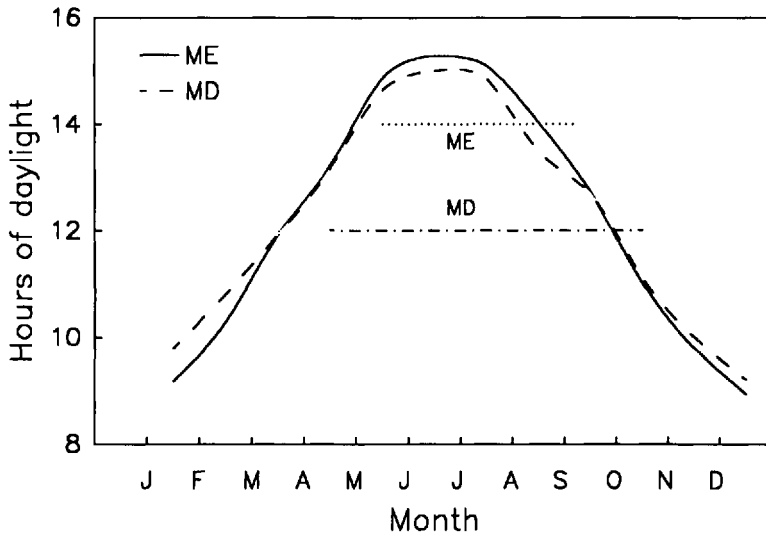
Figure 5. Hours of daylight for ME and MD locations (solid and dashed lines, respectively), and probable reproductive periods of *Coullana canadensis* (dotted line, 14-h y-intercept and dashed-dotted line, 12-h y-intercept, respectively). Mid-month daylengths were obtained from 1989 Tide Tables, NOAA.

Resting states may also occur in response to predation (Hairston and Munns, 1984), and as previously stated, invertebrate predation is important in regulating *Coullana canadensis* populations in Chesapeake Bay during summer, especially in August and September. Why then do copepods continue to reproduce at higher summer temperatures (25–28°C; Lonsdale, 1981b; Lonsdale and Levinton, 1986)? Selection for a resting stage earlier in the season in MD or other locales may not be strong because of unpredictable summer salinities, which when reduced will favor reproductive females of *C. canadensis* (Heinle et al., 1977). Continued summer reproduction may also be a consequence of physiological or chemical constraints at higher water temperatures which preclude a reproductive-resting state. Chemical analyses of a summer, encysted harpacticoid, however, also showed wax ester accumulation (Williams-Howze, 1992), and thus, this latter constraint is less probable.

We have established that the reproductive-resting trait in *Coullana canadensis* has a genetic basis, and that differences in its expression exist among populations. Seasonal changes in genotypic frequencies within a population cannot explain the geographic differences. *Coullana canadensis* was collected from 1983–1990 (except 1989) in ME over several months (May to July), under a variety of environmental conditions (13–20°C and 9–18‰), and a high percentage of copepods from all collections have expressed the reproductive-resting trait at 15°C and a 14:10 light : dark cycle (e.g., for the 1983 collection, the percentage of reproductive females ranged from 0–18%; Lonsdale and Levinton, 1986, 1989, this study). Prior to 1983 (1981 and 1982), the same temperature and light regime presented no problems for obtaining sufficient gravid ME females for experimentation (Lonsdale and Levinton, 1985), but reproductive percentage data are not available. For MD copepods, longer-term changes in genotypic frequencies have not adequately been documented, but the same life-history response of no reproduction at 15°C and a 12:12 h light : dark cycle was also observed in collections made from ~1976–

1978 (Lonsdale, 1981a). Overall, however, these findings suggest both short- and longer-term evolutionary stability regarding the expression of the reproductive-resting stage in *C. canadensis*.

Modulation of the reproductive response in *Coullana canadensis* by environmental factors has been shown in this study. The physiological control mechanisms of reproduction, however, remain unknown. In polychaetes at least, the endocrine system may control reproduction in several ways, including the regulation of gametocyte development and energy flow for reproduction (see Olive, 1985 for review). It is likely that the trait we studied in *C. canadensis* controls the sensitivity of the neuroendocrine system to daylength and temperature, which in turn regulates energy usage and ova production. Knowledge of the nature of the biochemical linkage between the genetic and physiological variation found among *C. canadensis* populations, and the relationship to individual fitness would contribute to our understanding of evolutionary processes in marine invertebrates (Koehn, 1987; Powers, 1987).

## LITERATURE CITED

Conover, D. O. 1991. The relation between capacity for growth and length of growing season: evidence for and implications of countergradient variation. Trans. Am. Fish. Soc. 119: 416–430.
——— and T. M. C. Present. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia 83: 316–321.
Coull, B. C. 1972. *Scottolana canadensis* (Willey, 1923) (Copepoda, Harpacticoida) redescribed from the United States East Coast. Crustaceana 22: 210–214.
——— and B. W. Dudley. 1985. Dynamics of meiobenthic copepod populations: a long-term study (1973–1983). Mar. Ecol. Prog. Ser. 24: 219–229.
——— and J. Grant. 1981. Encystment discovered in a marine copepod. Science 212: 342–344.
Donaghay, P. L. 1988. Role temporal scales of acclimation, food quality and trophic dominance in controlling the evolution of copepod feeding behavior. Bull. Mar. Sci. 43: 469–485.
Durbin, E. G., A. G. Durbin, T. J. Smayda and P. G. Verity. 1983. Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnol. Oceanogr. 28: 1199–1213.
Frost, B., M. R. Landry and R. P. Hassett. 1983. Feeding behavior of large calanoid copepods *Neocalanus cristatus* and *N. plumchrus* from the subarctic Pacific Ocean. Deep-Sea Res. 30: 1–13.
Gallager, S. M. and R. Mann. 1981. Use of lipid-specific staining techniques for assaying condition in cultured bivalve larvae. J. Shellfish Res. 1: 69–73.
Gonzalez-Villasenor, L. I. and D. A. Powers. 1990. Mitochondrial-DNA restriction-site polymorphisms in the teleost *Fundulus heteroclitus* support secondary intergradation. Evolution 44: 27–37.
Guillard, R. 1975. Culture of phytoplankton for feeding marine invertebrates. Pages 29–69 *in* W. L. Smith and M. H. Chanley, eds. Culture of marine invertebrate animals. Plenum Press, New York.
Hairston, N. G., Jr. and W.R. Munns, Jr. 1984. The timing of copepod diapause as an evolutionary stable strategy. Am. Nat. 123: 733–751.
——— and E. J. Olds. 1987. Population differences in the timing of diapause: a test of hypotheses. Oecologia 71: 339–344.
Heinle, D. R., H. S. Millsaps and C. V. Millsaps. 1977. Effects of tropical storm Agnes on standing crops and age structure of zooplankton in middle Chesapeake Bay. Chesapeake Research Consortium No. 54: 443–459.
Koehn, R. K. 1987. The importance of genetics to physiological ecology. Pages 170–185 *in* M. E.

Feder, A. F. Bennett, W. W. Burggren and R. B. Huey, eds. New directions in ecological physiology. Cambridge University Press, New York.

Lonsdale, D. J. 1981a. Influence of age-specific mortality on the life history traits of two estuarine copepods. Mar. Ecol. Prog. Ser. 5: 333–340.

———. 1981b. Regulatory role of physical factors and predation for two Chesapeake Bay copepod species. Mar. Ecol. Prog. Ser. 5: 341–351.

——— and S. H. Jonasdottir. 1990. Geographic variation in naupliar growth and survival in a harpacticoid copepod. Biol. Bull. 179: 113–120.

——— and J. S. Levinton. 1985. Latitudinal differentiation in embryonic duration, egg size, and newborn survival in a harpacticoid copepod. Biol. Bull. 168: 419–431.

——— and ———. 1986. Growth rate and reproductive differences in a widespread estuarine harpacticoid copepod. Mar. Biol. 91: 231–237.

——— and ———. 1989. Energy budgets of latitudinally separated *Scottolana canadensis* (Copepoda: Harpacticoida). Limnol. Oceanogr. 34: 324–331.

———, ——— and S. Rosen. 1988. Reproductive compatibility among populations of a widespread harpacticoid copepod. Hydrobiologia 167/168: 469–476.

Malone, T. C., L. H. Crocker, S. E. Pike and B. W. Wendler. 1988. Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. Mar. Ecol. Prog. Ser. 48: 235–249.

Marcus, N. 1979. On the population biology and nature of diapause of *Labidocera aestiva* (Copepoda: Calanoida). Biol. Bull. 157: 297–305.

———. 1984. Variation in the diapause response of *Labidocera aestiva* (Copepoda: Calanoida) from different latitudes and its importance in the evolutionary process. Biol. Bull. 166: 127–139.

Naess, T. and J. P. Nilssen. 1991. Diapausing fertilized adults. Oecologia 86: 368–371.

Ohman, M. D., J. M. Bradford and J. B. Jillett. 1989. Seasonal growth and lipid storage of the circumglobal, subantarctic copepod, *Neocalanus tonsus* (Brady). Deep-Sea Res. 36: 1309–1326.

Olive, P. J. W. 1985. Physiological adaptation and the concepts of optimal reproductive strategy and physiological constraint in marine invertebrates. Pages 267–300 *in* M. S. Laverack, ed. Physiological adaptations of marine animals. Symposia of the Society for Experimental Biology, No. 39. The Company of Biologists Limited, Cambridge, England.

Por, F. D. 1984. Canuellidae Lang (Harpacticoida, Polyarthra) and the ancestry of the copepods. Crustacean Suppl. 7. Studies on Copepoda II: 1–24.

Powers, D. A. 1987. A multidisciplinary approach to the study of genetic variation within species. Pages 102–130 *in* M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey, eds. New directions in ecological physiology. Cambridge University Press, New York.

Sargent, J. R. and R. J. Henderson. 1986. Lipids. Pages 58–107 *in* E. D. S. Corner, and S. C. M. O'Hara, eds. The biological chemistry of marine copepods. Clarendon Press, Oxford.

Smith, L. D. and B. C. Coull. 1987. Juvenile spot (Pisces) and grass shrimp predation on meiobenthos in muddy and sandy substrata. J. Exp. Mar. Biol. Ecol. 105: 123–136.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. WH Freeman, San Francisco. 859 pp.

Strathmann, R. R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12: 411–418.

Sullivan, B. K. and L. T. McManus. 1986. Factors controlling seasonal succession of copepods *Acartia hudsonica* and *A. tonsa* in Narragansett Bay, Rhode Island: temperature and resting egg production. Mar. Ecol. Prog. Ser. 28: 121–128.

Townsend, D. W. 1984. Comparison of inshore zooplankton and ichytyoplankton populations of the Gulf of Maine. Mar. Ecol. Prog. Ser. 15: 79–90.

Willey, A. 1923. Notes on the distribution of free-living Copepoda in Canadian waters. Contr. Can. Biol. (5), 1: 303–324.

Williams-Howze, J. 1992. Ecology and functional morphology of *Heteropsyllus nunni* (Crustacea: Copepoda), with a review of diapause in the orders of Cyclopoida, Calanoida, and Harpacticoida. Ph.D. Thesis, University of South Carolina. 184 pp.

——— and B. C. Coull. 1992. Are temperature and photoperiod necessary cues for encystment in the marine benthic harpacticoid copepod *Heteropsyllus nunni* Coull? Biol. Bull. 182: 109–116.

Wyngaard, G. 1988. Geographical variation in dormancy in a copepod: evidence from population crosses. Hydrobiologia 167/168: 367–374.

ADDRESS:  *(D.J.L., P.W.) Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, New York 11794-5000; (F.C.D.) Department of Oceanography, University of Hawaii at Manoa, Honolulu, Hawaii 96822.*