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Photoperiod and temperature regulation of diapause egg production in *Acartia bifilosa* from Southampton Water

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ABSTRACT: In Southampton Water the copepod *Acartia bifilosa* presents a diapause reproductive strategy, where there is a switch from subitaneous to diapause egg production around May, prior to the species' disappearance from the water column between June and October. The effect of temperature and photoperiod on the production of diapause eggs by *A. bifilosa* was studied in an attempt to determine the primary cues for its summer diapause. A parallel study on the effect of temperature on metabolic efficiency of *A. bifilosa* and the non-diapause species *A. discaudata*, defined by the species' 'scope for growth' (SfG), was examined as a potential, ultimate reason behind the diapause stage. Photoperiod was identified as the primary proximate cue that induced diapause in *A. bifilosa*, and this response was temperature-mediated. Diapause was triggered by a 13:11 h light:dark photoperiod (day length), corresponding to a late-April photoperiodic regime, and resting eggs were produced even at temperatures as low as 5°C. A very low number of diapause eggs were, however, also produced after 6 d at a 12:12 h light:dark photoperiod at elevated temperatures between 14 and 20°C, but the mean percentage produced was significantly less ($p < 0.05$) than under the longer day lengths. The ultimate cause of the over-summering strategy of *A. bifilosa* is currently unknown, but the SfG assay indicated that at 10°C, SfG was twice that at 20 or 5°C, and so it may diapause to avoid the higher temperatures in summer. This pattern contrasted with the SfG of *A. discaudata*, which suggested a simple, positive relationship with temperature. In the field, competition is greatly reduced in the winter months, so *A. bifilosa* has a better chance of survival, even with its lower SfG.

KEY WORDS: *Acartia bifilosa* · Diapause · Egg production · Photoperiod · Scope for growth

INTRODUCTION

The abundance and even presence in the water column of most copepod species varies on a seasonal basis, a pattern which is most typical in temperate coastal waters, where environmental fluctuations are greater than in tropical or open ocean waters (Grice & Marcus 1981). Diapause eggs have been documented in several temperate neritic copepods (Zillioux & Gonzalez 1972, Grice & Gibson 1975, 1977, Kasahara & Uye 1979, Johnson 1980) as a method of sustaining a population through adverse environmental conditions, and providing life-cycle stability by allowing a species' life-cycle to synchronise with the environment's seasonal rhythm (Alekseev & Starobogatov 1996).

Within Southampton Water, on the south coast of the UK, the calanoid copepod *Acartia bifilosa* appears in the water column from November/December to June (Conover 1957, Raymont & Carrie 1964), and diapauses over summer (Castro-Longoria & Williams 1999). It is common in many European estuaries (Irigoien & Castel 1995, Sautour & Castel 1995) and is also known to over-summer as the egg phase in the Baltic (Viitasalo 1992). Despite these studies, very little is known about its diapause behaviour, in particular the initial cue, and the ultimate reason behind this life-

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style strategy. Castro-Longoria & Williams (1999) reported that Southampton Water *A. bifilosa* produced 2 morphologically distinct egg-types: a smooth subitaneous egg and a diapause egg that is characteristically covered in spines. Subitaneous eggs are laid during the first months of the copepod's presence in the water column, with diapause eggs produced only in the 2 month period prior to the disappearance of *A. bifilosa* from the water column between June and October. It is unusual for a marine species to over-summer, because it is a costly strategy that removes the species from the water column at a time that is generally considered most advantageous in terms of food availability.

The ultimate cause of such seasonality is unknown, possibly it is an attempt to avoid higher temperatures, a food bottleneck (Santer & Lampert 1995), overcrowding (Ban & Minoda 1994) or predators (Slusarczyk 1995). The cause is unlikely to be food scarcity because the phytoplankton bloom starts in March/April and extends until autumn (Kifle 1992). *Acartia bifilosa* diapause coincides with the maximum population density of gelatinous predators *Aurelia aurita* and *Pleurobranchia pileus* in Southampton Water (Lucas & Williams 1995). *A. bifilosa* is less fecund than other members of the genus, and egg-hatching times are also longer (Castro-Longoria 1998), so *A. bifilosa* would clearly have a reduced capacity for recovery from predator impact. However, real-time cues, like the presence of predators in the water column, would come too late for *A. bifilosa* to avoid the threat by the production of diapause eggs. Clearly, it must rely on proximate cues that provide a reliable forecast of future conditions (Slusarczyk 1995). Temperature is one of the most commonly recognised cues for controlling copepod diapause (Uye 1985, Viitasalo 1992, Ban & Minoda 1994), but photoperiodic effect has only been studied comprehensively in *Labidocera aestiva* (Marcus 1979, 1980, 1982). In contrast to *A. bifilosa*, *L. aestiva* presents a conventional winter diapause. Laboratory investigations showed that short days (8:16 h light:dark) resulted in *L. aestiva* diapause egg production at all temperatures (Marcus 1979, 1980, 1982).

The potential for photoperiod to play a significant part in inducing *Acartia bifilosa* diapause egg production will be examined in this paper. Considering reproductive regulators, Castro-Longoria & Williams (1999) hypothesised that *A. bifilosa* fecundity might also be limited to some degree by temperature, such that adult intolerance of higher temperatures may be the cause of this species' unusual reproductive strategy. In an attempt to explain the ultimate cause behind *A. bifilosa* summer diapause, and as a way of understanding the copepod's winter performance, the ability of adult *A. bifilosa* to cope with temperature stress will be looked at using the scope for growth (SfG) assay, based on the energy equation of Winberg (1960). The SfG is a measure of the energy balance between the energy assimilated and the metabolic output of an individual. It is used to give an indication of how much energy is available for growth and reproduction, both key components of fitness (Bayne et al. 1985). This parallel study to the photoperiod investigation will highlight the copepod's metabolic performance and the amount of energy available to the copepod at temperatures reflecting the conditions of winter, spring/autumn and summer. The SfG results will be compared with those of *A. discaudata*, a species that has a very similar spatial distribution to that of *A. bifilosa* within Southampton Water. In contrast to *A. bifilosa*, it does not diapause over summer or winter, and is present in Southampton Water all year round, with maximum density in summer and very low numbers during winter (Castro-Longoria 1998). The SfG assay will indicate the optimum temperature for *A. bifilosa* and *A. discaudata*.

MATERIALS AND METHODS

Day length and temperature were tested as potential exogenous stimuli controlling the reproductive shift of *Acartia bifilosa* from subitaneous to diapause egg production. During March, *A. bifilosa* samples were collected from 1 to 5 m depth at Calshot buoy, at the mouth of Southampton Water, using a 120 µm mesh net. *Acartia* spp. do not show apparent diel vertical migration but are known to be highly concentrated in the surface layers in the afternoon (Checkley et al. 1992). Ambient temperature, light and salinity conditions were 6°C, a 12:12 h light:dark cycle, and 33 respectively, and females in the field were not producing diapause eggs (Castro-Longoria & Williams 1999).

Temperature and photoperiod regulation of *Acartia bifilosa* diapause egg production. The effect of temperature and photoperiod on *A. bifilosa* diapause egg production was investigated using experimental incubation regime combinations of 5, 10, 14 and 18°C and 12:12, 13:11 and 14:10 h light:dark photoperiods. Experiments were conducted at low light levels approximating field light-intensity levels. Experimental populations of 20 females were introduced immediately, without prior acclimation, into individual incubation chambers (Chinnery 2002) maintained at each of the light/temperature regimes. Individuals were not acclimated to experimental temperature and photoperiod conditions, as the intention was to examine the rapidity of any change to diapause egg production. The presence or absence of males did not influence fecundity or egg viability of female *A. clausi* collected from the field (Ianora et al. 1996), and so none were introduced to the incubation chambers. These cham-

bers were plastic cylinders closed at one end with a 200 µm mesh, and supported in a 1 l beaker filled with 600 ml of 26 µm filtered seawater (30 to 32 salinity). Three replicates were conducted for each incubation regime. Food was provided immediately and then at 2 d intervals in the form of 10 ml of *Isochrysis galbana* in its exponential growth phase to give excess food concentrations of 1×10^5 cells ml^{-1}. This concentration was comparable to other feeding studies (Ianora et al. 1996, Castro-Longoria & Williams 1999), and in excess of the maximum ingestion rates reported for *Acartia* sp. (Nival & Nival 1976). During the 8 d experimental period, eggs were collected every 2 d. The incubation cylinders containing the copepods were carefully removed, and the chamber water was filtered through a 45 µm mesh to capture the eggs. The cylinders were then put into another incubation chamber containing clean, preacclimated water and fresh food. Eggs were examined using a binocular microscope and the diapause:subitaneous egg ratio produced at each 2 d interval was recorded. This facilitated comparisons between samples and replicates. After collection, both subitaneous and diapause eggs were stored in large petri dishes in 20 ml of seawater (30 to 32 salinity) at 18°C for a further 8 d and hatch success was monitored.

Scope for growth assay. *Acartia bifilosa* and *A. discaudata* sub-samples were also immediately placed in feeding chambers, similar to the incubation chambers, at 5°C, which reflected field ambient-temperature, and at a light regime of 12:12 h light:dark. They were conditioned to the experimental *Isochrysis* food source of ~1×10^5 cells ml^{-1} d^{-1} for 24 h (Tester & Turner 1990). Individuals were taken from these sub-samples to determine individual dry weight for adult female *A. bifilosa* and *A. discaudata*. Before conducting any part of the SfG assay, all experimental animals were preacclimated for 24 h to the appropriate experimental temperature of 5 (winter), 10 (spring/autumn) or 20°C (summer) at a light regime of 12:12 h light:dark. The temperatures were taken from a year-long sampling programme conducted in Southampton Water (2001–2002), and each is representative of its season.

The 'cell count method' (Gauld 1951, Frost 1972) was used to determine feeding rate. Five replicates of *Acartia bifilosa* and *A. discaudata* were conducted for each experimental temperature. Five adult females were placed in individual 500 ml vials of 26 µm filtered seawater at 32 salinity with *Isochrysis* added to give an initial food concentration of ~1×10^5 cells ml^{-1}. The vials were maintained in constant darkness (Checkley et al. 1992, Irigoien et al. 1993, Irigoien & Castel 1995) and at experimental temperatures for 24 h on a plankton wheel set at 0.5 revolutions min^{-1}. The copepods were then carefully pipetted into fresh vials containing *Isochrysis* and run over 6 h. The concentration of algal cells in standard and running controls, together with the experimental vials, were determined and feeding rate was converted, by the derived calorific value of the algae 1.09×10^{-6} J $cell^{-1}$, to express feeding as J mg dry wt^{-1} d^{-1}.

Assimilation efficiency was calculated using the work of Conover (1966). Three replicate experimental populations of 25 *Acartia discaudata* or 25 *A. bifilosa*, preacclimated to experimental temperatures and experimental diet, were each placed in individual feeding chambers for 24 h, and samples of *Isochrysis* and faecal pellets were dried (60°C for 24 h), weighed and ashed (500°C for 3 h) to determine assimilation efficiency.

Respiration rates of preacclimated and fed experimental populations of either 5 *Acartia bifilosa* or *A. discaudata* females were measured over 6 h using the Winkler procedure (Crisp 1971, Lucas 1996) for determination of dissolved oxygen. Oxygen consumption, initially expressed as ml O_2 l^{-1} mg dry wt^{-1} d^{-1}, was converted (Crisp 1971, Bayne et al. 1985, Lucas 1996) to give J mg dry wt^{-1} d^{-1}. Five replicates were conducted for each species at each experimental temperature.

The copepods were assumed to be ammonotelic, such that the losses of organic matter through metabolism of protein were estimated from the rate of ammonium excretion (Abou Debs 1984, Checkley et al. 1992), as ammonia constitutes 80 to 90% of excreted nitrogen in *Acartia tonsa* (Kiørboe et al. 1985). The experiments were conducted in the light, as copepod ammonium excretion was maximal during the daytime (Checkley et al. 1992). Excretion rate was measured spectrophotometrically as dissolved NH_3-N following Solorozano (1969). Five experimental replicates of 10 preacclimated and fed adult females of both *Acartia bifilosa* and *A. discaudata* were run for 6 h. Excretion was initially expressed as µg NH_4-N mg dry wt^{-1} d^{-1} and then converted (Elliott & Davison 1975, Bayne et al. 1985, Lucas 1996) to J mg dry wt^{-1} d^{-1}.

Significance was tested by ANOVA and set at the $p < 0.05$ level. Assimilation efficiency was analysed by *t*-test and significance set at $p < 0.01$ level.

RESULTS

Temperature and photoperiod regulation of *Acartia bifilosa* diapause egg production

There was a clear threshold photoperiod of 13:11 h light:dark, equivalent to a late April natural photoperiod, which initiated an increase in the number of diapause eggs laid (Table 1). Copepods at the threshold photoperiod also produced far fewer eggs (45 to 97)

Table 1. *Acartia bifilosa*. Mean percentage of diapause eggs produced (±1 SD). Percentage data are not cumulative. –: no reading taken; n: total number of eggs produced from all replicates over each 2 d experimental period

Photoperiod (h light:dark)	Day	Temperature (°C)			
		5	10	14	18
12:12	2		–	–	–
	4		0 (n = 30)	0 (n = 72)	0 (n = 44)
	6		0 (n = 63)	1.1 ± 1.91 (n = 72)	1.43 ± 2.48 (n = 53)
	8		0 (n = 30)	23.0 ± 39.8 (n = 50)	14.4 ± 24.8 (n = 30)
Total number of eggs produced			123	194	127
13:11	2	31.9 ±18.0 (n = 28)	43.9 ± 2.84 (n = 36)	41.2 ± 5.12 (n = 49)	32.7 ± 18.9 (n = 30)
	4		–	–	–
	6	93.3 ± 11.5 (n = 14)	77.6 ± 25.4 (n = 39)	61.1 ± 9.64 (n = 11)	61.1 ± 9.64 (n = 9)
	8	93.3 ± 11.5 (n = 10)	100 ± 0.00 (n = 22)	83.3 ± 28.9 (n = 6)	91.7 ± 14.4 (n = 6)
Total number of eggs produced		52	97	66	45
14:10	2		13.7 ± 20.7 (n = 68)	3.7 ± 6.4 (n = 63)	10.6 ± 15.7 (n = 125)
	4		41.3 ± 11.5 (n = 76)	40.6 ± 4.9 (n = 55)	46.3 ± 11.6 (n = 22)
	6		60.2 ± 18.7 (n = 84)	63.9 ± 25.5 (n = 48)	54.9 ± 4.6 (n = 39)
	8		65.5 ± 14.2 (n = 158)	67.3 ± 19.9 (n = 54)	84.1 ± 9.3 (n = 43)
Total number of eggs produced			386	220	229

during the 8 d experimental period than experimental populations placed under a 12:12 (123 to 194) or 14:10 (220 to 386) h light:dark period. Even allowing for the missed sampling period on Day 4, the total number of eggs produced at a 13:11 h light:dark period at each temperature was less than a third of that at the other photoperiods. Adult mortality was negligible at all temperatures (<5%), and showed no particular pattern. *Acartia bifilosa* egg production rates in all of the replicates ranged from 0.15 eggs female^{-1} d^{-1} to 3.95 eggs female^{-1} d^{-1}.

The total number of eggs removed from an incubation chamber was determined for most, but not all, 2 d periods. The number of eggs produced in a 2 d period ranged from 6 to 158 eggs (Table 1), but there was no significant or consistent pattern with regard to the experimental incubation temperature, although the higher, 18°C, incubation usually produced the lowest number of eggs under each photoperiod tested. The range in egg numbers produced had no obvious influence on the proportion of subitaneous and diapause eggs. There was an immediate shift in egg-type production between the 12 and 13 h day lengths (Table 1), and after only 2 d there was a clear and significant ($p < 0.05$) difference between the mean percentage of diapause eggs produced under a 12:12 and 13:11 h light:dark period at all temperatures.

Temperature appeared to influence the egg-type response, as, even under a 12:12 h light:dark period, a small number of diapause eggs were produced after 8 d at the higher temperatures of 14 and 18°C (Table 1). The 2 d mean percentage of diapause eggs produced at this photoperiod was, however, significantly less ($p < 0.05$) than that produced under 13:11 and 14:10 h light:dark periods. At all temperatures tested, the percentage of diapause eggs produced at 13:11 and 14:10 h light:dark periods increased over the period of investigation, and after 8 d, diapause egg production was 100% in some replicates. After 8 d under a 13:11 h light:dark period, the difference in the mean percentages of diapause eggs produced at each temperature was not statistically significant (ANOVA $F_{3,8} < 1$). The diapause response was not overtly temperature-mediated at this sensitive photoperiod because spiny diapause eggs were produced even at temperatures as low as 5°C. Equally, although the mean percentages of photoperiod-collated diapause egg production after 8 d at 10, 14 and 18°C were 55.2 ± 44.6, 57.9 ± 37.9 and 63.3 ± 39.9%, respectively (Fig. 1), the results were not statistically significant (ANOVA $F_{2,24} < 1$).

The types of egg produced were affected primarily by photoperiod (Fig. 2), as there was no significant temperature effect within each photoperiod regime, even at the threshold day length of 13:11 h light:dark (Table 1). The temperature data were collated (Fig. 2) to highlight the significance of the photoperiod. The resulting mean percentage of diapause egg production over 8 d at light:dark photoperiods of 12:12, 13:11 and 14:10 h (temperature-collated data) were 12.4 ± 25.5, 92.1 ± 15.9 and 72.3 ± 15.8% respectively, and were significantly different (ANOVA $F_{2,24} = 5.61$, $p < 0.05$). The 13:11 h light:dark regime was clearly the threshold photoperiod, presenting the highest percentage of diapause eggs produced after 8 d, with similar results for the 14:10 h light:dark experimental programme.

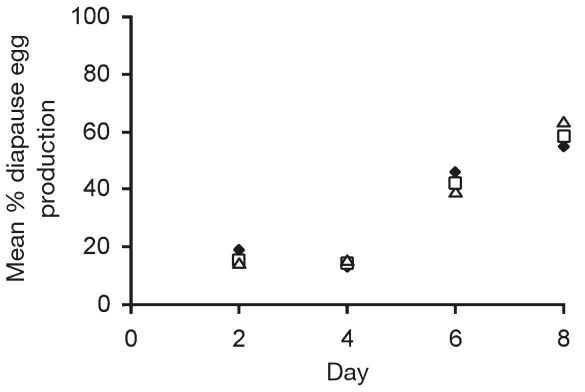

Fig. 1. *Acartia bifilosa.* Effect of temperature on diapause egg production (photoperiod-collated data). Photoperiod data (Table 1) have been combined and a single mean determined for each temperature (◆ = 10, □ = 14, and △ = 18°C)

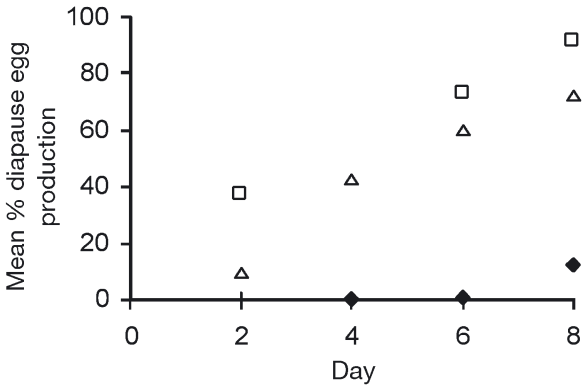

Fig. 2. *Acartia bifilosa.* Diapause egg production (temperature-collated data). Temperature data (Table 1) have been combined and a single mean determined for each photoperiod (◆ = 12:12, □ = 13:11, and △ = 14:10 h light:dark photoperiods)

Inevitably, a mixture of subitaneous and diapause eggs was collected from each incubation chamber, and so it was not clear whether, like *Labidocera aestiva* females (Marcus 1982), individual *Acartia bifilosa* females can produce both smooth and spiny eggs at the same time, or if the switch was a complete one with females either producing only diapause eggs or subitaneous eggs. There was no evidence, however, of the production of a spiny subitaneous egg as reported by Castro-Longoria & Williams (1999). The vast majority of subitaneous *A. bifilosa* eggs hatch within 5 d under good temperature and salinity conditions (>10°C and >25 salinity) (Castro-Longoria & Williams 1999). None of the 'spiny' eggs collected hatched within the 8 d observation period, suggesting the absence of this egg-type.

Scope for growth assay

Considering the metabolic performance of the congeners, *Acartia discaudata* ingestion rate clearly increased with increasing temperature (Table 2), suggesting that it was more active at higher temperatures, and its feeding rate at 20°C was significantly greater ($p < 0.05$) than at 5°C. In contrast, *A. bifilosa* ingested more food at 10°C, although the difference in feeding rate recorded at each experimental temperature was not significant. Comparing the 2 congeners, there was no difference in feeding rate at 5°C, but *A. bifilosa* had a significantly ($p < 0.01$) higher feeding rate at 10°C, whereas *A. discaudata* ingested significantly ($p < 0.01$) more food at 20°C.

There was a clear difference in the percent assimilation measured for *Acartia bifilosa* (Table 2), with a significantly ($p < 0.01$) lower assimilation percentage of 64.9% at 20°C, compared with the highest assimilation efficiency, 87.25%, recorded at 10°C. Although the percent assimilation of *A. discaudata* consistently increased with temperature, from 73.4% at 5°C to 82.5% at 20°C, there was no significant difference between these values. The only significant inter-species difference was at 20°C, where *A. discaudata* had the higher ($p < 0.01$) assimilation efficiency. Reflecting ingestion rate and assimilation efficiency patterns (Table 2), the amount of energy assimilated increased at each species' optimum temperature of 10 and 20°C for *A. bifilosa* and *A. discaudata* respectively (Fig. 3).

Table 2. *Acartia bifilosa* and *A. discaudata* scope for growth (mean ± 1 SD) at 5, 10 and 20°C. T: temperature; C: energy ingested; A (%): assimilation efficiency; A: energy assimilated; R: energy respired; U: energy excreted; P: scope for growth; T, A, R and U values all in J mg dry wt^{-1} d^{-1}

Species	T (°C)	C	A (%)	A	R	U	P
A. bifilosa	5	30.01 ± 9.27	79.3 ± 4.20	23.80	17.47 ± 8.18	0.043 ± 0.003	6.28
	10	38.76 ± 6.36	87.2 ± 5.09	33.80	18.08 ± 7.31	0.044 ± 0.003	15.67
	20	35.01 ± 12.7	64.9 ± 2.84	22.72	15.42 ± 6.48	0.058 ± 0.007	7.24
A. discaudata	5	27.85 ± 9.55	73.4 ± 3.80	20.44	15.66 ± 1.38	0.038 ± 0.001	4.74
	10	31.50 ± 7.26	80.8 ± 2.85	25.45	17.28 ± 4.26	0.046 ± 0.011	8.13
	20	42.50 ± 6.78	82.5 ± 2.61	35.06	12.97 ± 8.10	0.048 ± 0.003	22.04

For both species, respiration was responsible for the majority of the energy 'used' within the SfG definition (Table 2), representing 53.5 to 73.4% of the energy assimilated for *Acartia bifilosa*, and 37.0 to 76.6% for *A. discaudata*, corresponding to 15.42 to 18.08 and 12.97 to 17.28 J mg dry wt^{-1} d^{-1} respectively. Unusually, both congeners lost most energy through respiration at 10°C, and the least energy at 20°C. For *A. bifilosa* this followed a pattern reflecting its feeding and assimilation performance. However, there was no statistically significant difference between the intra- or interspecific respiration rates recorded at the experimental temperatures (Fig. 3).

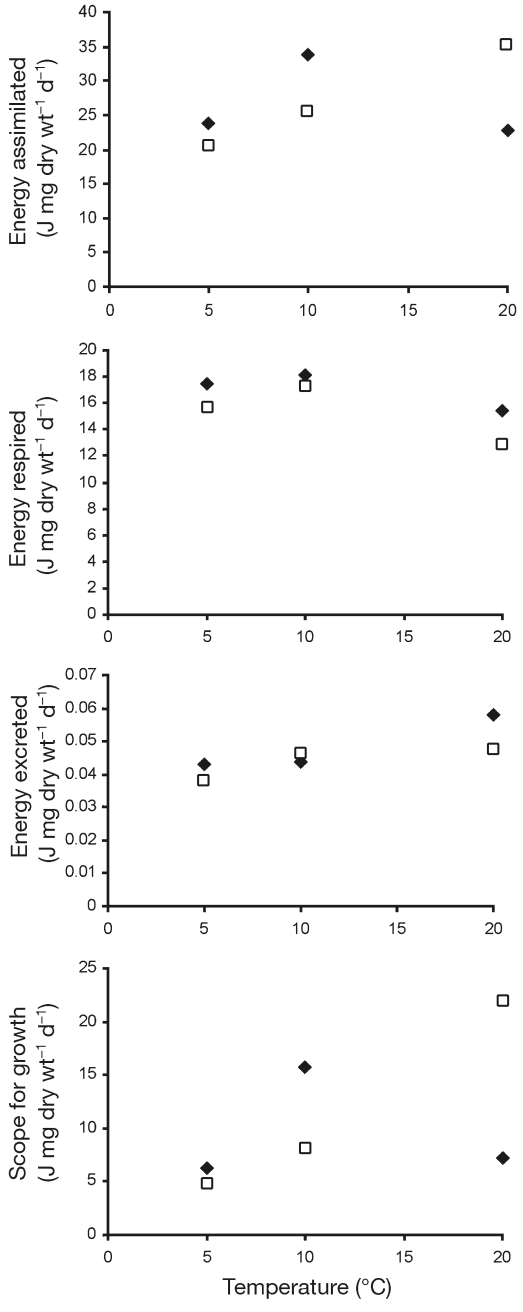

Fig. 3. *Acartia bifilosa* and *Acartia discaudata*. Scope for growth response to temperature (◆ = *A. bifilosa*, □ = *A. discaudata*)

As temperature increased, so did the excretion rate of both *Acartia bifilosa* and *A. discaudata* (Table 2), producing an increase of 0.043 to 0.058 J mg dry wt^{-1} d^{-1} and from 0.038 to 0.048 J mg dry wt^{-1} d^{-1} respectively. Again there were no inter- or intraspecific significant differences in the excretion rate measured at the 3 temperatures.

Considered as a single parameter, the SfG of an individual should increase as the 'environment' approaches optimum. Although it was not possible to test the results statistically, it was clear that *Acartia bifilosa* had a substantially higher SfG at 10°C, while the SfG of *A. discaudata* was consistently positively correlated with temperature, so that at 20°C it was higher than at 5 and 10°C (Fig. 3). With no significant differences within the excretion or respiration results, the SfG was obviously more dependent on the amount of food ingested, and ultimately the amount of energy assimilated by the individual. Indeed, the temperature-induced pattern of SfG most closely mirrored the pattern of the amount of energy assimilated (Fig. 3), suggesting that it, of all the energy-parameters measured, is the best indicator of stress.

DISCUSSION

There are a very limited number of critical experimental investigations into the effect of photoperiod on copepod diapause (Marcus 1979, 1980, 1982). The summer diapause response of Southampton Water *Acartia bifilosa* is triggered by a photoperiod change between 12:12 and 13:11 h light:dark schedules, and this is comparable to the light regime it would experience during the summer. *A. bifilosa* produces diapause eggs from May to July, before it disappears from the water column until November (Castro-Longoria 1998). Unlike *Labidocera aestiva*, which took 1 to 2 wk to fully adjust from subitaneous to diapause egg production (Marcus 1982), *A. bifilosa* diapause response is relatively rapid. Animals taken directly from the field, where they were subject to an ambient 11:13 h light:dark regime, and given no time to acclimatise to laboratory conditions, produce 37% diapause eggs within 2 d at a 13:11 h light:dark regime. Diapause egg production continues to increase dramatically over the period of the experiment, reaching 100% after only 8 d in some replicates. Some, if not all, of the *A. bifilosa* females can therefore switch the type of egg they produce very quickly. Total egg production, although low compared with some laboratory

studies, compares well with the *A. bifilosa* egg production rate values for March of 3.1 ± 1.3 eggs female^{-1} d^{-1} reported by Castro-Longoria (1998). Egg production was not monitored for individual females, and the eggs collected were a mixture of subitaneous and diapause types, so it is unclear whether, like *L. aestiva* (Marcus 1982) and *Anomalocera patersoni* (Ianora & Santella 1991), *A. bifilosa* has a transitional phase where both egg types are produced, or if it has an all-or-nothing response. The latter is unlikely however, as experimentally induced diapause (Table 2) was clearly influenced by a cumulative input of long day-length cycles, producing an increase in the number of diapause eggs over time.

Photoperiod is often accompanied by other, secondary factors governing diapause, and temperature generally plays a part in diapause induction in terrestrial insects, but its role for zooplankton is less consistent (Grice & Marcus 1981, Hairston et al. 1990). Marcus (1979) found a strong correlation between temperature and the type of egg produced by *Labidocera aestiva*, but the compensatory role of temperature is equivocal for *Acartia bifilosa.* At the threshold day length of 13:11 h light:dark, a low incubation temperature of 5°C does not inhibit diapause egg production (Table 1); however at a 12:12 h light:dark photoperiod, a small number of diapause eggs are produced after 6 d at higher incubation temperatures of 14 and 18°C. This suggests that if 'summer temperatures' come early, and so do associated changes in the pelagic ecosystem, leading to a phytoplankton bloom and increased zooplankton and gelatinous-predator numbers, then *A. bifilosa* can initiate production of diapause eggs, albeit in low numbers, to begin an egg pool to sustain the species. Such a flexible diapause response would help to ensure the maximum numbers of animals in next year's population.

Temperature clearly plays some role in initiating *Acartia bifilosa* diapause, but the ultimate reason behind its disappearance from the water column during the warmer summer period is likely to be predator avoidance (Lucas & Williams 1995, Slusarczyk 1995) or an attempt to avoid the higher temperatures. SfG was used to identify the basic metabolic response of *A. bifilosa* and *A. discaudata* to temperature, as an additional monitor of *A. bifilosa*'s egg production patterns. It is reported that the main limiting factor of crustacean SfG in excess food conditions is the amount of energy assimilated from the food ingested (Bayne et al. 1985, Naylor et al. 1989, Maltby et al. 1990). *A. bifilosa* certainly ingests more food at 10°C than at the other 2 experimental temperatures (Table 2), and it also consumes significantly more food at 10°C than *A. discaudata*. In contrast, *A. discaudata* ingests significantly more food at 20°C and its feeding rate is significantly greater than that of *A. bifilosa* at this higher temperature. These results correspond well with Mills (1997), who reported feeding rates of ~30 to 45 J mg dry wt^{-1} d^{-1} for *A. bifilosa* fed over a range of temperatures (5 to 20°C). In contrast, in terms of cells eaten mg dry wt^{-1} d^{-1}, the present results are 2 to 3 orders of magnitude larger than those reported for *A. clausi* (Ayukai 1987) and *A. tonsa* (Cowles et al. 1988). However, in both of these cases the initial food concentration was several orders of magnitude lower than in the present study, and copepod ingestion is known to increase linearly with food concentration (Frost 1972, Gaudy 1974). The current *Acartia* feeding rates should only be taken in the context of the SfG assay.

Reflecting the ingestion pattern, the assimilation efficiency for *Acartia bifilosa* is significantly higher at 10 than at 20°C (Table 2). Efficiency increases with increasing temperature in *A. discaudata*, but with no significant difference in measured assimilation efficiencies its energy intake depends solely on the amount of food ingested. The results are comparable to other copepod assimilation efficiencies reported (Gaudy 1974, Abou Debs 1984). *A. bifilosa* clearly shows optimal grazing and energy assimilation at 10°C, while *A. discaudata* energy assimilation simply reflects incubation temperature. This metabolic strategy would go some way to explaining why *A. discaudata* is present in Southampton Water all year round, while *A. bifilosa* diapauses over summer.

Although Hirche (1987) observed fluctuations in respiration rate with temperature for *Calanus glacialis*, *Metridia longa*, *C. hyperboreus* and *C. finmarchicus*, Lampitt & Gamble (1982) reported that respiration in *Oithona nana* was actually temperature-independent. For both *Acartia* congeners, respiration is responsible for up to 76.4 and 83.7% of energy 'lost' in *A. discaudata* and *A. bifilosa* respectively. For both species, respiration rate is lowest at 20°C and highest at 10°C, but the results are not statistically significant. The respiration rates are comparable but higher than those reported by Mills (1997), who recorded rates of ~2.5 J mg dry wt^{-1} d^{-1} for *A. bifilosa.* Abou Debs (1984) reported a range in respiration rate of 0.203 to 4.54 J mg dry wt^{-1} d^{-1} for *Temora stylifera*, but the experimental copepods were not fed to satiation prior to the investigation. *Acartia* populations in the current investigation were well fed and should not be unduly stressed by a lack of food during the experimental period (Kiørboe et al. 1985). Since respiration rate increases with food intake (Gaudy 1974, Abou Debs 1984, Kiørboe et al. 1985), the elevated respiration rates seen in *Acartia* are probably a reflection that the copepods were fed to satiation for 24 h prior to measurement.

There were no significant differences in excretion rate at the 3 experimental temperatures for either

copepod. The energy lost through this component is almost negligible when compared to the other factors of the SfG assay, at only ~0.05 J mg dry wt^{-1} d^{-1} for both species. These rates compare favourably with values for *Acartia bifilosa* (Mills 1997), *A. clausi* (Harris 1959) and *Temora stylifera* (Abou Debs 1984).

Reflecting the individual metabolic parameters, and driven by the pattern of energy assimilation, the SfG for each species is clearly influenced by temperature, with *Acartia bifilosa* SfG at 10°C > 20°C = 5°C and *A. discaudata* SfG at 20°C > 10°C > 5°C (Table 2). The SfG of *A. discaudata* increases virtually linearly with temperature (Fig. 3), suggesting a rather straightforward response to ambient field temperatures, whereas *A. bifilosa* SfG at 20 and 5°C are similar, but approximately half the SfG at 10°C. In Southampton Water the temperature ranges from 2°C in winter and fluctuates between 10 and 20°C from May to September. At the identified optimum temperatures, 10°C for *A. bifilosa* and 20°C for *A. discaudata*, the maximum amount of energy is available to the individual for gametic and somatic growth. The largest *A. bifilosa* adults in the Bothnian Sea were recorded in June, when the water temperature was ~8°C (Viitasalo et al. 1995). Once adult, available energy goes into reproductive effort and *A. bifilosa* clearly assimilates more energy at 10°C, and also produces more eggs, than at 20°C (Castro-Longoria 1998). A photoperiod of 13:11 h light:dark triggers diapause in Southampton Water *A. bifilosa*, and given its metabolic performance and greatly reduced SfG between 10 and 20°C, the ultimate reason behind its over-summer diapause strategy is likely to be an attempt to avoid the higher temperatures of this season. *A. bifilosa* can feed successfully on a wide spectrum of food, has a superior ability to switch its diet focus throughout the year and is known to supplement its diet with detritus over winter (Chinnery 2002). Competition is also greatly reduced during the winter months and so, even with its low SfG, it has a better chance of survival.

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