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Temperature effects on *Calanus helgolandicus* (Copepoda: Calanoida) development time and egg production

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Effects of temperature on aspects of the life cycle and physiology of the copepod Calanus helgolandicus (C. helgolandicus) were investigated in the laboratory. Development times (DTs) were determined for organisms reared individually at 9, 12 and 15°C under saturated food conditions. While animals were successfully reared from egg to adulthood at 12 and 15°C, at 9°C, all the individuals had died prior to entering NV. The cohorts were fed with a monoculture of Prorocentrum micans as food saturating conditions. The cohorts were fed with monocultures of Prorocentrum micans which should represent saturated food concentrations with a dinoflagellate diet used in many other experimental studies. However, the monoculture nature of the diet and/or the laboratory containment conditions may have caused the mortality rates encountered. Impacts of short-term temperature change on egg production (EP) and hatching success were also investigated over the course of 1 year on individuals collected from station L4 (Western English Channel) and incubated in the laboratory. DTs increased as temperature decreased, with longer DTs at comparable temperature with those in Thompson's study [(1982) Growth and development of Pseudocalanus elongatus and Calanus sp. in the laboratory. J. Mar. Biol. Ass. UK, ***62,*** *359–372.] Evidence is presented to suggest that in this other study a mix of Calanus finmarchicus and C. helgolandicus may have been used. Equiproportional development was observed for the nauplii, but no pattern was defined for the copepodites. At low temperatures, mortality rates in the incubations were higher, but adult condition factor was higher, the longer DTs allowed greater body mass to be accumulated. EP rate was correlated with temperature at station L4, but the short-term incubation temperature did not have a significant influence on EP when measured over a short timescale (24 h). Egg hatching success also did not differ between incubations with small temperature differences.*

INTRODUCTION

Temperature has a major influence on the physiology and life-cycle processes of copepods. Within species-specific physiological limits, egg production (EP), growth and development rates increase with increasing temperature (Uye, 1980, 1981). However, the degree of temperature and food dependence is not equal for all these processes. For example, development is less sensitive to food concentration than is growth (Ban, 1994; Bonnet and Carlotti, 2001), and the two are to some degree uncoupled (Kiørboe and Hirst, in press).

Calanus helgolandicus (*C. helgolandicus*) is an important copepod species in European continental shelf ecosystems, especially in the North Sea and Celtic Sea, accounting for up to 90% of the total mesozooplankton biomass in the latter region (Williams and Lindley,

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1980a, 1980b; Joint and Williams, 1985). It represents the main diet of the juvenile stages of some economically important fish and plays a significant role in the food web. Because of its importance in the ecosystem, there is a need to quantify physiological rates and life cycle in this species. Data on *C. helgolandicus* development time (DT) are scarce in the literature, and to our knowledge the study of Thompson (Thompson, 1982) is the only published assessment of *C. helgolandicus* stage DTs from egg to adult over a range of temperatures. However, *Calanus* for these experiments were collected from the southern North Sea, and no species identification checks were carried out (B. Thompson, personal communication), even though *C. helgolandicus* and *Calanus finmarchicus* (*C. finmarchicus*) co-occur in this region (Barnard *et al.*, 2004). Furthermore, the results do not match those of Corkett *et al.* (Corkett *et al.*, 1986) for *C. finmarchicus*, and seem closer to the embryonic duration times of *C. helgolandicus* from the English Channel (Corkett, 1972). Thompson's data have been used to predict *C. helgolandicus* DT (McLaren *et al.*, 1988; Kiørboe and Sabatini, 1995), but clearly there is some uncertainty about their validity.

Variations in EP and hatching rate have been shown to be closely related to changes in temperature (Uye, 1981; Abou Debs and Nival, 1983; Kiørboe *et al.*, 1988), but the ranges of temperatures as well as the duration of the exposure are also major factors to take into account. Experimentalists typically collect animals from the field and incubate them at temperatures chosen to represent those in the field at collection (Youn and Choi, 2007; Jónasdóttir *et al.*, 2008). However, given vertical stratification of temperature through the water column, the migration ambit of copepods, and the unknown thermal history of animals, there is clearly a problem choosing a single temperature reflecting those experienced *in situ*. The long time series of *C. helgolandicus* EP measurement at station L4, Western English Channel, has been run since 1992 on a weekly basis at a constant temperature of 15°C. In contrast field temperatures at L4 vary seasonally from ~6–18°C.

With these issues in mind, the purposes of this study were to:

(i) Examine how environmentally relevant temperatures impact stage-specific DT, adult size and condition of *C. helgolandicus*.
(ii) Explore critically the extent to which Thompson's data (Thompson, 1982) may represent *C. helgolandicus* (as commonly applied).
(iii) Determine the degree to which short-term (24 h) incubation temperature impacts experimental estimation of rates of fecundity and hatching success.

METHOD

Cultures

Calanus helgolandicus females were collected at station L4, Western English Channel, in October 2004. At this time the surface water temperature was 16°C. At this station, *C. helgolandicus* is the predominant *Calanus* species and *C. finmarchicus* only occurs in very low numbers at particular times of year (Russell, 1951; L4 data base, http://www.westernchannelobservatory.org.uk/l4/). *Calanus helgolandicus* copepodite stages CV and adult males and females can be identified by the concave internal surface of the basal segment of the fifth pair of swimming legs (Lindeque *et al.*, 2006). This feature was used to confirm the species identity of the animals collected. Females were incubated in filtered sea water for 9 h overnight at 15°C. Three replicates of 100 eggs were incubated individually (replicates) in 50 mL beakers at each of the three temperatures: 9, 12 and 15°C. These temperatures cover the main temperature range observed at L4 during the year (see http://www.npm.ac.uk/rsg/projects/observatory/l4_ctdf/). Egg to nauplius stage NII were incubated in filtered sea water (0.2 μm) as these early naupliar stages do not feed. From the first feeding stage, NIII, to adults, they were fed with *Prorocentrum micans* (*P. micans*) (27–28 μm ESD and 2803 pgC $cell^{-1}$; Rey *et al.*, 2001) at a concentration of 150 cells mL^{-1} (~420 μgC L^{-1}) which should represent non-limiting food concentrations. Green *et al.* (Green *et al.*, 1991) reported a critical food level threshold of 29 μgC L^{-1} for *C. helgolandicus* to develop from nauplii to copepodite stages, and concentrations of 150 and 200 μgC L^{-1} (Mullin and Brooks, 1970) have been reported to be sufficient for maximal growth rates of *C. helgolandicus* (which was probably in reality *Calanus pacificus*). In addition, the dinoflagellate *P. micans* is recognized as a good food source for nauplii and copepodite stages of *C. helgolandicus* (Paffenhofer, 1970; Rey-Rassat *et al.*, 2002a).

The T_0 of the experiment was considered as the time from which one hundred eggs had been placed in the individual beakers at each of the three temperatures tested. At this point all eggs were between 0 and 9 h old. Every morning each beaker was checked under a stereo microscope and development stage noted. The incubation seawater was then replaced. Any dead animals were removed and not considered in the calculation of the stage duration for the stage in which they had died.

Determination of stage durations

For each day of the experiment, the number of individuals in each stage allowed the contribution made by the

stage to the total numbers of animals to be determined (stage proportion). We estimated median DT (MDT) for each stage, defined as the time when 50% of the population had moulted to that stage, based on a total count of animals at each temperature each day (Peterson and Painting, 1990). For each developmental stage, MDT was calculated by fitting a model to cumulative abundance (P) of the stage at time (T). The duration of each developmental stage was estimated as the difference of the estimated MDTs between two successive stages.

The choice of the fitted model was made objectively in the 'model selection' framework using the small sample size corrected Akaike's Information Criterion (AICc; Burnham and Anderson, 2002). As data sets exhibit sigmoid shapes, two sigmoid functions were compared in order to choose the best one to infer MDT: the logistic function (i) (Ratkowsky, 1990), which is symmetrical with respect to its inflection point; and the cumulative Weibull (ii) function (Weibull, 1951), which does not exhibit such a symmetry. These two models encompass the range of possible shapes taken by the data sets.

$$P = \frac{c}{1 + \exp(-zT + f)} \quad (1)$$

$$P = c[1 - \exp(-zT^f)] \quad (2)$$

where c, z and f are fitted parameters: c controls the asymptote of the curve and z and f alter the curve shape in both equations [note that all three parameters interact and control the y-axis intersection in (1) and (2)].

The logistic function was selected as the best compromise as it respected the regression hypotheses (normality and homoscedasticity of the residuals) and provided the lowest AICc in the majority of cases. Once the logistic function was fitted to a data set, MDT was back calculated by inserting the parameters of the logistic function in the reciprocal equation (3) and for $P = 50$.

$$T = \frac{\log_c(c/P - 1) - f}{-z} \quad (3)$$

DT (D, in hours) within a single species is commonly assumed to be related to temperature (T, °C) described by the Bêlehràdek function:

$$D = a(T - \alpha)^{-b}$$

We set b at 2.05 as this value is commonly applied in the literature and regarded as representative for copepods (Corkett *et al.*, 1986). We then fitted this equation to the egg stage, for which we added complementary data from the literature to our own data set (see Hirst *et al.*, 2007 for full details) and this resulted in a value for α of -8.974. Subsequently, we fitted Bêlehràdek functions to the other stages, applying a b value of 2.05 and α of -8.974; hence we solved for a using the data available. A similar fitting was applied to the Thompson's data (Thompson, 1982), with $b = 2.05$ and $\alpha = -9.682$, as we could not assume that the data exclusively represented *C. helgolandicus* DT. A curve-fitting program (Lab Fit) was used in all cases. Comparison of the 'a' value between both studies was made using a paired t-test with Systat 11 software.

Condition factor

Adults produced at the end of the development experiments were kept for a further 3 days after moulting to adulthood. The total DT to this point was calculated from T_0 for each individual. Prosome lengths were measured, and they were pelleted individually in an ashed aluminium cup and frozen at −30°C for later carbon–nitrogen analysis (Carbo-Erba Elemental Analyser). Individual condition factor in terms of nitrogen (CF_N) was determined as the ratio of the body weight to the cube of the cephalothorax length (Durbin and Durbin, 1978). This CF_N index allows comparison of the relative condition of adults reared with the same diet but at different temperatures. For large copepods such as *Calanus*, the absolute weight within a specific stage is likely to change considerably (Rey-Rassat *et al.*, 2004). Comparing 3-day-old adults for a specific treatment allows us to highlight the large variability between organisms at a similar development point (equal adult age) in a population. As DT and growth have different temperature dependence (Bonnet and Carlotti, 2001) we hoped that the condition factor would give insight into the relationships between these two processes.

EP and hatching success

Calanus helgolandicus EP and hatching success at L4 were measured in the laboratory from September 2003 to September 2004, both at the *in situ* field temperature (9.8–18.8°C) measured during that period and at a constant 15°C. To measure EP, five groups of five females were placed for 24 h in 2L glass beakers of filtered sea water with a 300 μm-mesh filter on the bottom to prevent egg cannibalism. At the end of the incubation, the eggs from each treatment were counted and mixed together. Twenty four eggs were then incubated individually in a multi-well plate (well volume 2 mL) with

filtered sea water at their corresponding incubating temperature. Three replicates were run for each temperature, hence a total of 72 eggs per temperature were examined. Hatching success was monitored daily for a week. Eggs which did not hatch after 7 days were considered as not viable. EP and hatching success data sets at both 15°C and field temperature were compared in pairs using paired *t*-test with Systat 11 software.

RESULTS

Development

Of the eggs incubated at 12, and 15°C, only 4% and 20% respectively reached the adult stage, and all the resultant adults were female. At 9°C, animals did not survive beyond stage CII and stage durations beyond NV must be used cautiously because they are based on a single animal. Survival rates were always best at 15°C but quite similar to 9 and 12°C over stages NI to NIV–NV (~12 days) (Fig. 1). Time to 50% surviving was reached at 9.07 days at 12°C, 10.19 days at 9°C and 15.35 days at 15°C. This implies that temperature is an important parameter to explain *C. helgolandicus* population dynamics, as the 50% survival point of the population was 1.7 times later at 15°C than at 9°C.

Figure 2 shows the population progressing across stages. NI and NII stages had a short duration (of 3–6 days) while stage NIII had a much longer DT whatever the incubation temperature, of 8–11 days (Table I). At each temperature, the range between first and last appearance of the first two naupliar stages was ~6 days for each stage (apart for NI at 15°C when it was, 3 days), but from NII to CV the range was 8–22 days. Increased variability in DT was apparent after stage NIII. At 15°C, there was more spread in the time it took to reach adulthood, between 24 and 40 days. This high variability (16 days) in the total DT (egg to 3-day-old adult) was observed between the first and last adults collected at 15°C (Fig. 4a), while at 12°C, 3-day-old females appeared later than at 15°C (from 41 until 44 days after the start of the incubation). At 12°C adulthood was reached between 38 and 41 days. The average time to reach 3-day-old adults was 36.8 days at 15°C and 42.2 days at 12°C (i.e. 33.8 and 39.2 days, respectively, from egg hatching to moulting into adult). A comparison between our results and those of Thompson (Thompson, 1982) on cumulative stage duration from hatching to moulting to adult at (or ~) 9, 12 and 15°C is presented in Fig. 3. At the three temperatures, the main differences in DTs are in the late naupliar stages (NIII–NVI) and remain the same (at 12 and 15°C) or increase (at 9°C) during the copepodite stages (Fig. 3). Relationships between stage duration and temperature in our study and that of Thompson (Thompson, 1982) are compared in Table II. Comparison of the '*a*' value between both studies was made using a paired *t*-test with Systat 11 software and showed that the series were significantly different ($P < 0.05$).

Condition factor

There is a significant negative correlation ($R^2 = 0.294$, $P < 0.05$) between the prosome length of the females and the time taken to reach the 3-day-old adult stage

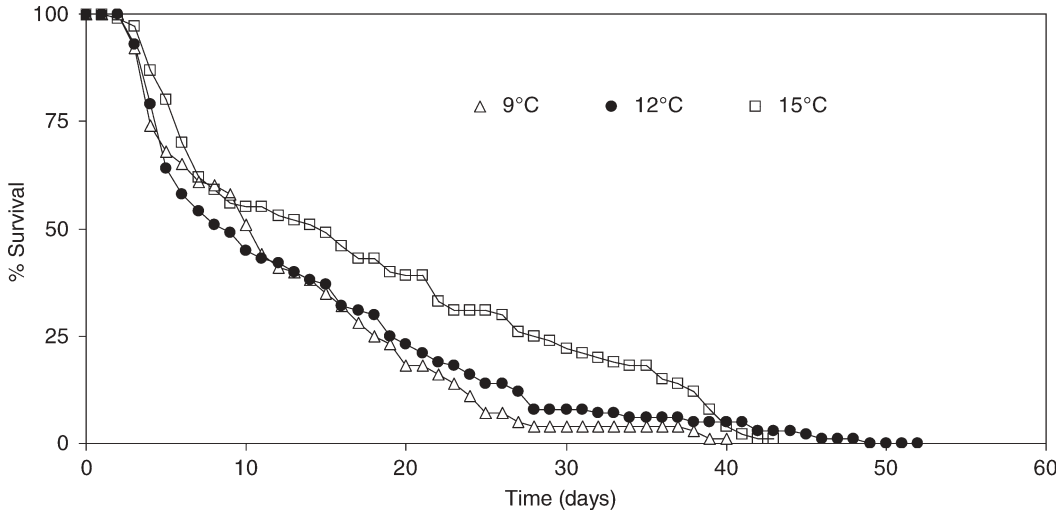

Fig. 1. Percentage survival through time of the 100 *C. helgolandicus* eggs and subsequent development stages incubated individually at 9, 12 and 15°C. The last data point at each temperature corresponds to the point when survival fell to 0% (at 9°C) or to a percentage which represents the proportion of individuals that have reached the adult stage (12 and 15°C).

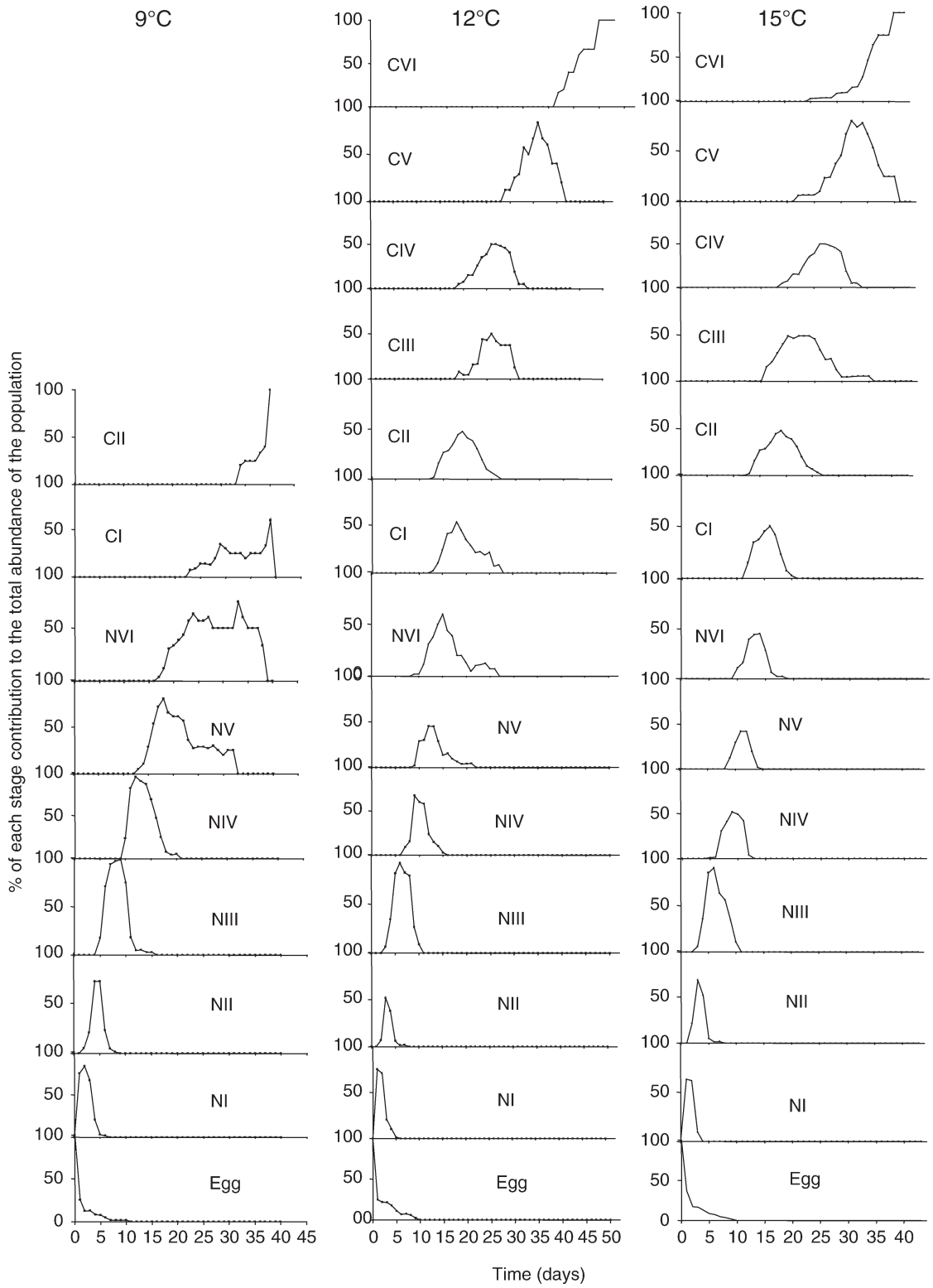

Fig. 2. Developmental sequence of the cohort of *C. helgolandicus*, illustrating the range in variability in DT for each developmental stage and stage progression.

Table I: Comparison of stage durations (in days) between this study and Thompson's study (Thompson, 1982)[a]

	Stages of development														
Temperature (°C)	Egg	NI	NII	NIII	NIV	NV	NVI	Nauplii DT NI–NVI	CI	CII	CIII	CIV	CV	DT NI–CV	Reference
4.5	3.89	2.06	6.1	10.32	4.96	1.22									Thompson (1982)
7.55	2.57	1.4	2.88	7.2	3.68	2.56	3.1	20.82	6.8	5.4	6.36	7.64	9.56	56.58	Thompson (1982)
9	1.28	2.78	2.04	4.79	5.46	6.02	(13.56)	35.93	(3.12)						This study
9.57	1.97	0.52	1.4	8.58	1.38	2.36	0.96	15.20	3.64	4.52	4.72	5.48	8.16	41.72	Thompson (1982)
11.5	1.72	0.6	1.9	4.02	1.68	1.8	0.68	10.68	2.64	3.52	3.72	4.64	7.4	32.60	Thompson (1982)
12	1.11	2.23	1.26	4.34	2.47	2.24	3.40	17.05	3.89	4.26	3.90	4.22	7.14	40.46	This study
13.3	1.39	0.54	1.22	3.96	1.72	1.7	0.6	9.74	2.42	2.66	3.44	4.04	5.5	27.80	Thompson (1982)
13.9									2.34	2.55	3.02	3.16	5.4		Thompson (1982)
15	1.16	1.68	1.68	4.10	2.19	1.42	1.88	14.11	2.69	3.30	4.99	4.54	7.06	36.69	This study
15	1.38	0.54	0.46	3.06	2.2	1.22	0.9	8.38	2.74	2.9	3.4	4.11	4.68	26.21	Thompson (1982)

[a]In all cases animals were reared in excess food and at the temperature given. Note our animals were *C. helgolandicus* whereas the identity of Thompson's reported as *C. helgolandicus* is questioned. Data in brackets are derived from a single organism.

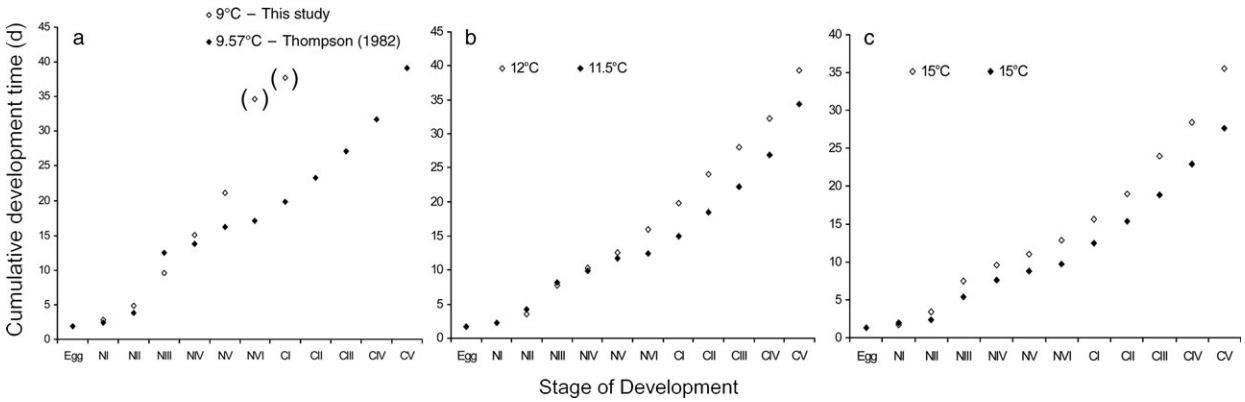

Fig. 3. Comparison between Thompson's (Thompson, 1982) (black diamonds) and our results (white diamonds) on cumulative stage durations (time to MDT in days) from hatching to emergence of adult according to temperature: (**a**) 9°C, (**b**) 12°C and (**c**) 15°C. CI was the latest stage obtained at 9°C in our study. Data into brackets are derived from a single organism.

Table II: C. helgolandicus – Bèlehràdek functions derived and applied in this study to determine stage-specific DTs (D, in hours)[a]

	Thompson (1982)		This study	
	a	*n*	*a*	*n*
Egg	21 291.11	6	13 905.75	15
NI	9945.34	6	25 850.91	3
NII	25 948.83	6	19 813.89	3
NIII	60 848.11	6	51 531.30	3
NIV	26 034.93	6	39 811.93	3
NV	22 580.54	6	37 900.91	2
NVI	16 033.30	5	74 292.34	2
CI	43 740.26	6	36 967.30	2
CII	44 304.53	6	51 049.35	2
CIII	50 500.49	6	57 675.45	2
CIV	59 738.99	6	57 632.30	2
CV	82 936.69	6	94 057.61	2

[a]Egg hatching times fitted to the equation $D = a(T-\alpha)^{-2.05}$ where α was determined as -8.974 in our study and -9.682 in Thompson's study (Thompson, 1982). These values were subsequently used for all later developmental stages when determining values for *a*. A curve-fitting program (Lab Fit) was used in all cases.

(Fig. 4b). At 15°C, the longer they took to reach the 3-day-old adult stage, the shorter the prosome length. Interestingly however this does not relate to the pattern in individual nitrogen weight (4C) or condition factor (4A), for which there is no significant relationship between age and these values ($P > 0.05$). Prosome lengths and nitrogen content weights were higher at 12°C (2119.31 ± 48.63 μm and 13.99 ± 1.32 μgN) than at 15°C (1955.57 ± 173.89 μm and 8.84 ± 3.66 μgN) for individuals with the same DT from egg to adult (between 40 and 45 days) ($P < 0.01$), hence the former had higher condition factors.

EP and hatching success

EP increased from December to June when it reached its maximum value (~40 eggs female^{-1} day^{-1}) before decreasing over the autumn to reach a minimum during winter (~2 eggs female^{-1} day^{-1}). Hatching success was usually very high throughout the year (between 80 and

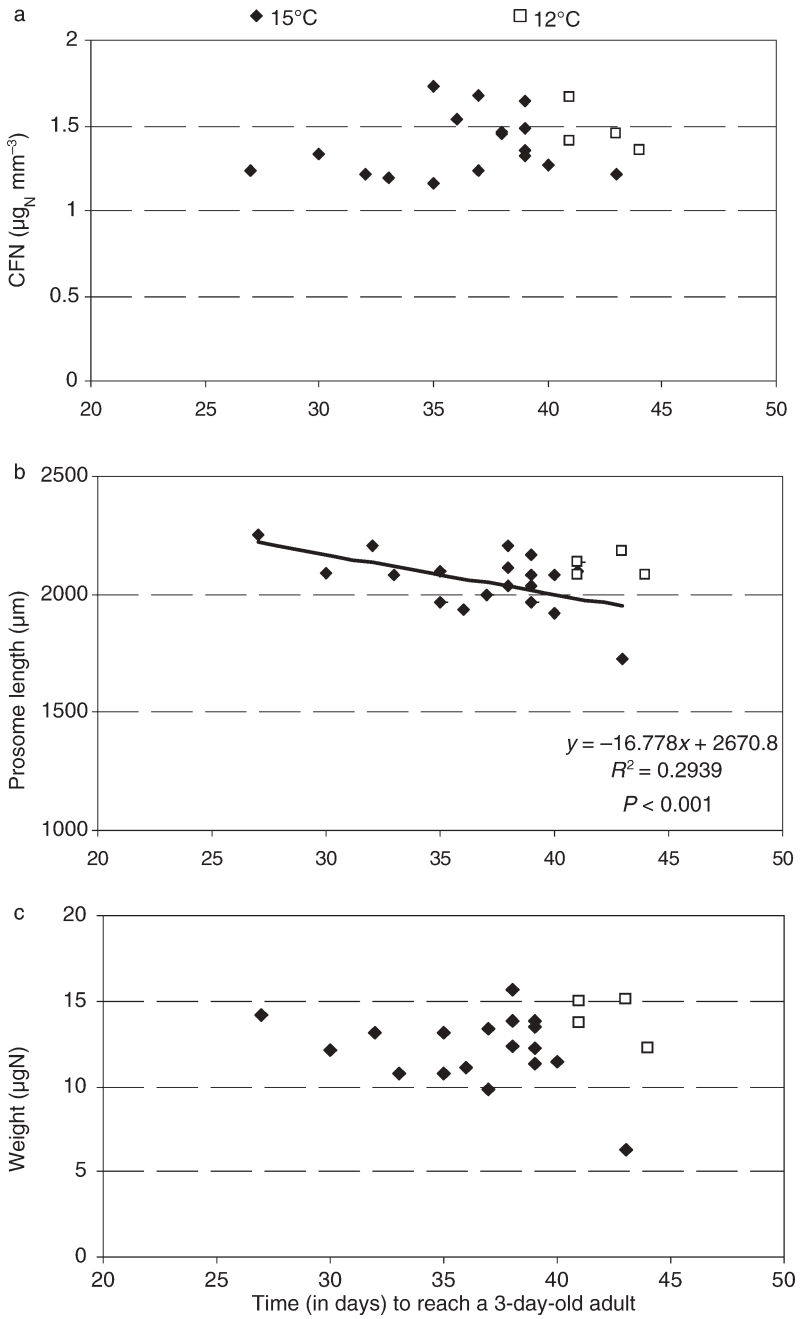

Fig. 4. (**a**) Condition factor (CF_N) calculated for adult females as the ratio of the nitrogen content (µgN) to the cube of the prosome length (Durbin and Durbin, 1978). (**b**) Prosome length (regression corresponds to individuals reared at 15°C). (**c**) Nitrogen content of adult females according to the temperature they were reared at and the time they took to reach the 3-day-old adult status.

100%). However, lower values (<50%) were observed in February and June 2004. EP was not significantly different between incubations at 15°C and *in situ* field temperature (EPf) (paired *t*-test, $P > 0.05$; Fig. 5a). When the 15°C EP rates are expressed as a percentage of field temperature EP rates and regressed against the differences in temperature (15°C − field incubation temperature), no significant relationships were observed ($P > 0.05$) (results not shown). There was also no significant difference between the success of egg hatching at field temperature and eggs incubated at 15°C ($P > 0.05$, Fig. 5b) and no significant correlation between hatching success and temperature ($P > 0.05$). The mean EP percentage, i.e. $(EP_{15}/EP_f) \times 100$, when field temperature is

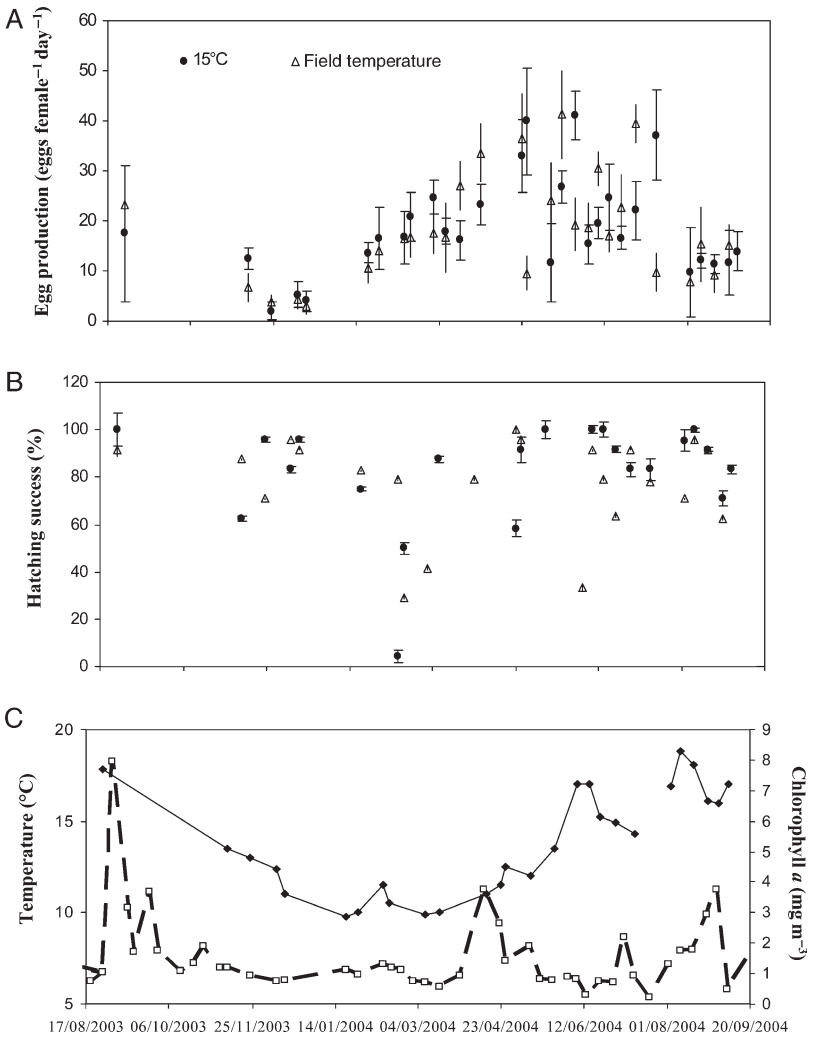

Fig. 5. Changes from August 2003 to September 2004 in (**a**) *C. helgolandicus* EP and (**b**) hatching success. Measurements were made at both a constant 15°C and at the measured *in situ* water column average field temperature at station L4. Error bars represent the 95% confidence intervals. (**c**) Temperature (continuous line) and Chl *a* concentration (dash bold line) at the location where females were collected (L4 station).

<15°C is 111.12 ± 15.93 (95% confidence interval), and 100.34 ± 16.64 for temperature ≥ 15°C. The mean hatching success for temperature <15°C is 106.68 ± 24.75 (95% confidence interval), and 133.75 ± 42.58 for temperature ≥ 15°C.

DISCUSSION

Development

As demonstrated for many copepods, DTs decreased with increasing temperatures (McLaren, 1978; McLaren *et al.*, 1989). Our results clearly indicate that development is not isochronal for *C. helgolandicus*, and NI and NII have shorter stage durations than NIII (Table I). The longer duration of stage NIII in comparison with NI and NII is probably due to this being the first feeding stage. This observation was also noted by Lebour (Lebour, 1916) for *C. helgolandicus* (probably misidentified as *C. finmarchicus*). NIII was the commonest stage in the cultures which she concluded was because the nauplii spent a long time in this stage. Each copepodite stage duration is longer than for any of the naupliar stages, with CV being the longest. Peterson's review (Peterson, 2001) showed that for *Calanus* spp., copepodites versus nauplii cumulative duration ratio was ~1.97, meaning that cumulative copepodite DT was nearly twice as long as that for nauplii. Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002a) also found non-isochronal DTs when studying copepodite stages of *C. helgolandicus* reared at different food concentrations.

Table III: C. helgolandicus – proportion of full DT taken to achieve the MDT for each stage, where DT is defined as the time from egg hatching to moult into adult

	Thompson (1982)					This study	
	7.55°C	9.57°C	11.5°C	13.3°C	15°C	12°C	15°C
NI	0.04	0.05	0.05	0.05	0.05	0.03	0.03
NII	0.07	0.06	0.07	0.07	0.07	0.08	0.08
NIII	0.12	0.09	0.12	0.11	0.09	0.11	0.12
NIV	0.24	0.29	0.24	0.24	0.20	0.22	0.23
NV	0.30	0.32	0.29	0.30	0.28	0.28	0.29
NVI	0.34	0.37	0.34	0.36	0.32	0.34	0.33
CI	0.40	0.39	0.36	0.38	0.35	0.42	0.38
CII	0.51	0.48	0.44	0.46	0.45	0.52	0.46
CIII	0.60	0.58	0.54	0.56	0.56	0.62	0.55
CIV	0.71	0.69	0.65	0.67	0.68	0.72	0.68
CV	0.84	0.81	0.78	0.81	0.83	0.82	0.81
CVI	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Thompson (Thompson, 1982) suggested a non-conformist development (i.e. development which does not conform to the equiproportional rule; see Peterson and Painting, 1990) while Corkett *et al.* (Corkett *et al.*, 1986) observed equiproportional development for *C. helgolandicus*. Our results presented in Table III and the data review from the literature listed in Table IV show that the proportion of time spent in the naupliar stages is very similar in each study, regardless of temperature, indicating that from egg to NV, the development is equiproportional. However, copepodites seem to show different patterns, supporting a non-conformist development as suggested by Thompson (Thompson, 1982). Nevertheless, because of the sparse literature data on copepodite DT (Table IV) and the lack of replication of our experiments, we cannot be certain regarding the copepodites.

Temperature is the main control of DT. For example, Uye (Uye, 1988) observed a positive relationship between longevity and temperature in *Calanus sinicus*. Recent work has investigated, in the laboratory, the importance of temperature and diet on naupliar DT and survival in both congeneric species *C. helgolandicus* and *C. finmarchicus* (Cook *et al.*, 2007). However, Cook *et al.*'s experimental work was carried out with mass cultures, providing an average duration for each naupliar stage within the culture population. NI and NII stage durations were not correlated with temperature or the food type and concentration used. Our survival rates were higher at 15°C than at 12 and 9°C. This supports the Cook *et al.*'s (Cook *et al.*, 2007) study reporting that survival of nauplii fed with *P. micans* increased with temperature.

In our study, the *C. helgolandicus* incubated at 9°C all had died by stage CI. However, Thompson (Thompson, 1982) and Diel and Klein Breteler (Diel and Klein Breteler, 1986) were successful in raising cultures (to adult) at even lower temperatures (Tables I and IV). We suggest three possible explanations. The first being that both cited studies used a mixture of *C. helgolandicus* and *C. finmarchicus* and that *C. finmarchicus* has a colder thermal niche than *C. helgolandicus* (Fig. 13 in Bonnet *et al.*, 2005). The second argument relates to the fact that it is important to keep in mind the natural temperature range of the population being studied. Both Thompson (Thompson, 1982) and Diel and Klein Breteler (Diel and Klein Breteler, 1986) incubated individuals collected in the southern North Sea, while our copepods were from the Western English Channel, where the minimum temperature reaches 8°C in February. Winter temperatures in the southern North Sea are frequently <8°C. The lower temperature limits for the Western English Channel population are probably higher than those of the North Sea population. Similarly, data from the Continuous Plankton Recorder (CPR) show that in the Mediterranean, the range of temperatures at which *C. helgolandicus* is found varies from 12 to 23°C, while in the Atlantic and the North Sea *C. helgolandicus* has a thermal niche from 3 to 22°C (Fig. 6). Therefore, even if both the Thompson (Thompson, 1982) and Diel and Klein Breteler (Diel and Klein Breteler, 1986) studies were carried out on *C. helgolandicus*, those populations may well be adapted to living in these different thermal regimes. Finally, the mortality rates encountered during the experiment could have been induced by containment artefacts and/or the food diet offered. *Prorocentrum micans* has been often used as a food source when growing *C. helgolandicus* (Paffenhöfer, 1976). Naupliar (Cook *et al.*, 2007) and copepodite (Rey-Rassat *et al.*, 2002a) development have been successful at high and low concentrations of *P. micans* and Irigoien *et al.* (Irigoien *et al.*, 2000) have obtained up to 25% males when rearing cultures fed with *P. micans*, indicating that the cohorts were developing very well. However, a monospecific diet is quite an unnatural diet for copepods and may lack essential dietary requirements necessary for optimal survival.

EP and hatching success

The trend observed in EP, an increase from December to April–May with a low value in March and a decrease during autumn, is typical for station L4 (Pond *et al.*, 1996; Irigoien *et al.*, 2000; Rey-Rassat *et al.*, 2004). Maximum EP is observed in April and May and

Table IV: Literature review of temperature effect on DT (days) of C. helgolandicus reared in excess of food

Reference	Temperature (°C)	Food source	Rearing method	DT (days)												Total time to adults (days)
				Egg	NI	NII	NIII	NIV	NV	NVI	CI	CII	CIII	CIV	CV	
Crockett (1972)	0.7		Cohort	6.91												
Corkett (1972)	3.9		Cohort	4.20												
Diel and Klein Breteler (1986)	5	RH + IG + PM + OM (500 μg CL^{-1})	Cohort													95–110
Corkett (1972)	7.4		Cohort	2.41												
Cook *et al.* (2007)	8	Without food –non-feeding stages	Cohort		2.2	3.4										
Cook *et al.* (2007)	8	IG-H[a]	Cohort				5.1	13.6								
Cook *et al.* (2007)	9	IG-L[a]	Cohort				5.3									
Cook *et al.* (2007)	8	PM-H[a]	Cohort				6.1	10.9								
Cook *et al.* (2007)	9	Without food –non-feeding stages	Cohort		1.7											
Diel and Klein Breteler (1986)	10	RH + IG + PM + OM (500 μgCL^{-1})	Cohort													39
Cook *et al.* (2007)	12	Without food –non-feeding stages	Cohort		1.5	2.4										
Cook *et al.* (2007)	12	IG-H[a]	Cohort				3.5	8.4	11.3							
Cook *et al.* (2007)	13	IG-L[a]	Cohort				3.5									
Cook *et al.* (2007)	12	PM-H[a]	Cohort				3.6	6.4	8.4	9.7	12.7					
Cook *et al.* (2007)	13	PM-L[a]	Cohort				3.9	7.9	11.7	17.1	19.1					
Corkett (1972)	14.2		Cohort	1.37												
Cook *et al.* (2007)	15	Without food –non-feeding stages	Cohort		1.2	2.0										
Cook *et al.* (2007)	15	IG-H[a]	Cohort				3.0	6.2	8.6							
Cook *et al.* (2007)	15	IG-L[a]	Cohort				3.0									
Cook *et al.* (2007)	15	PM-H[a]	Cohort				2.9	4.7	5.8	7.0	8.1					
Cook *et al.* (2007)	15	PM-L[a]	Cohort				2.6	4.6	5.9	7.4	9.3					
Rey-Rassat *et al.* (2002a)	15	PM-L[a]	Cohort			Egg to CI: 12.0						2.9	2.6	4.3	7	28.8
Rey-Rassat *et al.* (2002a)	15	PM-H[a]	Cohort			Egg to CI: 11.9						2.2	2.9	2.6	4.8	24.4
Rey *et al.* (2001)	15	RB (>360 μgC L^{-1})	Cohort	1.7	NI–NII: 1.2		2.9	1.6	1.5	–						
Rey *et al.* (2001)	15	IG (>360 μgC L^{-1})	Cohort	1.5	NI–NII: 1.3		2.1	1.8	1.4	1.4						
Rey *et al.* (2001)	15	PM (>360 μgC L^{-1})	Cohort	1.3	NI–NII: 1.5		2.3	1.4	1.3	1.5						
Rey *et al.* (2001)	15	PC (>360 μgC L^{-1})	Cohort	1.3	NI–NII: 1.5		2.3	2.1	1.7	–						
Rey *et al.* (2001)	15	TW (>360 μgC L^{-1})	Cohort	1.2	NI–NII: 1.6		2.1	1.9	1.5	–						
Shreeve *et al.* (1998)	15	TW (>300 μgC m^{-3})	Mean of 3 methods[b]								3.4	1.35	3.8	3.5		
Shreeve *et al.* (1998)	15	IG (>300 μgC m^{-3})	Mean of 3 methods[b]								1.35	1.65	2.75	3.35		
Shreeve *et al.* (1998)	15	PM (>300 μgC m^{-3})	Mean of 3 methods[b]								3.15	2.65	1.75	2.65		

[a]L, low concentration ($L = 77.5$ μgC L^{-1}); H, high concentration ($H = 278$ μgC L^{-1}); [b]Mean of the three methods: cohort, sorted stage method and sieve fractionating method. IG, *I. galbana*; PM, *P. micans*; RB, *Rhodomonas baltica*, PC, *Pleurochrysis carterae*; TW, *Thalassiosira weissflogii*, RH, *Rhodomonas* sp.; OM, *Oxyrrhis marina*.

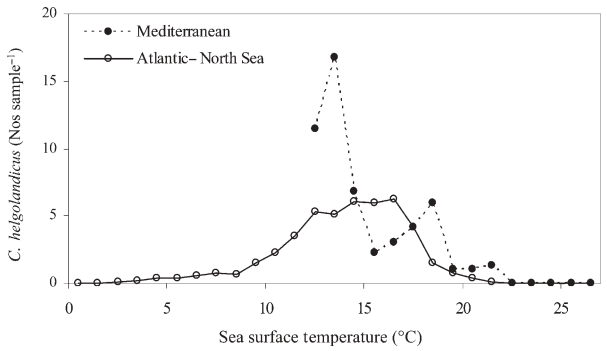

Fig. 6. Distribution of *C. helgolandicus* in the Mediterranean and in the Atlantic/North Sea derived from CPR data expressed as a function of sea surface temperature.

reaches ~42 eggs female^{-1} day^{-1}. EP was correlated with field temperature ($P < 0.05$).

No significant influence of the incubation temperature on *C. helgolandicus* EP or egg hatching success on a short timescale (24 and 72 h respectively) was observed (paired *t*-test, $P < 0.05$ testing temperature–EP pairs). Our results are in agreement with Laabir *et al.* (Laabir *et al.*, 1995), who found that EP rates of field collected *C. helgolandicus* incubated for 24 h at 5, 15.7 or 22.3 $\pm$ 0.5°C were not significantly different and that hatching success was also not affected by temperature during a short incubation period (observation for 72 h). These authors did however find that beyond 72 h the food type supplied to the adults had a significant effect on hatching rate of the eggs produced.

We found little evidence of changes in EP or hatching success when incubation temperature were from 3.8°C greater to 5.2°C lower than the field. This has practical implications for the procedures we can apply for EP or hatching success experiments. For example, when monitoring these variables on the long term, we could use a constant average of temperature over the year for incubations. Our results show that this is true at least for temperatures doubling between the winter and the summer period. This means that the historic L4 data are representative of the *in situ* EP rates during the last 15 years. Higher variations in the range of temperature may need further investigations, even if the results of Laabir *et al.* (Laabir *et al.*, 1995) show no difference for a 17°C temperature range. However, no information on the field temperature at the time of their incubation was mentioned.

Condition factor

Individual rearing in this study, enabling animals to be sampled 3 days after reaching the adult stage, and the similarity of their feeding history, allowed us to compare the single impact of temperature on condition factor. Campbell *et al.* (Campbell *et al.*, 2001) suggest that CF_N is dependent on food concentration but not on temperature. However as mentioned by Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002a), Campbell *et al.* (Campbell *et al.*, 2001) did not distinguish between animals that were sampled at the beginning or at the end of each developmental stage (they looked at the condition factor for stages NIII–CV) and therefore obtained an average value. Our results show no significant difference between condition factors at 12 and 15°C (*t*-test, $P > 0.01$), however, due to the small numbers of individuals which reached the adult stage at 12°C, we suggest that additional experiments on the influence of temperature on CF_N need to be run on many more animals, but also to include observations when they become adults.

At 15°C, the appearance of 3-day-old adults was spread over 16 days. Most individuals reached this point 35–40 days after the eggs were laid, but some early and late developers were present (Fig. 4). Prosome lengths of early developers are greater than late developers ($R^2 = 0.294$, $P < 0.001$). However, CF_N seems to follow a different pattern with early and late developers having a lower CF_N than females of mid-size which have developed in ~36.8 days. The early and late arrivers had low weights in our experiments. Similarly both Hygum *et al.* (Hygum *et al.*, 2000) and Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002a) have observed latecomers with a low body weight when rearing mass cultures of *Calanus*.

Fewer individuals reached a 3-day-old adult stage at 12°C than at 15°C. However, these individuals had a greater CF value at the former temperature mainly due to the fact that they were heavier. This is in agreement with Vidal (Vidal, 1980a) who suggested that late copepodites must attain a greater body weight at low temperature, since the growth rate of late stages is similar at all temperatures, but stage duration is longer at low temperature (Vidal, 1980b). As a consequence, we suggest that at low temperatures, mortality rates are higher, but those surviving have a higher condition factor due to longer DT but a growth rate which is by comparison relatively less reduced.

Comparison with the results of Thompson (Thompson, 1982)

Thompson (Thompson, 1982) sampled *Calanus* off Flamborough Head, in the North Sea, between March 1974 and August 1976. As she states, "no attempt was made to separate *Calanus finmarchicus* and *C. helgolandicus* in the laboratory, it is probable that the samples of live

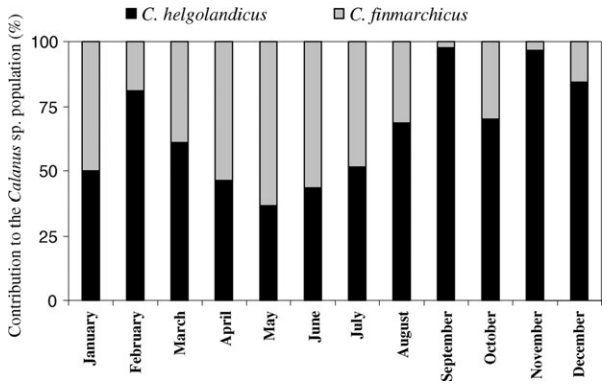

Fig. 7. Monthly percentage composition of the *Calanus* sp. populations in the area D2 (west southern North Sea) of the CPR survey. Values are averages of each month from January 1974 to December 1976.

plankton . . . contained both species". To assess the species composition of the *Calanus* population off Flamborough during Thompson's study, we analysed the semi-quantitative data (surface data) from the CPR surveys in the area D2 (west southern North Sea) between January 1974 and December 1976. Monthly averages of the *Calanus* sp. population composition are presented in Fig. 7. Results show that *C. helgolandicus* predominates in this area; however, both species are present throughout the year with a ratio ~1:1 in January, April, May, June and July. This suggests Thompson's results on *Calanus* DT may well be for a mix of *C. finmarchicus* and *C. helgolandicus*. This is further supported when Thompson's results are compared with DT from published data on *C. finmarchicus* and from our results on *C. helgolandicus*, (Tables I and IV). For example, in our study at 12°C, it took *C. helgolandicus* 12.5 days to develop from NIII to NIV, while Thompson measured a duration of 8.2 days and Campbell *et al.* (Campbell *et al.*, 2001; from their Table III) and Cook *et al.* (Cook *et al.*, 2007) observed a duration of 6.6 and 7.1 days for *C. finmarchicus* between NIII and NVI respectively. *Prorocentrum micans* was used as the food source in our experiment whereas Thompson (Thompson, 1982) used a mixture of *Isochrysis galbana* (*I. galbana*) and *Skeletonema costatum*. Temperature is considered a primary factor influencing DT, however several other factors are also likely to have an effect on development rate: including food quality and quantity (Bonnet and Carlotti, 2001; Rey *et al.*, 2001), past feeding history (e.g. biochemical composition of the eggs, Rey-Rassat *et al.*, 2002b), rearing conditions (Shreeve *et al.*, 1998) and even the methods to calculate the duration of stage of development (Cook *et al.*, 2007).

Our study of temperature effects on *C. helgolandicus* DT is less complete than that of Thompson (Thompson, 1982) in terms of the range of temperatures tested. However, all the individuals were collected at the same time from the field and cultures were run in parallel, meaning a similar feeding history of the individuals incubated for EP. In addition, the copepods used were undoubtedly *C. helgolandicus*. Consequently, we provide in this paper the first relationships between temperature and DT for all *C. helgolandicus* stages of development.

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