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Naupliar development times and survival of the copepods *Calanus helgolandicus* and *Calanus finmarchicus* in relation to food and temperature

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We measured egg hatching times and stage specific naupliar development times of the key calanoid copepods Calanus helgolandicus and C. finmarchicus in response to temperature, food quantity and food type. Calanus helgolandicus development times decreased with increasing temperature and when fed Isochrysis galbana (4.4 μm ESD) rather than Prorocentrum micans (29.5 μm ESD). Nauplii needed higher food carbon concentration to develop past the first feeding stage (N3) when fed I. galbana compared with P. micans. At low food carbon concentrations nauplii developed more slowly past N3 than at more saturated levels. The survival of nauplii fed P. micans increased with temperature, but starved nauplii survival decreased at higher temperatures. We parameterized a temperature-dependent model of development for both species which fitted the observed stage durations under non-limiting food extremely well and demonstrated that C. finmarchicus develops faster than C. helgolandicus. Further data are needed to clarify the effect of food-temperature interactions on development rates.

INTRODUCTION

Zooplankton play a pivotal role in the functioning of marine ecosystems as they form a link in pelagic food webs between producers and secondary consumers (Banse, 1995). The accurate measurement of marine zooplankton secondary production is therefore essential to a full understanding of the transfer of energy to higher trophic levels. Secondary production measurements in marine systems rely on estimates of growth rates (Kimmerer, 1987; Huntley and Lopez, 1992; Poulet *et al.*, 1995; Aksnes *et al.*, 1997; Hirst and Bunker, 2003), and typically focus on copepods as they usually dominate the mesozooplankton. To interpret field estimates of copepod growth, information is needed on the typical characteristics of growth and development for individuals of the population, and on the causes of variability (Carlotti and Nival, 1991) such as food and temperature.

Mortality rates of copepod eggs and nauplii can be both high and very variable (Ohman *et al.*, 2004). Development rates and mortality are closely associated, for example delayed development can lead to animals staying in more vulnerable stages for longer (Lopez, 1996). The interplay between development, growth and mortality has rarely been described in the early stage copepods (Lopez, 1996) that experience most of the population mortality, although see Lopez (Lopez, 1991). We aim here to explore these issues further using the important species *Calanus helgolandicus* and *C. finmarchicus*.

The most important factors controlling stage durations of copepods are temperature, food quantity and

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food quality. Some studies have concluded that food is rarely limiting for nauplii in nature (McLaren, 1978) and therefore models using only temperature are frequently used. Campbell *et al.* (Campbell *et al.*, 2001) use a Belehrádek function to explain temperature effects at constant food levels and an Ivlev function to describe food effects at a constant temperature, but do not combine the two into a single function. Speirs *et al.* (Speirs *et al.*, 2005, 2006) describe a model that includes both the effects of temperature and food level on the development rates of *C. finmarchicus*. However, no such function has been described for *C. helgolandicus*.

The aim of this study is to investigate how stage durations of *Calanus* spp. eggs and nauplii vary with temperature, food quantity and food type and to parameterize the development rate model of Speirs *et al.* (Speirs *et al.*, 2005) for *C. helgolandicus*.

METHOD

Table I lists the abbreviations used to describe the experiments undertaken.

Table I: Summary of abbreviations used to describe experiments

Abbreviation	Experiment type	Algal species	Food level
EH	Egg hatch	None	None
S	Nauplii development	None	Starved
IG-L	Nauplii development	*I. galbana*	Low
IG-H	Nauplii development	*I. galbana*	High
PM-L	Nauplii development	*P. micans*	Low
PM-H	Nauplii development	*P. micans*	High
PI-L	Nauplii development	*I. galbana* + *P. micans*	Low
PI-H	Nauplii development	*I. galbana* + *P. micans*	High

Phytoplankton cultures

Cultures of the prymnesiophyte *Isochrysis galbana* (CCAP code 927/1, 4.1–5.2 μm ESD) and the dinoflagellate *Prorocentrum micans* (CCAP code 1136/8, 23.2–33.9 μm ESD) were obtained from the Culture Collection of Algae and Protozoa, Oban. Cultures of the diatoms *Cylindrotheca closterium* and *Lithodesmium* spp. were isolated from water samples taken off Stonehaven. The batch cultures of algae were grown in 0.2 μm filtered natural seawater enriched with f/2 (*I. galbana* and *P. micans*) (Guillard and Ryther, 1962) or f/2 with silicate media (*C. closterium* and *Lithodesmium* spp.). They were incubated in a plant growth chamber set at 10°C and greater than saturating light intensity ($>200\ \mu Em^{-2}\ s^{-1}$) on a 12:12 h light: dark cycle. Cell concentrations in the cultures were monitored using a Coulter Multisizer II.

Female *Calanus* maintenance

Zooplankton were collected by vertical hauls using a 200 or 350 μm mesh ring net fitted with a non-filtering cod-end. *Calanus helgolandicus* females were collected from the north-western North Sea (56°57.8′N 02°06.2′W). *Calanus finmarchicus* females were collected from Loch Etive on the west coast of Scotland (56°27.3′N, 5°11.3′W). Females were kept in several gently aerated 30 L containers (maximum 25 individuals per litre) of freshly collected 95 μm filtered seawater in a controlled temperature room set at *in situ* sea temperature. They were fed once a day in excess with a mixture of *P. micans*, *C. closterium* and *Lithodesmium* spp., and kept on a 12:12 h light: dark cycle. Females were acclimated to laboratory temperature and food conditions for at least 1 week before the eggs they produced were used in experiments, and were kept for a maximum of 4 weeks. To collect eggs, females were transferred to several 1 L egg production chambers (Hay, 1995) (maximum 40 individuals per litre) filled with freshly collected 95 μm filtered seawater.

Egg hatch experiments

Egg hatch experiments were carried out on *C. helgolandicus* eggs at 8, 12 and 15°C, and a *C. helgolandicus* versus *C. finmarchicus* comparison was carried out at 9°C (Table II). Eggs collected over 2 h were considered to be a cohort, with the mid-point of the incubation period as age zero. Up to 240 healthy eggs were placed individually in 0.2 μm filtered seawater and incubated in a controlled temperature laboratory. The eggs were inspected every 2 to 4 h until all eggs had hatched or were decomposing. Eggs that did not hatch were disregarded when calculating egg hatch times.

Nauplii development experiments

Development of *C. helgolandicus* nauplii was determined at 8, 12 and 15°C and comparisons made of starved, low level and high level single algal diets of *I. galbana* (IG) and *P. micans* (PM) (Table II), with the exception of a low level PM diet at 8°C. These food species were chosen as they are reliable in culture and to provide a contrast in size. We did not measure other species differences which may affect their quality as food. Development of *C. finmarchicus* nauplii at 12°C was investigated at low and high levels of a mixed PM and IG (PI) diet. A further series of development times at 10°C were measured using a mixture of *C. finmarchicus*

Table II: Summary of food and temperature conditions and final survival in egg hatch and nauplii development experiments

Egg hatch experiments	Temperature (°C)	No. eggs incubated	% hatch success	Experiment duration (days)			No. replicate experiments
C. helgolandicus	8	240	76.9	6			EH expts 3 and 4 (Nov)
	12	120	62.5	5			EH expt 2 (Sep)
	15	156	68.2	5			EH expt 2 (Sep)
C. helgolandicus	9	60	30.0	3.5			EH expt 1 (Jun)
C. finmarchicus	9	60	68.3	3.5			EH expt 1 (Jun)
Nauplii development experiments	**Temperature (°C)**	**Food regime**	**Mean food level in μMC (in μgCL^{-1})**	**Experiment duration (days)**	**Final % survival**	**Final stage**	**No. replicate experiments**
C. finmarchicus	12	PI-L	23.2 (278.9)	11	48.8	C1	Expt 2 (Aug)
	12	PI-H	88.0 (1057.0)	11	57.5	C1	Expt 2 (Aug)
C. helgolandicus	8	S	0.95 (11.4)	8	47.5	N3	Expts 3 and 4 (Aug)
	8	IG-L	25.3 (303.9)	12.5	27.5	N3	Expt 4 (Aug)
	8	IG-H	81.8 (982.9)	14	36.3	N4	Expt 4 (Aug)
	8	PM-H	37.2 (446.8)	21.5	25.0	C1	Expt 9 (Oct)
	12	S	1.5 (17.6)	11	8.8	N3	Expts 3 and 4 (Aug) & 6 (Sep)
	12	IG-L	26.1 (312.9)	13	27.5	N3	Expts 4 (Aug) and 6 (Sep)
	12	IG-H	91.4 (1097.2)	13	38.8	N5	Expts 4 (Aug) and 6 (Sep)
	12	PM-L	12.0 (143.6)	19	10.0	C1	Expt 7 (Sep)
	12	PM-H	46.2 (554.6)	17.5	42.5	C1	Expt 7 (Sep)
	15	S	3.8 (45.4)	8	2.5	N3	Expt 6 (Sep)
	15	IG-L	29.3 (352.5)	12	20.0	N3	Expt 6 (Sep)
	15	IG-H	99.5 (1195.3)	10.5	62.5	N5	Expt 6 (Sep)
	15	PM-L	8.9 (106.6)	11	70.0	C1	Expt 8 (Oct)
	15	PM-H	37.2 (446.3)	9.5	70.0	C1	Expt 8 (Oct)
Mixed *C. finmarchicus* and *C. helgolandicus*	10	IG-H	103.9 (1248.5)	9.5	30.0	N4	Expt 1 (Jun)
	10	PI-H	80.4 (966.1)	9.5	35.0	N5	Expt 1 (Jun)
	10	PM-H	63.1 (757.4)	9.5	52.5	N5	Expt 1 (Jun)

See Table I for description of food regime abbreviations. Approximately 5×10^3 cells L^{-1} of *P. micans* = 1 μMC = 12.011 μgCL^{-1}, 7×10^5 cells L^{-1} of *I. galbana* = 1 μMC = 12.011 μgCL^{-1}.
Final % survival and final stage determined from the final sample taken in an experiment.

and *C. helgolandicus* eggs at a high level of single PM, single IG and mixed PI diets.

Eggs collected over a 12 h period were considered a cohort and were incubated in groups of 40 in 250 mL bottles. Incubation food regimes were set up in 0.2 μm filtered seawater (Table II). Cell counts of batch cultures were made using a Coulter Multisizer II. Cell carbon values were calculated using published carbon to volume relationships for thecate (*P. micans*) and athecate (*I. galbana*) dinoflagellates (Menden-Deuer and Lessard, 2000). To maintain suspension bottles were incubated on a rotating wheel or a rocking incubator (2–3 rpm, 160°) in a controlled temperature room. Experiments were sampled every 12 h by sacrificing one bottle from each treatment. The water was retained for triplicate algal cell counts. Nauplii were staged, measured (total length, see Rey *et al.*, 2001) and assessed for morbidity using an inverted microscope.

The survival of fed nauplii was modelled using generalized linear models (McCullagh and Nelder, 1989) assuming over-dispersed binomial errors. A 'full' model that included temperature, food type and concentration was first fitted to the data, in which the proportion of nauplii that survived in each incubation bottle was a linear logistic function of time over the experimental duration. The model was then simplified in a backwards stepwise selection procedure. The relationship between the length of each naupliar stage and temperature, food type and concentration was investigated using linear mixed models (McCulloch and Searle, 2001), essentially analysis of variance for unbalanced data with between-individual and between-bottle variance components.

Calculation of median development time and moulting period duration

Median development time (MDT) (Peterson and Painting, 1990) for a given developmental stage was calculated by fitting a sigmoidal Hill function (Hill, 1913) to a plot of the percentage of the population in earlier developmental stages against time since the eggs were laid [equation (1)]. The Hill function is

$$y = \frac{Mx^p}{(c^p + x^p)} \tag{1}$$

where y is the percentage of the cohort in earlier developmental stages, x is time since eggs were laid (days), and M is the highest y-axis value (i.e. 100% when the entire cohort is in earlier developmental stages). The fitted coefficients P and c correspond to the gradient of the curve (between 75% and 25%) and the x value that produces 50% of the highest y-axis value (i.e. the MDT for that stage), respectively. Moulting period duration (MPD) (Campbell *et al.*, 2001) of a stage was calculated from the fitted Hill function as the time interval between the start of stage (95% of the cohort remaining in previous stages, Fig. 1) and end of stage (5% of the cohort remaining in previous stages, Fig. 1). As they are non-feeding stages, all N1 and N2 development data at a given temperature were pooled to calculate the MDT and MPD for these stages.

Model of *Calanus* development

Demographic models of zooplankton populations involving representation of life-history stages clearly require explicit sub-models of the developmental process. In their spatial population model of *C. finmarchicus*, Speirs *et al.* (Speirs *et al.*, 2005) combined the assumption that the temperature dependence of development time follows a Belehrádek function, with the assumption of equiproportional development. The result fitted the available data well but was sufficiently simple to be incorporated into a computationally efficient spatial population model. As a step towards a related population model for *C. helgolandicus*, we apply the same approach here.

The Belehrádek function assumes that the time from egg to the end of the ith stage, ES_i, is a non-linear function of temperature

$$\mathrm{ES}_i = \frac{1}{b_i(T + T_0)^{2.05}} \tag{2}$$

where T is temperature (°C), T_0 is a characteristic temperature and b_i is a stage-dependent coefficient. Corkett *et al.* (Corkett *et al.*, 1986) have shown that the exponent, 2.05, characterizes the temperature response of a large range of copepod species, and has been widely used in other studies. If we further assume that the characteristic temperature T_0 is the same for all developmental stages, then all the stages respond to temperature in the same way, and the proportion of the total egg-adult time spent in any given stage is temperature invariant. Thus, if PR_i is the proportion of egg-adult development time spent in stage i, we can express the stage duration of the ith stage, SD_i, as

$$\mathrm{SD}_i = \mathrm{PR}_i \times \mathrm{ES}_{\mathrm{adult}} \tag{3}$$

where $\mathrm{ES}_{\mathrm{adult}}$ is the egg-adult time obtained from equation (2).

To fit this model to our data (Table III), we used equation (2) to derive values for b_i for each developmental stage and an overall value for T_0. The MDT of a given stage occurs when half the cohort has developed to the end of the next stage, and is our best estimate of

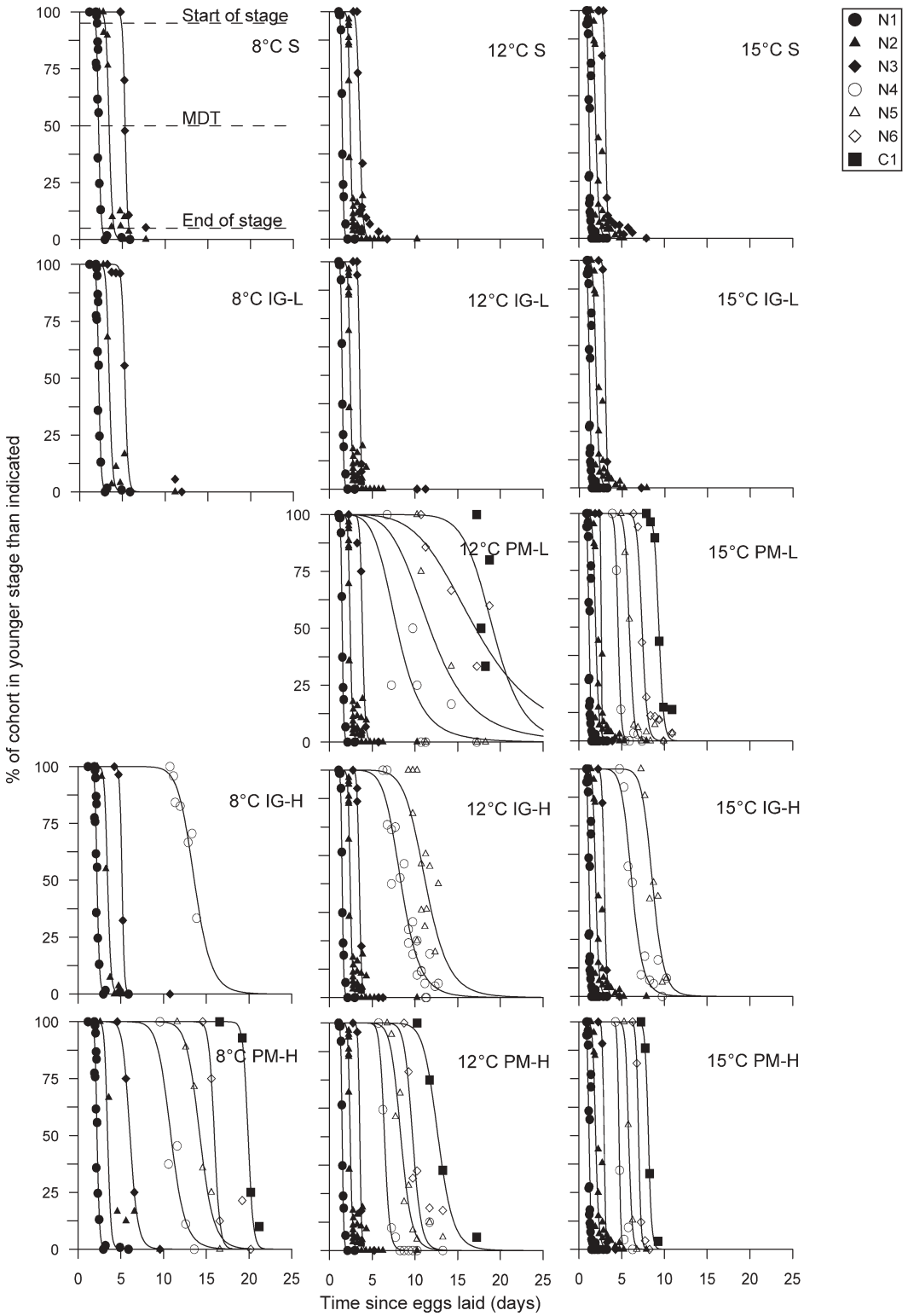

Fig. 1. *Calanus helgolandicus* nauplii development at different temperatures and fed different diets fitted with Hill functions. N1 data come from egg hatch experiments at each temperature. N2 Hill functions are fitted to data from all experiments at each temperature. See Table I for description of abbreviations.

time to end of stage. Therefore, the MDT of stage $i+1$ gives time to end of stage i (ES_i) which we use here. The parameters were estimated by Quasi-Newton minimization of the sum of squares. As this model assumes equiproportional development, which only applies when food is non-limiting, the model was fitted to all non-feeding N1 and N2 data but only to N3 to C1 data from the PM-H incubations. Having thus derived T_0, we use a food saturated *C. helgolandicus* egg to adult female development time of 24 days at 15°C taken from Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002) in equation (2) to yield the adult female value of b_i. This is equivalent to the value of b_{adult} needed in equation (3) (Table IV). The derived values of b_i and T_0 were also used in equation (2) to predict stage durations at 15°C, which in turn were used with the derived b_{adult} value in equation (3) to derive the proportion of egg to adult development time spent in each stage (PR_i) (Table IV). Figure 2 from Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002) also allowed us to estimate the proportional development of stages C1 to C5 (Table IV) to give a complete temperature-dependent model of *C. helgolandicus* development that assumes non-limiting food conditions. The same model was also fitted to *C. finmarchicus* development data from Campbell *et al.* (Campbell *et al.*, 2001).

The model of *C. finmarchicus* development from Speirs *et al.* (Speirs *et al.*, 2006) also contained a component describing the effects of food such that

$$ES_i = \frac{1}{(1 - e^{F/F_i})b_i(T + T_0)^{2.05}} \qquad (4)$$

where F is the food concentration and F_c is a characteristic food value. The food-dependent response seen in our data (see Results) is not straightforward and could not be satisfactorily described by this model, and so we do not include a food-dependent component in our current model.

RESULTS AND DISCUSSION

Naupliar development times and survival

For all diets, as temperature increased the development times decreased (Table III, Fig. 1). At all temperatures, the development of starved nauplii and those on an IG-L diet was effectively arrested at N3 (Table III) while all other diets successfully supported development past N3. Also at all temperatures, fastest development times and shortest MPDs were found in nauplii fed the PM-H diet (Table III, Fig. 1). The IG-H fed nauplii always took longer to develop compared to nauplii on the PM-H diet (Table III, Fig. 1). At 12°C, nauplii fed a PM-L diet developed more slowly, and with longer MPDs, than the PM-H diet (Table III, Fig. 1). In comparison, at 15°C, nauplii fed a PM-L diet developed at a rate similar to nauplii fed a PM-H diet (Table III, Fig. 1).

The overall patterns for *C. helgolandicus* nauplii development described here are decreasing development times with increasing temperature, food cell size and cell concentration. The underlying pattern of decreasing development times with increasing temperature has been found previously for *C. helgolandicus* (Thompson, 1982; Diel and Klein Breteler, 1986) and for many other copepod species (Peterson, 2001) and holds to the point at which nauplii begin to feed. After this, the pattern of development is also moderated by food quantity and quality. The extent to which the previous feeding history of females might affect the embryonic and post-embryonic development of *Calanus* was not examined as the females used in our experiments were all acclimated to excess food concentrations.

The effects of cell size and cell concentration on naupliar development can vary with temperature. Development was arrested at concentrations of small cells less than 30 μMC (360 μgCL^{-1}) at all temperatures, but similar low concentrations of large cells (PM-L) gave reduced development rates at 12°C and supported near optimal development rates at 15°C. This may reflect the effects of temperature on the food species as well as on the feeding nauplii. Phytoplankton growth rates, although varying with species, generally increase with temperature to some optimal temperature, (Berges *et al.*, 2002) and this may have been so in our incubations. We note that Kiørboe (Kiørboe, 1989) found copepod ingestion rates, in terms of nitrogen, increased with algal growth rate.

When fed at low food concentrations or unsuitable food types (e.g. *I. galbana*), the variability between individual development rates was increased which reduced the accuracy of development time estimates. It is widely accepted that low food concentrations will increase development times and the variability in development time between individuals (Mullin and Brooks, 1970; Vidal, 1980; Campbell *et al.*, 2001; Rey-Rassat *et al.*, 2002). Even at high food levels, when cell size is small, as in the IG-H experiments, the development times were longer and more variable than at high concentrations of large cell food (PM-H experiments). *Calanus* spp. copepodites select food particles based on size and are unable to feed efficiently on particles less than 10 μm (Frost, 1972). Evidence points to the fact that nauplii are similarly selective (Fernandez, 1979; Irigoien *et al.*, 2003) and may even be less efficient at feeding on

Table III: Experimentally derived C. helgolandicus naupliar median development times (MDTs) and moulting period durations (MPDs)

Nauplii development experiments			MDT of stage (days) (and fitted coefficient *P* in parentheses)							MPD of stage (days)						
Species	Temperature (°C)	Food regime	N1	N2	N3	N4	N5	N6	C1	N1	N2	N3	N4	N5	N6	C1
C. finmarchicus	9		1.4 (−83.1)							0.1						
	12	PI-L	ND	ND	3.5 (−13.7)	7.3 (−11.6)	8.4 (−16.0)	**10.6 (−8.5)**		ND	ND	1.5	3.8	3.1	**7.5**	
	12	PI-H	ND	ND	2.9 (−6.6)	5.6 (−30.1)	7.1 (−19.7)	8.7 (−17.2)	10.0 (−26.1)	ND	ND	2.7	1.1	2.1	3.0	2.3
C. helgolandicus	8		2.2 (−16.7)	3.4 (−18.9)						0.8	1.1					
	8	S			5.3 (−36.1)							0.9				
	8	IG-L			5.3 (−28.1)							1.1				
	8	IG-H			5.1 (−39.6)	13.6 (−13.8)						0.8	5.8			
	8	PM-H			6.1 (−13.8)	10.9 (−13.2)	14.2 (−17.6)	16.0 (−50.0)	19.9 (−67.2)			2.6	4.9	4.8	1.9	1.7
	9		1.7 (−12.7)							0.8						
	12		1.5 (−18.3)	2.4 (−18.6)						0.5	0.8					
	12	S			3.5 (−20.5)							1.0				
	12	IG-L			3.5 (−40.4)							0.5				
	12	IG-H			3.5 (−28.0)	8.4 (−7.8)	**11.3 (−8.5)**					0.7	6.5	**7.9**		
	12	PM-L			3.9 (−24.9)	**7.9 (−5.3)**	**11.7 (−4.8)**	**17.1 (−4.6)**	**19.1 (−10.7)**			0.9	**9.2**	**15.3**	**23.6**	**10.6**
	12	PM-H			3.6 (−36.2)	6.4 (−22.1)	8.4 (−13.4)	9.7 (−19.7)	12.7 (−14.3)			0.6	1.7	3.7	2.9	5.3
	15		1.2 (−9.3)	2.0 (−11.0)						0.8	1.1					
	15	S			3.1 (−32.5)							0.6				
	15	IG-L			3.0 (−30.9)							0.6				
	15	IG-H			3.0 (−22.3)	6.2 (−9.7)	8.6 (−13.2)					0.8	3.8	3.9		
	15	PM-L			2.6 (−131.2)	4.6 (−26.3)	5.9 (−22.7)	7.4 (−25.2)	9.3 (−34.3)			0.1	1.0	1.5	1.7	1.6
	15	PM-H			2.9 (−31.3)	4.7 (−44.6)	5.8 (−30.0)	7.0 (−48.5)	8.1 (−42.2)			0.6	0.6	1.1	0.8	1.1
C. finmarchicus and *C. helgolandicus*	10	IG-H			9.1 (−25.1)							2.1				
	10	PM-H			3.8 (−15.5)	7.1 (−15.3)	**9.5 (−11.9)**					1.4	2.8	**4.7**		
	10	PI-H			4.2 (−16.2)	7.1 (−20.9)	8.9 (−25.3)					1.5	2.0	2.1		

MDTs, *P* and MPDs of naupliar stages derived from fitted Hill functions [equation (1), see Method). ND, no data. Numbers in bold indicate values predicted from Hill functions with an r^2 of less than 0.8.

Table IV: Fitted parameters of C. helgolandicus and C. finmarchicus temperature-dependent development models under non-limiting food conditions and predicted stage durations (SD_i, days)

	C. helgolandicus			*C. finmarchicus*		
T_0	6.01			9.75		
b_{adult}	8.106×10^{-5}			6.186×10^{-5}		
b_i						
Egg	2.016×10^{-3}			1.505×10^{-3}		
N1	1.211×10^{-3}			9.122×10^{-4}		
N2	7.259×10^{-4}			5.724×10^{-4}		
N3	4.119×10^{-4}			3.034×10^{-4}		
N4	3.176×10^{-4}			2.414×10^{-4}		
N5	2.780×10^{-4}			2.024×10^{-4}		
N6	2.227×10^{-4}			1.700×10^{-4}		
C1	1.663×10^{-4}			1.437×10^{-4}		
C2	1.381×10^{-4}			1.215×10^{-4}		
C3	1.169×10^{-4}			1.018×10^{-4}		
C4	9.878×10^{-5}			8.165×10^{-5}		
C5	8.121×10^{-5}			6.186×10^{-5}		
PR_i						
Egg	4.027×10^{-2}			4.109×10^{-2}		
N1	2.680×10^{-2}			2.672×10^{-2}		
N2	4.478×10^{-2}			4.025×10^{-2}		
N3	8.532×10^{-2}			9.582×10^{-2}		
N4	5.848×10^{-2}			5.238×10^{-2}		
N5	3.644×10^{-2}			4.940×10^{-2}		
N6	7.256×10^{-2}			5.811×10^{-2}		
C1	0.1236			6.679×10^{-2}		
C2	0.0997			7.861×10^{-2}		
C3	0.1064			9.876×10^{-2}		
C4	0.1277			0.1496		
C5	0.1778			0.2425		
Temperature (°C)	8	12	15	8	12	15
SD_i						
Egg	2.2	1.3	1.0	1.8	1.2	0.9
N1	1.5	0.9	0.6	1.2	0.8	0.6
N2	2.5	1.5	1.1	1.8	1.2	0.9
N3	4.7	2.8	2.0	4.3	2.8	2.2
N4	3.2	1.9	1.4	2.3	1.5	1.2
N5	2.0	1.2	0.9	2.2	1.4	1.1
N6	4.0	2.4	1.7	2.6	1.7	1.3
C1	6.8	4.1	3.0	3.0	2.0	1.5
C2	5.5	3.3	2.4	3.5	2.3	1.8
C3	5.9	3.5	2.5	4.4	2.9	2.2
C4	7.0	4.2	3.1	6.6	4.4	3.4
C5	9.8	5.8	4.3	10.8	7.1	5.5

Fitted parameters T_0, b_{adult}, b_i and PR_i of *C. helgolandicus* and *C. finmarchicus* development models derived from equation (2). Predicted stage durations at 8, 12 and 15°C derived from equation (3) using data from this study, Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002) and Campbell *et al.* (Campbell *et al.*, 2001).

small particles (Paffenhöfer and Lewis, 1989). Thus, when sub-optimal food is available in the field, the range of development rates is likely to be greater than when food is optimal. This emphasizes the importance of taking food quality/size into account when estimating or modelling secondary production of copepods in the field.

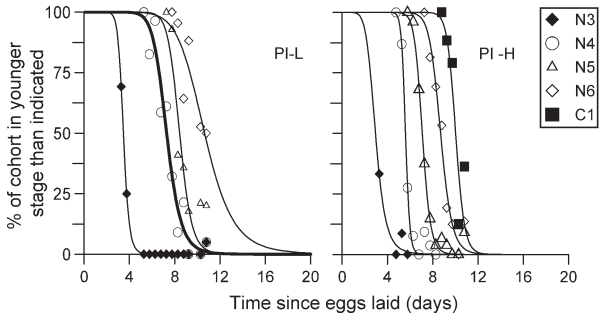

Fig. 2. *Calanus finmarchicus* nauplii development at 12°C fed a mixed *I. galbana* and *P. micans* diet (PI) at different levels fitted with Hill functions. See Table I for description of abbreviations.

It is important to note that there are also taxonomic differences between *I. galbana* and *P. micans* that may contribute to their differing suitability as food to support development rates in nauplii. *Prorocentrum micans* is a dinoflagellate covered with cellulose plates or thecae whereas *I. galbana* is a naked prymnesiophyte. The carbon to volume relationships of Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000) suggest that thecate dinoflagellates have, on average, 35% more carbon than similarly sized athecate species. Studies have also shown that different groups of marine phytoplankton have significantly different major nutrient (carbon, nitrogen and phosphorous content) and trace element composition (Quigg *et al.*, 2003). This means that the biochemical composition, and therefore food quality, of the two food species used in this study is probably completely different.

The egg hatch times from this study compare well with published egg hatch times for *C. helgolandicus* (Corkett, 1972; Thompson, 1982; Rey *et al.*, 2001). Rey *et al.* (Rey *et al.*, 2001) present a comprehensive study on the effect of *I. galbana* and *P. micans* diets on *C. helgolandicus* nauplii at 15°C. Their reported development times with *I. galbana* (43.3 μMC or 520 μgCL^{-1}) were consistently shorter than those in our study at 15°C with 100 μMC (1201 μgCL^{-1}) and shorter than those found by López *et al.* (López *et al.*, 2007) at 15°C and 66 μMC (800 μgCL^{-1}). This may indicate that the concentrations in our IG-H experiment were too high since Peterson (Peterson, 1986) found high mortality of *C. marshallae* nauplii with dense concentrations of phytoplankton (greater than 5×10^4 cells mL^{-1} of flagellates). This may be caused by larger changes in the oxygen levels and pH of incubation water with increased phytoplankton concentration, or by clogging of the naupliar mouthparts. However, *C. helgolandicus* N4-5 nauplii have shown saturated ingestion rates at about 66 μMC (800 μgCL^{-1}) of *I. galbana* (López *et al.*, 2007). Development times with *P. micans* (42.0 μMC, or

505 μgCL^{-1}) at 15°C from Rey *et al.* (Rey *et al.*, 2001) were almost identical to our 15°C PM-L development times (8.9 μMC or 107 μgCL^{-1}).

Egg hatch success was generally between 60 and 80% except on one occasion when only 30% of *C. helgolandicus* eggs hatched at 9°C (Table II). This result came from an experiment with animals collected in the field in June, a time of year when low hatch rates have been measured at this site (personal observation). It is possible that low fertilization frequency of these females occurred since they were acclimated to the same food conditions as in later experiments.

Confirming expectations, starved nauplii survival decreased as temperature increased (Table II); virtually no nauplii survived in the final incubation bottles sacrificed after 11 days at 12°C and 8 days at 15°C. Due to a shortage of eggs, the starved incubation at 8°C only ran for 8 days, by which time just less than 50% of nauplii were alive. The reduced metabolic rate as temperature decreases explains the longer survival of starved nauplii at lower temperatures. Overall, for fed nauplii, food type had a highly significant effect on survival ($P < 0.0001$). However, this effect involved interactions with the other explanatory variables and interpretation was simplified by modelling survival for the two diets, IG and PM, separately. For IG, the number of nauplii that survived in successive sacrificed incubation bottles decreased as incubation time increased ($P < 0.0001$), but there was no significant effect of temperature or concentration ($P > 0.05$). For PM, survival only decreased significantly with time at 12°C and low concentration ($P < 0.001$). For PM at the other combinations of temperature and concentration, survival increased with temperature ($P < 0.0001$), but there was no significant effect of time or concentration ($P > 0.05$). Other studies have found a significant decline in the condition and survival of *Calanus* sp. with decreasing food concentration (Paffenhöfer, 1970; Campbell *et al.*, 2001), although Paffenhöfer (Paffenhöfer, 1976) found total egg to C1 mortality of less than 4% for *C. pacificus* nauplii at 15°C fed only 5.9 μMC (70 μgCL^{-1}) of *P. micans*.

Length at stages N1 and N2 did not depend on any of the explanatory variables ($P > 0.05$). Temperature, food type and concentration all affected the length ($P < 0.05$) of later stages, but the relationships were complicated (i.e. involved interactions) and changed with naupliar stage. Although statistically significant, it was not possible to attribute any biological significance to these inconsistent relationships. A trend of increasing body size with increasing food concentration, and the effects of some food types on the size of copepodites and adults of *Calanus* species have been documented (Mullin and Brooks, 1970; Paffenhöfer, 1976; Vidal, 1980; Hygum *et al.*, 2000; Rey-Rassat *et al.*, 2002), but effects on nauplii are less clear. Previous studies have shown that the effect of temperature on size of copepod nauplii is not seen until the last naupliar or first copepodite stage (Twombly and Tisch, 2000; Campbell *et al.*, 2001; Lee *et al.*, 2003), which could explain why we found no clear differences in nauplii length between treatments. It is also possible that length differences between treatments are masked by our use of average length of nauplii, as the true differences would be seen in the final lengths of nauplii in a given stage.

The *C. finmarchicus* development times from our study agree with those published in Campbell *et al.* (Campbell *et al.*, 2001). For *C. finmarchicus* fed the PI-L diet, development times were slower and MPDs longer (Table III, Fig. 2) than nauplii fed the PI-H diet. For nauplii at 10°C fed either a PM-H diet or a PI-H (mixed) diet, development times (Table III) were very similar. This indicates that incubations with nauplii fed on PM diets are comparable with those fed a PI diet. The low survival of *C. helgolandicus* nauplii fed the PM-L diet at 12°C was not evident for *C. finmarchicus* fed the PI-L diet at 12°C, however the mean carbon food level in the *C. finmarchicus* incubation was nearly double than that in the *C. helgolandicus* incubation.

Model of *Calanus* development

Table IV gives the temperature-dependent development model parameters derived from equations (2) and (3) using data for non-limiting food conditions for *C. helgolandicus* and *C. finmarchicus*. The stage durations predicted by the models show that, at each temperature, *C. finmarchicus* develops more rapidly than *C. helgolandicus*.

The food and temperature-dependent *C. finmarchicus* development model of Speirs *et al.* (Speirs *et al.*, 2005; Speirs *et al.*, 2006) could not be fitted to the *C. helgolandicus* development data from this study. The delay in development produced by low food at 12°C violated the assumption of equiproportionality, so it became inevitable that the model failed when food effects were included. Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002) found a food-limited increase in development times at 15°C at a food level of about 11.7 μMC (140 μgCL^{-1}) of *P. micans*, which implies a critical food concentration of 6.5 μMC (78 μgCL^{-1}), but a model using this value does not fit the experimental data from this study. This leads to the conclusion that there is currently not enough data on food and temperature effects on *C. helgolandicus* development to understand or formulate functions to represent what appears to be a food-temperature interaction.

The *C. finmarchicus* food and temperature-dependent development model of Speirs *et al.* (Speirs *et al.*, 2005, 2006) gives a development rate threshold food concentration of 2.4 μMC (29.2 μgCL-1). Although we were unsuccessful in determining a critical food concentration for *C. helgolandicus* development, our data suggests that *C. helgolandicus* is more sensitive to low food concentration at temperatures that would prevail when the two species co-occur in the field. In the field this sensitivity, combined with the faster field development of *C. finmarchicus*, means that the more northern species may face lower natural and predation mortality than its southern congener *C. helgolandicus*, over equivalent development stages.

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