

# Effects of temporary food limitation on development and mortality of *Macoma balthica* larvae

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**ABSTRACT:** Long-term observations (1973 to 2001) of populations of the intertidal bivalve *Macoma balthica*, in the western Wadden Sea, have suggested that larval mortality is strongly density-dependent. In addition, density-independent factors may affect larval mortality. One possible source for such an effect is food limitation. In laboratory experiments, *M. balthica* larvae were reared under food limiting conditions, both quantitatively (high or low food level) and temporally (starvation during the first, second or third week). The results indicated that larvae offered high food levels grew significantly faster ( $6.9 \mu\text{m d}^{-1}$ ), and metamorphosed earlier (16.5 d) and at greater length (264  $\mu\text{m}$ ) than larvae subjected to low food level ( $4.4 \mu\text{m d}^{-1}$ , 19.3 d and 244  $\mu\text{m}$ , respectively). For both food levels, starvation in the first week resulted in a late metamorphosis at a large size, starvation in the second week resulted in an early metamorphosis at a small size, and starvation in the third week yielded intermediate results. Larval mortality was always lower in the low food condition, but the timing of starvation had no impact on mortality. This suggests that larvae, *in vivo*, do not die directly from food limitation alone. The results are discussed with reference to models of metamorphosis.

**KEY WORDS:** Bivalve larvae · Metamorphosis · Density dependence · Food limitation

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## INTRODUCTION

Fecundity of the intertidal bivalve *Macoma balthica* is proportional to adult stock density (Honkoop & Van der Meer 1997) (Fig. 1). However, a stock–recruitment relationship is lacking for this species (Van der Meer et al. 2001) (Fig. 1), where recruitment is defined as the number of 0 year-class individuals (>1 mm) present per  $\text{m}^2$  at the time of sampling in spring, 1 yr after spring spawning. Such an absence of a stock–recruitment relationship has also been demonstrated for other bivalve species (Loosanoff 1966). Thus, there appears to be a limiting factor (carrying capacity) in the larval or juvenile stages that determines maximum recruit density (Van der Meer et al. 2001). The mortality of *M. balthica* larvae or juveniles during the egg-to-recruit phase must thus be strongly density-dependent (Fig. 1) (Van der Meer et al. 2001, Philippart et al. 2003). Small variations in such density-dependent mortality rates

may explain the observed year-to-year variation in recruit densities (Van der Meer et al. 2001).

Many mechanisms exist which result in density-dependent mortality of larvae or juveniles, including intra-specific competition (Bertness 1989), diseases (Renault et al. 2000), size selective/aggregative predation (Elmgren et al. 1986, Hiddink et al. 2002) and competition for space (Roughgarden et al. 1985). Another important factor that may determine density-dependent mortality is food availability (Fenaux et al. 1994). A reduction of food levels may be due to competition between larvae, but competition with the adult stock is more probable, which may result in both density-dependent food limitation through a reduction of the food source, and in density-dependent predation of larvae through accidental ingestion. For *Macoma balthica* larvae, the filtration rates of adult *M. balthica* (Hummel 1985) are probably less important than those of dominant filter feeders such as cockles *Cerastoderma edule*

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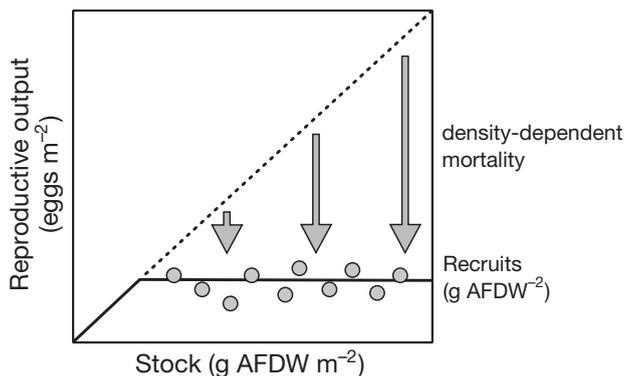


Fig. 1. *Macoma balthica*. Reproductive output of the bivalve stock. Output is proportional to stock density (Honkoop & Van der Meer 1997), but a stock-recruitment relationship is lacking (horizontal line) (Van der Meer et al. 2001); hence, the mortality during the egg-to-juvenile (pre-recruit) phase must be strongly density-dependent. AFDW = ash-free dry wt

and mussels *Mytilus edulis*, which more or less co-vary in abundance with *M. balthica* (Beukema et al. 2001).

In addition to density-dependent factors, density-independent factors may contribute to the egg-to-recruit mortality, such as match/mismatch of larvae and their food source (Philippart et al. 2003), the water temperature or off-shore transport due to effects of winds (Young et al. 1998). In our study area, the Wadden Sea, the food concentration may vary through time due to wind-driven turbidity and temperature (Cadée 1986).

Food availability will determine both when a larvae metamorphoses and at what size, i.e. excess food will generally result in earlier metamorphosis with a large size being attained with the converse being true when food is scarce (e.g. review in Morey & Reznick 2000). Differences in food availability may lead to differences in mortality, dispersal ability, age and size at first reproduction, and reproductive output (Smith 1987). For example, in amphibian research metamorphic characters of larval amphibians have attracted great attention because of their high plasticity and their strong influence on fitness (Wilbur & Collins 1973). Several models of amphibian metamorphosis have been developed to explain size patterns in relation to the timing of metamorphosis (Wilbur & Collins 1973, Leips & Travis 1994, Hentschel 1999). In general, metamorphosis models suggest that a minimum size threshold must be attained after which metamorphosis is possible, and that a maximum size exists at which metamorphosis occurs, regardless of environmental conditions (Wilbur & Collins 1973, Leips & Travis 1994, Hentschel 1999). The models differ primarily in the degree to which larval development remains flexible with increasing age. Wilbur & Collins (1973) propose that development remains flexible throughout the larval period, i.e.

larvae will initiate metamorphosis when mass-specific growth rates fall below a specific level. In contrast, Leips & Travis (1994) and Hentschel (1999) propose that development becomes fixed late in the larval period, i.e. after fixation, age at metamorphosis cannot be influenced by environmental circumstances (review in Twombly 1996).

For *Macoma balthica*, only a few observations have been made on the age and size at which metamorphosis occurs (Drent 2002). No observations have been made in relation to food availability. *M. balthica* larvae probably feed mainly on small unicellular phytoplankton species, as has been demonstrated for the larval Atlantic surfclam *Spisula solidissima similis* (Walker et al. 1998), in which densities *in situ* usually vary by several orders of magnitude ( $10^2$  to  $10^5$  cells  $\text{ml}^{-1}$ ; Cadée 1986). However, phytoplankton densities used during feeding experiments are usually 5 to  $20 \times 10^4$  cells  $\text{ml}^{-1}$  (e.g. Loosanoff & Davis 1963), indicating that *M. balthica* larvae in nature may experience sub-optimal food conditions during, at least, part of their development. This may have consequences for the age and size at metamorphosis.

The aims of this study were (1) to examine the *in vitro* effects of various food levels, both quantitatively and temporally, on mortality in *Macoma balthica* larvae; (2) to measure the size and age at which *M. balthica* larvae metamorphose under various food levels, both quantitatively and temporally; (3) to compare the observed results with those predicted by existing metamorphosis models. It was envisaged that low food levels would result in higher larval mortality and slower development compared to high food levels. In addition, early starvation should result in a higher mortality and a slower development rate compared to late starvation, since larvae would not be able to build up energy reserves.

## MATERIALS AND METHODS

**General methods.** Mature adult *Macoma balthica* were collected from the Mok Bay, Texel, The Netherlands ( $53^{\circ}00' \text{N}$ ,  $4^{\circ}45' \text{E}$ ) in March 2001 and stored at  $5^{\circ}\text{C}$  in aerated basins ( $50 \times 40 \times 10$  cm), ~100 individuals per basin, prefilled with sandy sediment. The bivalves were fed weekly with a mixture of concentrated algae (*Isochrysis* sp. and *Tetraselmis* sp., Reed Mariculture). To provide food for the developing *M. balthica* larvae, fresh *I. galbana* from a continuous culture system was used.

**Production and maintenance of larvae.** *Macoma balthica* were induced to spawn at the beginning of May 2001 following the procedure used by Honkoop et al. (1999). Fertilisation was carried out by pipetting

eggs of several females into a beaker and adding 1 to 3 ml of sperm suspension derived from 1 or more males. The resultant mixture was left undisturbed overnight at 15°C. Fertilised eggs (diameter approx. 100 µm) were then separated from all other matter, by rinsing them over stacked sieves of 120 and 50 µm. Subsequently, they were transferred into 2 l conical cylinders containing filtered (1 µm) and ultra violet irradiated seawater (UVFS) (salinity: 23 to 25) dosed with  $1.5 \times 10^{-5}$  g l<sup>-1</sup> penicillin G potassium salt and  $2.5 \times 10^{-5}$  g l<sup>-1</sup> streptomycin sulphate (Drent 2002). The larval cultures were gently stirred by means of air-bubbles and maintained at 15°C. At Day 3, the majority of the larvae had reached the D-stage.

**Experimental set-up.** Three treatments at 2 food levels were applied to the D-larvae (5 replicates per treatment). The food level was high ( $80 \times 10^3$  cells ml<sup>-1</sup> of *Isochrysis galbana*) or low ( $4 \times 10^3$  cells ml<sup>-1</sup>). The treatments consisted of a starvation period in the first week, the second week, or the third week (Table 1). The replicates consisted of 80 ml glass-bottles. The initial density of the larvae was 2 ml<sup>-1</sup>. To mix the water, all treatments were placed on a rollertable (4 to 5 rpm) and the water (UVFS) was changed 3 times per week.

**Mortality.** Larval densities were measured for each replicate (3 sub-samples per replicate) at Days 8, 15 and 22. The density at Day 8 was considered as the initial density. After the analysis, larvae were rinsed back into the bottles. The mortality rate was obtained from fitting a regression line through the natural logarithms of the densities through time, according to:

$$\ln(N_t) = \ln(N_0) + rt,$$

where  $N_t$  is the density of larvae at time  $t$  (larvae ml<sup>-1</sup>),  $N_0$  is the initial density at Day 8 (larvae ml<sup>-1</sup>),  $r$  is the mortality rate (d<sup>-1</sup>) and  $t$  is age (d) of the larvae.

Table 1. *Macoma balthica*. Overview of experimental set-up of 2-factor larval growth experiment over 3 wk period, whereby starvation period was introduced in first, second or third week. High (H) = 80 000 cells *Isochrysis galbana* ml<sup>-1</sup>, Low (L) = 4000 cells ml<sup>-1</sup>, 0 = 0 cells ml<sup>-1</sup> (starvation). (e.g. 0HH = starvation in Week 1 with high food levels in Weeks 2 and 3, H0H = high food levels in Weeks 1 and 3 and starvation in Week 2)

Food level	Starvation week	Code
High	1	0HH
	2	H0H
	3	HH0
Low	1	0LL
	2	L0L
	3	LL0

**Growth.** The shell length of 15 to 25 individuals per replicate was measured at Days 5, 10, 15, 17, 20 and 24 to the nearest 0.10 µm. The initial shell length at Day 3 (D-larvae) was  $160 \pm 1.5$  µm for all treatments. Growth rate (µm d<sup>-1</sup>) was calculated with the linear model:

$$SL = 160 + b(\text{Age} - 3),$$

where SL = shell length (µm), 160 is the initial shell length (µm) at Day 3, and  $b$  is the growth rate (µm d<sup>-1</sup>).

**Development.** The absence or presence of a foot was scored to determine larval development status. Larvae that showed a clearly visible foot bulging out of the shell (pediveligers) were considered to have metamorphosed. Average values for age at metamorphosis were calculated for each treatment with the logistic regression:

$$\ln[p(1-p)^{-1}] = \alpha + b\text{Age},$$

where  $p$  is the probability of passing metamorphosis. Average values for shell length at metamorphosis were calculated with the logistic regression:

$$\ln[p(1-p)^{-1}] = \alpha + bSL$$

To take into account the effects of food level, starvation period or any interactions, various logistic regression models were fitted to the data on absence or presence of a foot. The models were of the form:

$$\ln[p(1-p)^{-1}] = lp,$$

where  $lp$  is the linear predictor, calculated as the sum of a constant  $\alpha$  and the effects of independent factors and interactions of these factors. The model with the lowest sum of the deviance and  $\zeta$  times the number of estimable parameters should be used, where  $\zeta$  is a penalty for adding extra parameters to the model. By convention,  $\zeta$  ranges between 2 and 6 (Atkinson 1981). The age and size at which 50 % of the larvae had metamorphosed (reaction norm) were calculated by setting  $p$  at 0.5 in the best fitting logistic regression model, yielding  $lp = \ln[0.5/(1-0.5)]$ , i.e.  $lp = 0$ .

## RESULTS

### Mortality

The initial density (i.e. at Day 8) was  $0.88 \pm 0.03$  larvae ml<sup>-1</sup> (mean  $\pm$  SE). At the high food levels, the larval density was reduced by 69% to  $0.28 \pm 0.01$  larvae ml<sup>-1</sup> at Day 22 (Fig. 2). In contrast, at the low food levels, the density was reduced by 58% to  $0.37 \pm 0.04$  larvae ml<sup>-1</sup> (Fig. 2). Analysis of the mortality rates, using analysis of variance (ANOVA), revealed that at the high food levels ( $0.036 \pm 0.001$  d<sup>-1</sup>, mean  $\pm$  SE), mortality rates were higher than at the

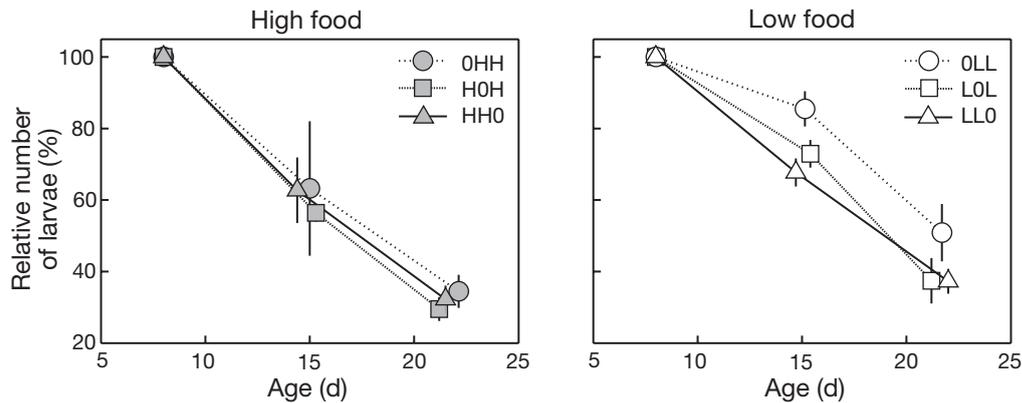


Fig. 2. *Maccoma balthica*. Relative number of larvae (mean  $\pm$  SE from Days 8 to 22 at high and low food levels). For clarity, data are slightly offset along x-axis. See Table 1 for treatment codes

low food levels ( $0.023 \pm 0.003 \text{ d}^{-1}$ ) (Tables 2 & 3). The timing of the starvation period (first, second or third week) had no effect on larval mortality rates (Table 3).

### Growth

The initial shell length at Day 3 was  $160 \pm 1.5 \mu\text{m}$  (mean  $\pm$  SE) for all treatments. At Day 24, larvae in the low (L) food level treatments had a shell length between  $228 \pm 18 \mu\text{m}$  (OLL) and  $266 \pm 8 \mu\text{m}$  (LLO), while larvae in the high (H) food levels had a shell length between  $292 \pm 19 \mu\text{m}$  (OHH) and  $319 \pm 15 \mu\text{m}$  (H0H) (Fig. 3, Table 2).

On average, larvae grew at a rate of  $5.7 \pm 0.7 \mu\text{m d}^{-1}$  (mean  $\pm$  SE) (Table 2). Analysis using ANOVA revealed that larvae at the high food levels grew faster ( $6.9 \pm 0.6 \mu\text{m d}^{-1}$ ) than larvae reared at the low food levels ( $4.4 \pm 0.5 \mu\text{m d}^{-1}$ ) (Table 3). Pairwise comparisons using Bonferroni's method revealed a signifi-

cantly lower growth rate of larvae starved in the first week ( $4.4 \pm 0.8 \mu\text{m d}^{-1}$ ) compared to growth rate of larvae starved in the second ( $6.2 \pm 0.5 \mu\text{m d}^{-1}$ ) and third week ( $6.4 \pm 0.6 \mu\text{m d}^{-1}$ ) (Fig. 3, Table 3). Correlation of larval growth rates and larval mortality rates revealed a positive correlation ( $r^2 = 0.76$ ,  $p = 0.01$ ) (Fig. 4).

### Development

The average age at metamorphosis was  $17.9 \pm 1.1 \text{ d}$  (mean  $\pm$  SE) at a length of  $254 \pm 6 \mu\text{m}$  (mean  $\pm$  SE) (Table 2). For the high food levels, metamorphosis occurred at an earlier age ( $16.5 \pm 1.5 \text{ d}$ ), and at greater length ( $264 \pm 6 \mu\text{m}$ ), than for the low food levels ( $19.3 \pm 1.2 \text{ d}$  and  $244 \pm 6 \mu\text{m}$ , respectively) (Fig. 5, Table 2). Furthermore, larvae starved in the second week metamorphosed earlier and at smaller length than larvae starved in the first or third week (Fig. 5). Larvae starved in the third week metamorphosed latest and at greatest lengths (Fig. 5).

Table 2. *Maccoma balthica*. Larval mortality rates between Days 8 and 24, shell length (SL) at Day 24, growth rates between Days 3 and 24, age at metamorphosis and shell length at metamorphosis. Data are means  $\pm$  SE. Treatment codes in Table 1

Code	Mortality ( $\text{d}^{-1}$ )	SL at Day 24 ( $\mu\text{m}$ )	Growth ( $\mu\text{m d}^{-1}$ )	Age at metamorphosis (d)	SL at metamorphosis ( $\mu\text{m}$ )
OHH	$0.035 \pm 0.003$	$291.9 \pm 19.3$	$5.51 \pm 1.09$	$19.4 \pm 1.3$	$274.8 \pm 3.1$
H0H	$0.038 \pm 0.003$	$319.2 \pm 15.3$	$7.61 \pm 0.45$	$14.3 \pm 0.2$	$252.5 \pm 0.5$
HH0	$0.035 \pm 0.007$	$312.2 \pm 19.7$	$7.69 \pm 0.78$	$15.8 \pm 0.3$	$264.6 \pm 8.2$
OLL	$0.021 \pm 0.005$	$227.5 \pm 17.7$	$3.28 \pm 0.87$	$21.7 \pm 0.8$	$255.2 \pm 3.3$
LOL	$0.030 \pm 0.006$	$258.2 \pm 5.1$	$4.87 \pm 0.17$	$17.6 \pm 0.7$	$234.1 \pm 2.7$
LLO	$0.030 \pm 0.003$	$265.5 \pm 7.7$	$5.17 \pm 0.37$	$18.5 \pm 0.9$	$244.0 \pm 2.5$
High	$0.036 \pm 0.001$	$307.8 \pm 8.2$	$6.94 \pm 0.63$	$16.5 \pm 1.5$	$264.0 \pm 6.4$
Low	$0.027 \pm 0.003$	$250.4 \pm 11.6$	$4.44 \pm 0.45$	$19.3 \pm 1.2$	$244.4 \pm 6.1$
Overall mean	$0.031 \pm 0.003$	$279.1 \pm 14.3$	$5.69 \pm 0.69$	$17.9 \pm 1.1$	$254.2 \pm 5.9$

Table 3. ANOVA of the effects of Food level and Starvation week on the mortality rate and growth rate of *Macoma balthica* larvae. SS = sum of squares, df = degrees of freedom, MS = mean squares, Week 1 = starvation period during Week 1, Week 2 = starvation period during Week 2, Week 3 = starvation period during Week 3. ns = not significant; \*p ≤ 0.05; \*\*p ≤ 0.01; \*\*\*p ≤ 0.001

Source	Mortality rate (d <sup>-1</sup> )				Growth rate (µm d <sup>-1</sup> )			
	SS (×10 <sup>-3</sup> )	df (×10 <sup>-3</sup> )	MS	F	SS	df	MS	F
Food level	5.853	1	5.853	5.51*	46.89	1	46.89	19.24***
Starvation week	1.893	2	0.946	0.89 <sup>ns</sup>	25.19	2	12.59	5.17***
Starvation week × Food level	0.962	2	0.481	0.45 <sup>ns</sup>	0.33	2	0.17	0.07 <sup>ns</sup>
Error	23.369	22	1.062		58.48	24	2.44	
Pairwise comparisons of starvation week	(Week 1 = Week 2 = Week 3)				Week 1 < (Week 2 = Week 3)			

Analysis of the data revealed that the model with the lowest sum of the deviance and ζ times the number of estimable parameters was  $lp = \alpha + (\text{week} \times \text{age}) + (\text{week} \times \text{length})$  (Table 4). According to this model the probability of passing metamorphosis is a function of shell length and age. Furthermore, the

value of the function (*lp*) differs per starvation period (Fig. 6).

*A posteriori* models used for pairwise comparisons of starvation weeks showed that starvation in Week 1 versus starvation in Weeks 2 and 3 resulted in a lower sum of the deviance and ζ times the number of estimable parameters than any other combination of starvation weeks (Table 4). This implies that an early starvation period leads to metamorphosis at a significantly larger size and higher age.

**DISCUSSION**

The current experiment revealed that at high food levels, *Macoma balthica* larvae grew faster (6.9 µm d<sup>-1</sup>) and metamorphosed earlier (16.5 d) at greater size (264 µm), than at low food levels (4.4 µm d<sup>-1</sup>, 19.3 d and 244 µm, respectively). In addition, it was determined that the probability of passing metamorphosis is a function of both age and size, where the value of the function is determined by the timing of the starvation period. Similar observations have been made for a variety of taxa, such as insects, amphibians and crustaceans (reviews in Twombly 1996, Morey & Reznick 2000). In particular, most species show a reduced age at metamorphosis (or maturity) with increasing growth conditions, often accompanied by a larger size at the transition.

The average growth rate recorded (5.7 µm d<sup>-1</sup>) compares well to that found by Drent (2002) of 5.2 µm d<sup>-1</sup> for *Macoma balthica* larvae at 15°C, as do the average age and size at metamorphosis, i.e. 20.6 d

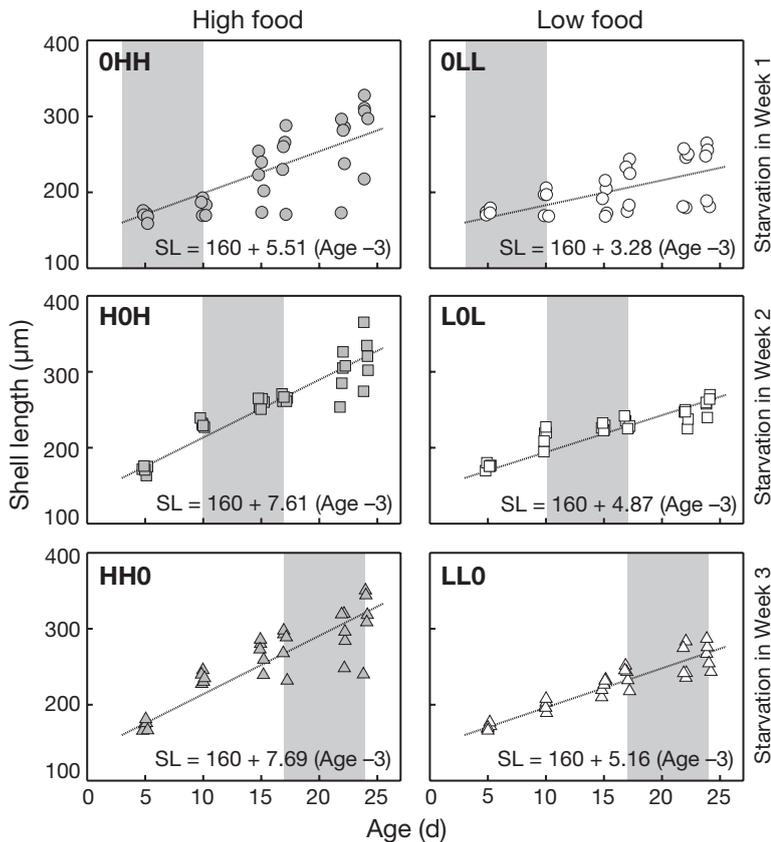


Fig. 3. *Macoma balthica*. Larval shell length per replicate for each treatment. Shaded blocks indicate weeks without food. Regression lines show growth rates (µm d<sup>-1</sup>) and are forced through the initial shell length of 160 µm at Day 3. For clarity, data are slightly offset along x-axis. See Table 1 for treatment codes

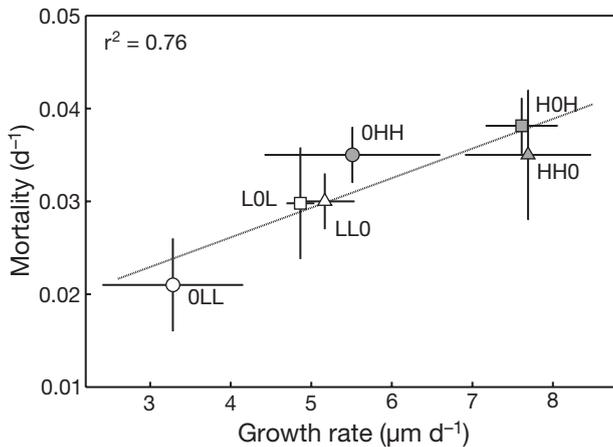


Fig. 4. *Macoma balthica*. Larval mortality (mean ± SE) plotted against growth rate. See Table 1 for treatment codes

and 254 µm (Drent 2002). In contrast, the recorded mortality rates (0.02 to 0.04 d<sup>-1</sup>) in the experiments were slightly lower than the egg-to-recruit mortality rate of 0.05 d<sup>-1</sup> determined by Philippart et al. (2003) and much lower than the range of natural daily mortality rates of meroplanktonic larvae of 0.13 to 0.28 d<sup>-1</sup> (review in Rumrill 1990). However, such results arise from the absence of predators, diseases, etc. in *in vitro* conditions.

Although it was predicted that the effect of timing of starvation on mortality would be greatest when the larvae started feeding, i.e. directly after the lecitho-

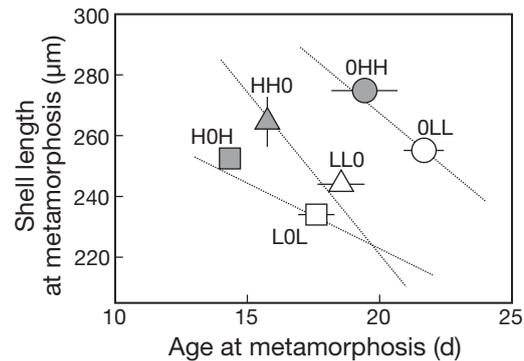


Fig. 5. *Macoma balthica*. Larval age and shell length (mean ± SE) at metamorphosis for different treatments, calculated with simple logistic models (see 'Materials and methods'). For comparison, regression lines of Fig. 6 are included. See Table 1 for treatment codes

trophic phase, mortality rates in early starvation periods were no different from mortality rates in later periods (Table 4). The length of the starvation period (7 d) was clearly not long enough to directly affect larval mortality. Larvae may have used energy reserves in the form of lipids to survive these periods. These reserves could have been built up in the period when larvae were provided with food or, for larvae that did not receive food in the first week, remained from the eggs (Videla et al. 1998). For instance, unfed surfclam larvae (*Spisula solidissima solidissima*) survived up to 15 d (Walker et al. 1998).

Table 4. Goodness-of-fit (in terms of deviance, Dev.) of the various models and the sum of the deviance and  $\zeta$  times the number of estimable parameters (No. est) for  $\zeta = 2, 4$  and 6 (see 'Materials and methods'). Model with the lowest sum (\*) is preferred. Age = age of *Macoma balthica* larvae (d), Length = shell length of larvae (µm), Week = starvation week (Week 1, Week 2 and Week 3), Food = food level (high and low), Treatment = combination of Week and Food

Mod. No.	Model	df	Dev.	No. est	Deviance		
					( $\zeta = 2$ )	( $\zeta = 4$ )	( $\zeta = 6$ )
1	Constant	2580	3219	1	3221	3223	3225
2	Age	2579	1993	2	1997	2001	2005
3	Length	2579	1528	2	1532	1536	1540
4	Age + Length	2578	1210	3	1216	1222	1228
5	Food × Age	2577	1880	4	1888	1896	1904
6	Food × Length	2577	1457	4	1465	1473	1481
7	Week + Age + Length	2576	1025	5	1035	1045	1055
8	Week × Age	2575	1653	6	1665	1677	1689
9	Week × Length	2575	1425	6	1437	1449	1461
10	(Food × Age) + (Food × Length)	2575	1199	6	1211	1223	1235
11	(Week × Age) + (Week × Length)	2572	994	9	1012	1030*	1048*
12	(Food × Age) + (Food × Length) + (Week × Age) + (Week × Length)	2569	987	12	1011	1035	1059
13	(Treatment × Age) + (Treatment × Length)	2491	801	90	981*	1161	1341

Post-hoc cross comparisons between treatments effects (i.e. timing of starvation) using Model 11 as the base, which is considered as the best model (lowest sum at '4' and '6' levels)

Week 1 < (Week 2 = Week 3)  
(p < 0.05)

In the current experiment the minimum size at which metamorphosis occurred was approximately 200  $\mu\text{m}$  (Fig. 6) and the relation between age and size at metamorphosis (reaction norm) was negative, i.e. fast growing larvae metamorphosed earlier at larger size than slow growing larvae. These results support both fixed and flexible development models. Furthermore, starvation in the second week led to an earlier metamorphosis at smaller size than starvation in the third week (Fig. 5). These results support the flexible development model of Wilbur & Collins (1973), in which at rapid growth rates metamorphosis is delayed to capitalise on this growth and at slow growth rates metamorphosis is initiated to leave the deteriorating habitat (Wilbur & Collins 1973).

The results demonstrated that larvae metamorphosed successfully in all treatments and that larvae adjusted their age and size at metamorphosis to different food limiting conditions, while mortality rates were not increased at the low food levels or in early starvation periods. The ecological meaning of the results is not clear yet, since the diet of the *Macoma balthica* larvae consisted of *Isochrysis galbana* and not of a natural phytoplankton assemblage. In another experiment (Bos et al. 2006) in which *M. balthica* larvae were reared on natural phytoplankton, however, larvae often did not metamorphose due to food limitation, suggesting that some minimal energy intake is required to complete metamorphosis. The mortality rate also did not increase with decreasing food levels, as observed in this experiment. *M. balthica* larvae in nature are therefore supposedly adapted to varying food supplies to a certain extent, by possessing sufficient energy reserves to survive temporary or longterm food limitation, as has been reported for *Crassostrea virginica* larvae (Loosanoff 1974).

The results suggest that direct mortality through food limitation is not likely as a mechanism causing strong density-dependent or density-independent larval mortality. The observed egg-to-recruit density-dependent mortality of *Macoma balthica* in the Western Wadden Sea (Van der Meer et al. 2001) may, however, be the result of a combination of factors. Assuming that larvae and adults compete for the same food source, and that larvae are accidentally ingested by the adult stock (André & Rosenberg 1991), then larvae will be

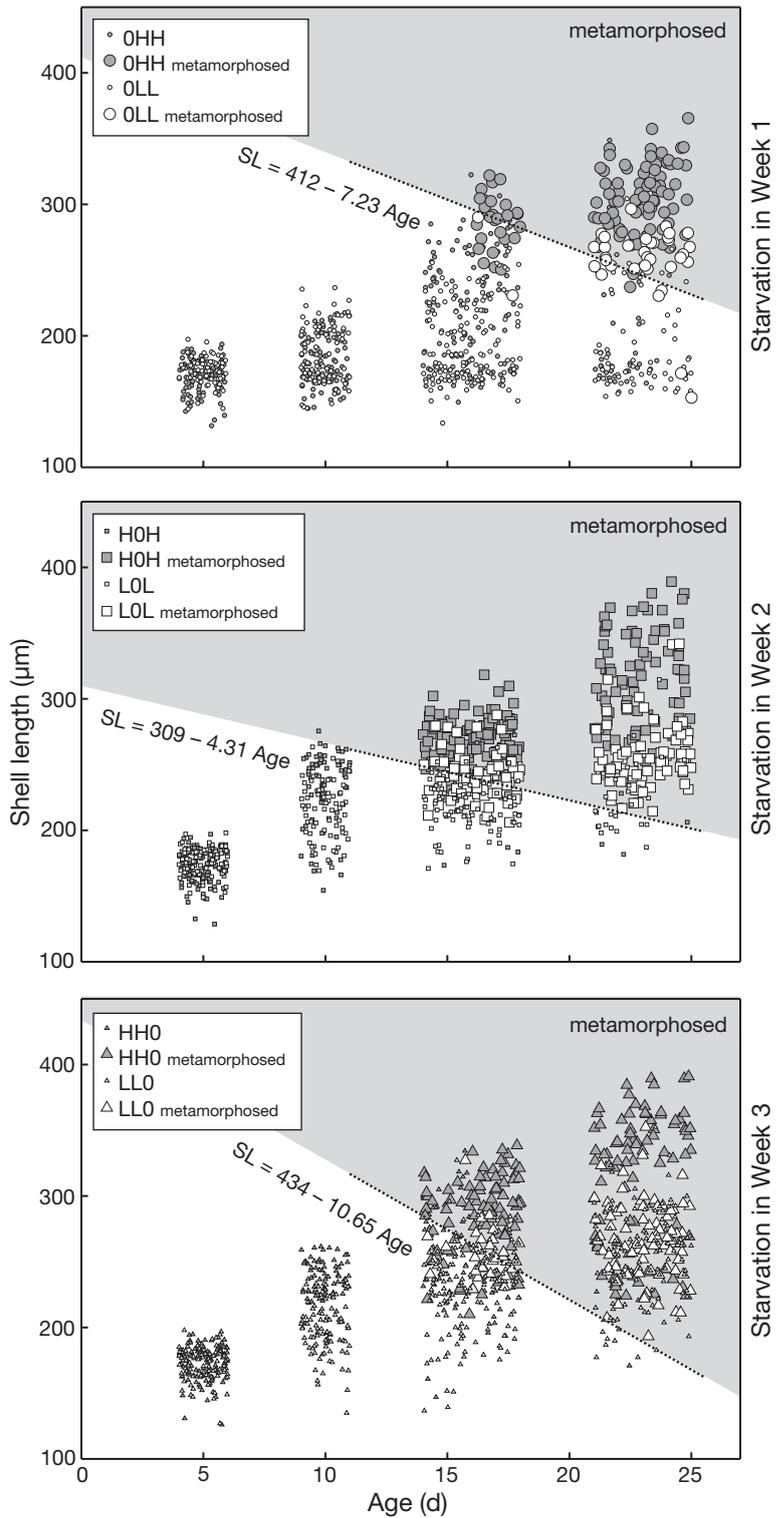


Fig. 6. *Macoma balthica*. Age (d) and shell length (SL) of individual larvae starved in the first, second or third week. Small symbols indicate non-metamorphosed larvae, large symbols metamorphosed individuals. Regressions indicate the transition to metamorphosis (reaction norms) and are calculated from the best fitting logistic model (see Table 4). For clarity, data are offset along x-axis. See Table 1 for treatment codes

more food limited at higher adult stock densities, resulting in a longer pelagic period, with an increased risk of being predated (Philippart et al. 2003). Food limitation also results in a smaller size at settlement, which make the juveniles more likely to be predated upon by size-selective shrimp (e.g. Hiddink et al. 2002). Such shrimp predation may be density-dependent, characterised by some density level below which predation is no longer profitable. Finally, food limitation in the larval phase may be followed by a poor juvenile condition in the post-settlement phase, resulting in increased post-settlement mortality (Phillips 2002).

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