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# Reproductive investment in the intertidal bivalve *Macoma balthica*

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**Abstract**

Bivalve eggs generally contain large amounts of lipids which, in comparison with proteins and carbohydrates, have high energy contents and are thus costly in energetic terms. As lipid contents vary between species, comparisons of reproductive investments should not only include numbers and sizes of eggs, but also their energy content. We estimated the investment in egg material of mature females of the Baltic tellin *Macoma balthica* (L.) in terms of both mass and energy content. All mass below a minimum body mass (below which no eggs are produced) was defined as structural mass. This threshold amounts to a body mass index (BMI) of 5.6 (ash-free dry mass per cubic shell length in mg $cm^{-3}$). More than half (55%) of the mass above the structural mass was invested in egg material and 45% in extra somatic tissue and tissue for production and storage of gametes. This means that the amount of eggs spawned ranged from 0 (at BMI = 5.6 mg $cm^{-3}$) to 33% of the total ash-free dry mass (at a high BMI value of 14 mg $cm^{-3}$). Eggs contained a relatively large amount of lipids, about 30% of their ash-free dry mass, whereas non-egg material contained only about 7% lipids. Eggs of two other bivalves in the Wadden Sea, the cockle *Cerastoderma edule* and the mussel *Mytilus edulis*, were smaller and contained only about 11% and 20% lipids, respectively. Energy content of *M. balthica* eggs amounted to ~0.006 J, in the other two species to ~0.002 J. The function of the more expensive eggs in *M. balthica* may be related to its early spawning in spring, causing slower larval development until first feeding. 

*Keywords:* marine bivalves; reproduction; total lipid; fecundity; egg size; egg mass; spawning

## 1. Introduction

In temperate areas most bivalve species produce pelagic larvae. The larvae of about 25% of the species living at such latitudes are completely dependent on stored nutrients until they reach metamorphosis (lecithotrophic development). The larvae of the other species are mainly dependent on planktonic food (planktotrophic development) (Thorson, 1946; Ockelmann, 1962; Mileikovsky, 1971), although they also feed on stored nutrients until their first-feeding stage (Loosanoff and Davis, 1963; Sprung, 1984; Gallager et al., 1986). Thus, for both types of development, larvae are dependent on their own nutrient storage for at least part of the time till settlement.

In contrast to the main energy source in adult bivalves, which is carbohydrate, especially glycogen (Bayne, 1976; Navarro et al., 1989), the main energy source for their larvae consists of lipids (Helm et al., 1973, 1991; Holland and Spencer, 1973; Bayne, 1976; Chu and Webb, 1984; Gallager et al., 1986; Whyte et al., 1992). The size of larval lipid stores

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appears to be of critical importance for survival, because in some species the proportion of larvae that reach the first-feeding stage was positively related to the initial lipid content of the eggs (Helm et al., 1973; Gallager and Mann, 1986).

For some species, a considerable proportion (up to 94%) of the total energy intake can be transferred to the gametes during gametogenesis (Bayne, 1976). During this period, carbohydrates are converted into lipids (in the gonads (mantle) or in the eggs) and stored in the ripening gametes (Gabbott, 1975; Bayne, 1976; Zandee et al., 1980; Bayne et al., 1982; Pipe, 1985, 1987; De Gaulejac et al., 1995; Galap et al., 1997). Costs of lipid synthesis are high, due to the high energy content of these compounds and to an extra conversion step in which glycogen is converted into lipids (Gabbott, 1975; Galap et al., 1997).

In spite of the relatively high costs of lipid synthesis, lipid content in bivalve eggs is high compared with other tissues. Lipid content of eggs of different species or different populations within a species is generally positively correlated to the length of the larval period until first feeding (Helm et al., 1973; Gallager and Mann, 1986). Eggs of species producing lecithotrophic larvae are generally larger than eggs of planktotrophic larvae-producing species (Vance, 1973; Strathmann, 1985) and contain more energy-rich substances, such as lipids (Crisp, 1974), often also per volume-unit (Strathmann, 1985). For a number of bivalve species, published records of eggs are listed in Table 1, whereas lipid content values of the total soft tissue of a number of bivalves can be found in Beukema (1997).

Our study presents data about the spawned egg mass in the bivalve *M. balthica*. Using these data, together with fecundity data published earlier (Honkoop and Van der Meer, 1998), we estimated which proportion of the total body mass of *M. balthica* consists of the egg material. We also measured the lipid contents of the total body excluding egg and gonadal material, and of the eggs. These data were used to calculate the energy content of the total egg mass and the energy content of the other tissues together.

We compare egg sizes and lipid contents in *M. balthica* with such values in two other common bivalves with a different life history, the cockle *Cerastoderma edule*, and the mussel *Mytilus edulis* (Honkoop and Van der Meer, 1998). The differences in reproductive investment between these species are discussed and related to differences in spawning season.

## 2. Materials and methods

### *2.1. Experimental design*

In 1995, an experiment was performed to study differences in reproductive output of three bivalve species, *M. balthica*, *M. edulis*, and *C. edule*. Groups of the first two species were collected at Balgzand, a tidal flat area in the southwestern part of the Dutch Wadden Sea, whereas *C. edule* were collected in the Mok, a tidal area at the southern tip of the island of Texel. During the winter until the spawning period in spring, animals of each species were kept in the same set-up, using two replicates per temperature level at two different temperatures, cold (C) and mild (M). In each of the four plots, two tidal treatments (sub-plots) were performed, tidal (t) and subtidal (s). Thus, four treatments were performed, Cs, Ct, Ms, and Mt.

Table 1
Lipid content in eggs (% of dry mass) of different bivalves

| Species | Type of larval development | Egg lipid content (% dry mass) | References |
|---|---|---|---|
| *Nucula turgida* | lecithotrophic | 47 | Davis and Wilson, 1983 |
| *Crassostrea virginica* | planktotrophic | 17 | Gallager and Mann, 1986 |
| *Mercenaria mercenaria* | planktotrophic | 6–20 | Gallager et al., 1986; Gallager and Mann, 1986 |
| *Mytilus edulis* | planktotrophic | 15–22 | Gabbott, 1975; Bayne et al., 1975, 1978 |
| *Mytilus galloprovincialis* | planktotrophic | 22 | Sedano et al., 1995 |
| *Patinopecten yessoensis* | planktotrophic | 20.6 | Whyte et al., 1987 |
| *Teredo navalis* | planktotrophic | 35.5 | Mann and Gallager, 1985 |

The set-up was placed outdoors. Fresh sea water from the Marsdiep, the southwestern tidal inlet from the Wadden Sea, was pumped into the set-up at a rate of 8 $dm^3$ $min^{-1}$. To maintain differences in water temperature, the water was continuously cooled (by 2.5ºC) for the cold treatment, or remained untreated for the mild treatment. The average temperature during January, February, and March was 3.1ºC and 5.7ºC for the cold and mild treatments, respectively. For more details about the experimental set-up (split-plot design) see Honkoop and Beukema (1997).

### 2.2. Collection and treatment of lipid samples

To study mass and lipid allocation in the experimental groups of *M. balthica*, individuals were removed from the set-up just prior to spawning at the beginning of April 1995. For each of the eight sub-plots, shells of some of the collected animals were opened and sex was determined. Gonads (including gametes) were removed, which can be done relatively easily in *M. balthica*. Non-gonadal (i.e. somatic) tissues from eight females per sub-plot were pooled and stored at −35ºC. Prior to lipid analysis, frozen samples were freeze-dried for four days and these freeze-dried samples were ground to powder in a ball-mill.

Due to the fact that gonadal tissue of *M. edulis* (and to a lesser extent of *C. edule*) is intertwined between digestive organs, it is almost impossible to separate the tissues from each other. Therefore, for these two species, only data on the lipid content of freshly spawned eggs will be presented. The eggs of these species were treated as the eggs of *M. balthica*.

Whenever gametes were needed, about 100 individuals of *M. balthica*, *C. edule*, and *M. edulis* were stimulated to spawn. Therefore, groups of individuals were stored at 4ºC for a period of one to three days. To initiate spawning, individuals were transferred to beakers containing 100 $cm^3$ fresh seawater with a temperature of 12ºC (one individual per beaker), thus experiencing a temperature shock of 8ºC. Each 30 min the seawater was changed. After the first replacement the first individuals, mostly males, started to spawn. For more details see Honkoop and Van der Meer (1997, 1998). All spawns of simultaneously spawning females were pooled and concentrated by means of a hand-centrifuge. The pellets thus obtained were removed with a pipette and for later lipid analysis stored in Eppendorf cups at −35ºC. Prior to the lipid analysis, frozen egg-samples were also freeze-dried for four days.

### 2.3. Determination of ash-free dry mass

Because the ash content of the tissues is variable (Beukema and De Bruin, 1977), the lipid content has to be expressed as a percentage of the ash-free dry mass (AFDM). To determine the AFDM, a part of the tissue powder and of the eggs was placed in pre-weighed ($W1$) porcelain and platinum cups, respectively. Cups containing the freeze-dried tissues were weighed ($W2$), incinerated for 4 h at 580ºC, cooled to room temperature and weighed again ($W3$) and the percentage AFDM of total dry mass $\left[(W2 - W3)/(W2 - W1) \times 100\%\right]$ was calculated. The rest of the tissue powder was used for lipid measurements, using 5 mg AFDM per analysis if sufficient material was available.

Egg sizes of *M. balthica*, *C. edule*, and *M. edulis* were determined as described earlier (Honkoop and Van der Meer, 1997, 1998). Freshly spawned eggs were photographed on slides using a Zeiss camera fitted to a Zeiss binocular. After development, the slides were projected on a transparent screen and the eggs were measured, using a digital calliper.

To determine the mass per individual egg, mature *M. balthica* were collected at Balgzand just prior to spawning, and spawning initiated as described above. The clutches of eggs were fixed with a few drops of 40% formaldehyde, pooled, and a sub-sample was taken to count the total number of eggs, using a binocular (Honkoop and Van der Meer, 1997). The total clutch of fixed eggs was divided into four equal aliquots. The eggs were concentrated, transferred to clean glass tubes and dried for 4 days at 60ºC in a ventilated stove. After drying, the tubes were weighed ($W1$) and incinerated at 580ºC for 4 h, cooled to room temperature and weighed again ($W2$). The AFDM was calculated ($W1 - W2$) and the average ash-free dry mass per egg was determined.

To calculate the body mass at a standard shell length for each species per sub-plot, the AFDM of ten individuals of known shell length was determined. Therefore, shortly after collection, the animals were immersed in boiling water to kill them and open their shells. Subsequently, the bodies were

completely removed from the shell, transferred to porcelain cups, and dried for 4 days at 60ºC in a ventilated stove. The length of each shell was measured with a digital calliper to the nearest 0.01 mm along the anterior–posterior axis. The cups containing the dried flesh were weighed to the nearest 0.1 mg ($W1$). Subsequently the dried flesh was incinerated for 4 h at 580ºC, cooled to room temperature, weighed again ($W2$) and the ash-free dry mass (AFDM) was determined ($W1 - W2$). The body mass index (BMI) is defined as the AFDM/shell length$^3$ (mg cm$^{-3}$). The BMI value was a useful tool to compare the AFDM of animals with a different shell length, of groups of animals from different populations, or groups of animals which were exposed to different experimental treatments.

### 2.4. Lipid analysis

The analysis of the lipid content was based on the method of Zöllner and Kirsch (1962) and adapted for small sample aliquots (containing about 0.5 mg lipids). The following procedure was used: a sample containing about 5 mg AFDM was weighed (to the nearest 0.00001 g) in a clean and dry glass tube and 2 cm$^3$ concentrated sulphuric acid was added. About 45 glass tubes containing sample material (somatic tissue, gonadal tissue or eggs) and 3 glass tubes containing 0.5 mg lipid as standard solution were placed for 10 min in a water bath at 100ºC. Then the tubes were cooled in cold water for 5 min. From each tube, 50 mm$^3$ was placed in polyethylene tubes using a micropipette and during thorough mixing, 1 cm$^3$ colour reagent (containing 11.9 mole dm$^{-3}$ phosphoric acid and 8 mmole dm$^{-3}$ vanillin (4-hydroxy-3-methoxybenzaldehyde)) was added. After 35 min the absorbance at 530 nm was measured with a Hitachi UV–VIS type 181 spectrophotometer, using 50 mm$^3$ concentrated sulphuric acid and 1 cm$^3$ colour reagent as a blank. Using the absorbance of the standard solution, containing a known amount of lipids, the lipid content of the AFDM could be calculated. When the lipid content of more than one sample per sub-plot was determined, the mean value per sub-plot was calculated and this value was used for statistical analyses. Data from the split-plot design were statistically analysed with the appropriate ANOVA procedures in SYSTAT (Wilkinson, 1990).

To compare the lipid analyses according to the colorimetric method of Zöllner and Kirsch (1962) with the more frequently used gravimetric analyses based on the method described by Blight and Dyer (1959), we determined the lipid content of freeze-dried and powdered *M. edulis*. For the colorimetric method, the mussel meat was treated as described above and for the gravimetric lipid analyses we used the method of Blight and Dyer (1959) as modified by Booij and Van den Berg (1994). Both types of analysis were performed five times. The average lipid content of the powdered tissues of *M. edulis* determined colorimetrically was 7.8% (SD 0.3), and determined gravimetrically 6.0% (SD 0.2) of the ash-free dry mass. The standard deviation of the mean was small in both cases, but the average value determined colorimetrically was higher than the average value found gravimetrically. Thus both methods were accurate, but the colorimetric method has the advantage that it is suited for small sample sizes (containing 0.5 mg lipids).

## 3. Results

### 3.1. Total egg mass per female

Based on numbers of spawned eggs, egg size and density of eggs, it was possible to estimate the amount of egg material, expressed as mass unit, spawned by *M. balthica* females over a range of BMI values. Honkoop and Van der Meer (1997) showed that the number of eggs spawned per female was linearly related to the body mass index (BMI in mg cm$^{-3}$) of the female at the beginning of the spawning season: i.e. body mass ($m$ in mg) divided by cubic shell length ($l$ in cm):

$$\mathrm{BMI} = m \times l^{-3} \quad (1)$$

For BMI values lower than 5.6 mg cm$^{-3}$ (which is equivalent to an ash-free dry mass of 18.9 mg for a 15 mm standard female) no eggs were produced. This body mass can be considered as the structural body mass, i.e. the minimal body mass, necessary for a functional normal life (Van der Meer and Piersma, 1994). At higher BMI values, the number of eggs ($y$) was proportional to the additional mass, i.e. to (BMI − 5.6) (Honkoop and Van der Meer, 1997).

For a standard individual of 15 mm, the following relationship was obtained:

$$y = 7739(\mathrm{SE} = 1463) \times [\mathrm{BMI} - 5.6(\mathrm{SE} = 2.2)] \quad (2)$$

Eq. 2 was based on six field groups (each group contained at least seven empty females) and twenty experimental groups (each group contained at least four empty females) and described earlier in Honkoop and Van der Meer (1997). Egg sizes differed slightly between these 26 groups, and in order to estimate the relationship between total egg mass spawned per female and BMI, we first assessed, for each group separately, the total mass of spawned egg by multiplying the total number of freshly spawned eggs by the size of these eggs (expressed in volume units) and a constant conversion factor for the ash-free dry mass per volume-unit (density). For the density, one overall estimate was obtained by using a test sample. For this test sample (four replicates, each containing about $10^5$ eggs obtained from a field group), the average diameter was 107.8 μm (SE = 1.2) (which is equivalent to a volume of 655927 $\mu m^3$ (SE = 12647), assuming that the egg is a sphere). The average ash-free dry mass per egg was 0.257 μg (SE = 0.015). Thus the calculated average density was 0.257 μg/655927 $\mu m^3$ = 0.39 g $cm^{-3}$ (SE = 0.02). This density was used for all 26 groups of *M. balthica* females in which BMI and egg number were estimated. The thus calculated values of total ash-free dry mass (in mg) spawned as eggs by 15-mm standard females are shown in Fig. 1A as open points. The best linear fit ($R^2 = 0.58$, $P < 0.001$) for these data points was:

$$\text{egg mass} = 1.869(\mathrm{SE} = 0.329) \times [\mathrm{BMI} - 5.6(\mathrm{SE} = 2.0)] \quad (3)$$

which revealed that above the structural body mass (the mass at 5.6 BMI units), 1.869 mg ash-free dry mass per extra BMI unit was spawned as egg material. In standard-length females, the total ash-free dry body mass increase per BMI unit increase amounts to $1.5^3 = 3.375$ mg (from Eq. 1). Thus the proportion of all mass over the structural body mass of 18.9 mg put into eggs amounts to 1.869/3.375 = 0.55 or 55%. Using Eq. 1 it is also possible to calculate the investment in egg mass spawned at a range of BMI values as a percentage of total ash-free dry mass

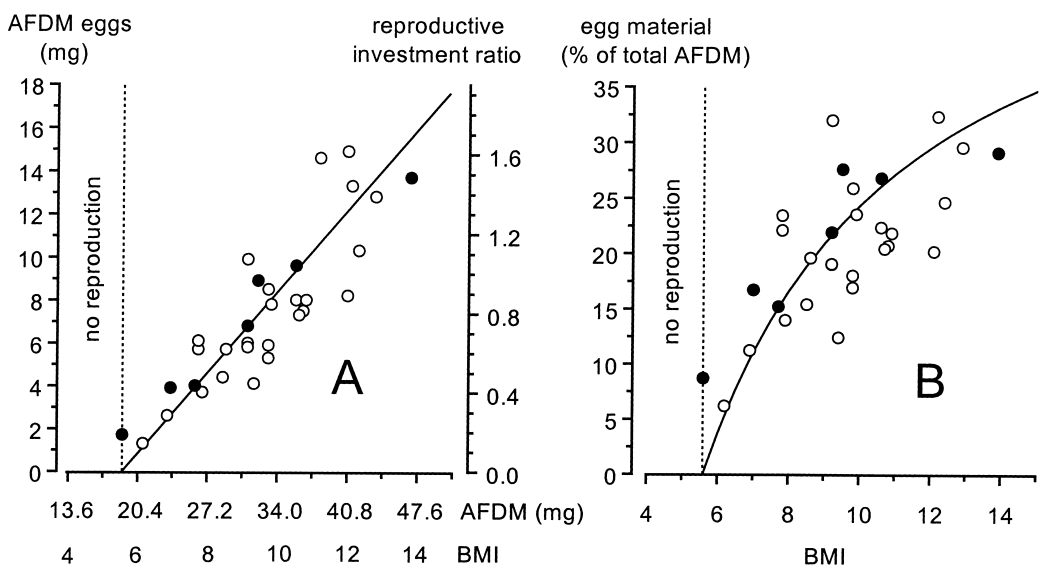



Fig. 1. (A) Investment in egg material by female *M. balthica* with a standard shell length of 15 mm at a range of BMI values, expressed both in weight of egg mass (mg AFDM) (left *Y*-axis), and in relative reproductive investment, i.e. energy in gonads, eggs and extra somatic tissue divided by the energy content of the structural body mass (right *Y*-axis). BMI is AFDM divided by cubic shell length (mg $cm^{-3}$). (B) Reproductive investment in egg material by female *M. balthica* as a function of the total body mass. The lines represent the best fit through our data (circles). Dots represent investment in egg mass calculated from measurements made by De Wilde and Berghuis (1978).

(Fig. 1B shows the results as open points). At a BMI range of 5.6 to 14 mg cm$^{-3}$, between 0 and ~33% of the total ash-free dry mass is released as egg material during the spawning season.

Per BMI unit increase (above BMI = 5.6), 7739 eggs are produced (Honkoop and Van der Meer, 1997), weighing 1.869 mg (see Eq. 3). This means that the average mass of an individual egg in the experimental and field groups was $(1.869/7739) \times 10^3 = 0.24$ μg. This would be equivalent to a 106.0 μm diameter (at a density of 0.39 g cm$^{-3}$). This value is close to the mean diameter of eggs of the experimental and field groups as described by Honkoop and Van der Meer (1997, 1998).

### 3.2. Validation of reproductive investment

Our data on egg mass can be compared with data published by De Wilde and Berghuis (1978). They collected adult individuals of *M. balthica* at Balgzand and weighed the clutch of eggs of empty spawned females within a range of shell lengths and BMI values. We recalculated these data to a standard *M. balthica* and present the spawned egg mass (in mg) as solid points in Fig. 1. The best linear fit (Fig. 1A, $R^2 = 0.97$, $P < 0.0001$) for these data was:

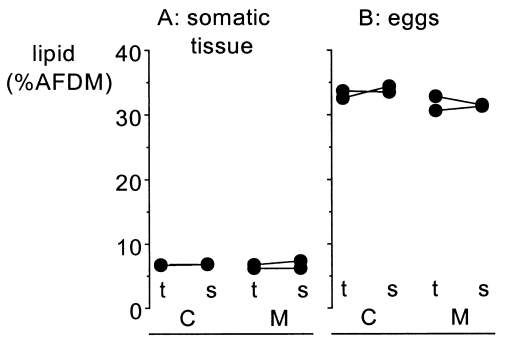
$$\text{egg mass} = 1.495(\text{SE} = 0.117) \times [\text{BMI} - 4.5(\text{SE} = 0.8)] \quad (4)$$

Statistical analysis revealed that the slopes of the lines of the two data sets available (described by Eqs. 3 and 4, respectively) were not significantly different (test on the homogeneity of slopes, $F_{[1,29]} = 0.62$, $P > 0.1$), neither were the intercepts (ANCOVA, $F_{[1,29]} = 0.08$, $P > 0.1$).

### 3.3. Energy value of reproductive mass

Mean lipid contents of non-gonadal (i.e. somatic) tissues of mature females of *M. balthica* are shown in Fig. 2A. The mean values of the eight different groups (one from each sub-plot) were relatively low, around 7% of the AFDM. The lipid content of freshly spawned eggs was substantially higher, about 32.5% of their AFDM (Fig. 2B).

Because no significant differences in lipid content in somatic tissue was observed between experimental groups subjected to different treatments (Fig. 2A),




| | df | A: somatic ss | A: somatic F | B: eggs ss | B: eggs F |
|---|---|---|---|---|---|
| C vs. M | 1 | 0.05 | 0.1 | 7.69 | 10.6* |
| t vs. s | 1 | 0.08 | 1.6 | 0.15 | 0.2 |
| Interaction | 1 | 0.02 | 0.3 | 0.65 | 0.7 |
| Plot | 2 | 0.71 | | 1.45 | |
| Error | 2 | 0.11 | | 1.94 | |

Fig. 2. Lipid contents (as percentage of the total ash-free dry mass) just prior to spawning of (A) somatic tissue of eight pooled individuals per sub-plot per treatment, and (B) freshly spawned eggs of experimental groups of female *M. balthica*. Each line connects the lipid percentage of the tidal (*t*) and subtidal group (*s*) for each plot at two temperature treatments, cold (*C*), and mild (*M*). At the bottom of each part of the graph, an analysis of variance is given. Significance levels are indicated by ** if $P < 0.05$, and by * if $P < 0.10$.

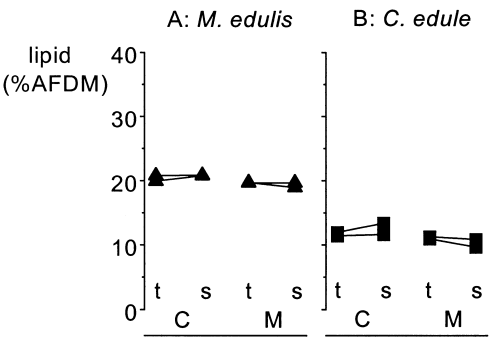
mean lipid percentages could be calculated for this part of the body. The differences between treatments in lipid content of the eggs (Fig. 2B) were small too, and again an average value for the egg lipid content was calculated. The lipid content of somatic tissues amounted to 6.7% (SE = 0.1), and of eggs to 32.5% (SE = 0.7) of the AFDM. The relatively small standard errors indicate that the lipid percentages in the various body compartments (eggs and somatic tissues) differed significantly.

The value of the X-axis intercept of the line represented by Eq. 3, BMI = 5.6 mg cm$^{-3}$ equalling 18.9 mg AFDM for a standard female, is used as the weight of the structural body mass. Using caloric values for lean mass (total mass minus lipid and ash mass) and lipids in *M. balthica* of 18.8 and 36.1 kJ g$^{-1}$, respectively (Beukema and De Bruin, 1979) and lipid percentages of the different tissues (somatic tissue and eggs), the energy content of the structural body mass of a standard female was 377.2 J (18.9 mg AFDM containing 6.7% lipid and 93.3% lean mass). Furthermore it was possible to calculate the energy

content per extra BMI unit (3.375 mg AFDM) above the structural BMI, containing 55% eggs (1.869 mg AFDM) and 45% other tissue (1.506 mg AFDM). Per extra BMI unit, the energy content of the total animal increases by 75.7 J, i.e. 45.6 J in eggs (1.869 mg containing 32.5% lipid and 67.5% lean mass) and 30.1 J in other tissues (1.506 mg containing 6.7% lipid and 93.3% lean mass). Because 7739 eggs are produced per extra BMI unit, the energy content per egg amounts to $45.6/7739 = 5.9 \times 10^{-3}$ J. By dividing the extra energy (all energy above the energy present in the structural body mass) by the energy content of the structural body mass it was possible to calculate the relative investment in extra somatic tissue, gonads, and eggs in animals with various BMI values. The energy value of the extra mass at any BMI value is $(\text{BMI} - 5.6) \times 75.7$. An individual with a high BMI of 14 thus contains $635.9/377.2 = 1.7$ times more energy in its extra mass than in its structural mass. These values are shown on the right-hand axis in Fig. 1A.

### 3.4. Comparison with C. edule and M. edulis

The lipid contents of freshly spawned eggs in *M. edulis* (20.0% of AFDM, Fig. 3A) and *C. edule* (11.4% of AFDM, Fig. 3B) were lower than the lipid content of eggs spawned by *M. balthica* (32.5% of AFDM, Fig. 2B). Per unit of weight, *M. balthica* eggs contained 2 to 3 times more lipids. If the density of eggs of *C. edule* and *M. edulis* is the same as for eggs of *M. balthica*, e.g. 0.39 g AFDM per $cm^3$, and the caloric value of lipids and lean mass are similar in the three species (18.8 and 36.1 kJ $g^{-1}$), then it is possible to calculate the energy content per egg. The results (Table 2) indicate that the eggs of *M. balthica* contained about three times more energy ($5.9 \times 10^{-3}$ J $egg^{-1}$) than the eggs of *C. edule* or *M. edulis* ($1.97 \times 10^{-3}$ and $1.78 \times 10^{-3}$ J $egg^{-1}$, respectively).




| | df | ss | F | ss | F |
|---|---|---|---|---|---|
| C vs. M | 1 | 1.92 | 21.2** | 3.96 | 4.1 |
| t vs. s | 1 | 0.34 | 0.4 | 0.00 | 0.1 |
| Interaction | 1 | 0.53 | 6.1 | 1.42 | 5.1 |
| Plot | 2 | 0.18 | | 1.94 | |
| Error | 2 | 0.18 | | 0.55 | |

Fig. 3. The lipid contents (as percentage of the total ash-free dry mass) of freshly spawned eggs of (A) *M. edulis* and (B) *C. edule*, in experiments performed in 1995. Each data point is the mean of pooled eggs spawned by two to seven females. Each line connects the lipid percentage of the tidal (*t*) and subtidal group (*s*) for each plot at two temperature treatments, cold (*C*), and mild (*M*). At the bottom of each part of the graphs, an analysis of variance is given. Significance levels are indicated by ** if $P < 0.05$ and by * if $P < 0.10$.

## 4. Discussion

### 4.1. Reproductive investment of M. balthica

The body of a *M. balthica* can be divided into different parts, structural mass and stores. The structural part is defined as the minimum body mass at which a normal life is possible but no stores are

Table 2
The calculated energy content per egg of freshly spawned eggs of *C. edule*, *M. balthica*, and *M. edulis* as observed in 1995

| Species | Size (μm) | Volume ($\times 10^{-7}$ $cm^3$) | Mass (μg) | Lipid (%) | Lipid (μg) | Lean mass (μg) | Energy ($\times 10^{-3}$ J $egg^{-1}$) |
|---|---|---|---|---|---|---|---|
| *C. edule* | 77.5 | 2.44 | 0.096 | 11.4 | 0.011 | 0.085 | 1.97 |
| *M. balthica* | 105.6 | 6.17 | 0.242 | 32.5 | 0.078 | 0.164 | 5.93 |
| *M. edulis* | 73.2 | 2.05 | 0.080 | 20.0 | 0.016 | 0.064 | 1.78 |

Egg size is measured as the egg diameter (μm) (Honkoop and Van der Meer, 1997). The lipid percentage of the eggs is the average value of eight experimental sub-plots from which data are shown in Fig. 2.

available for growth or reproduction (Van der Meer and Piersma, 1994). However, the structural part does contain some reserves, not in the sense of stores such as glycogen and lipids, but in the sense of, for example, muscle material that can be mobilised during periods of starvation. The structural body mass is thus higher than the body mass at which the animal dies. In *M. balthica* females, the structural body mass at 15 mm shell length amounts to 5.6 mg ash-free dry mass per cubic cm (Honkoop and Van der Meer, 1997), equalling 18.9 mg AFDM or 377 J. The comparable mass value calculated from data published by De Wilde and Berghuis (1978) is lower, although not significantly different (Eq. 4). As BMI values below 4 to 5 mg $cm^{-3}$ can be found only in moribund animals, the threshold for recovery to normal life will be about this value.

If the body mass is higher than the structural body mass (BMI = 5.6), the extra mass can be considered as stores available for reproduction, and will primarily be present in the form of reproductive material. In the field, BMI values in spring range from 5 to 14 mg $cm^{-3}$. At the high BMI value (14), 636 J is invested in gonadal material, eggs and extra somatic tissue. The eggs released at BMI = 14 (about 15 mg eggs, Eq. 4) contain 383 J. The real costs of reproduction include an unknown amount of energy for production and storage of gametes (overhead costs). If 45% of the AFDM per extra BMI unit is considered as gonadal tissue and somatic tissue necessary to support gametogenesis (overhead tissue) (Bayne et al., 1982; Pipe, 1987), an estimate of the overhead costs could be made using the total energy content per BMI unit (if BMI > 5.6), 75.6 J, and the energy content of the overhead tissue per BMI unit, 30.3 J, thus $30.3/75.6 = 0.40$ or 40% are overhead costs. Because we have no data available on the amount of gonadal tissue, and it is impossible to separate somatic tissue into a part necessary for reproduction and a part that is not, it is not possible to estimate the overhead costs directly.

In fact, the costs of reproduction can be even higher than estimated. In this study important costs are neglected, namely the costs of synthesising eggs and gonadal tissues. No data are available to estimate these costs in *M. balthica*, but it is acknowledged that during gametogenesis a large part of energy from ingested food can be transferred to the gonads (Bayne, 1976; Bayne et al., 1983; Iglesias and Navarro, 1991).

### *4.2. Why does M. balthica produce such expensive lipid-rich eggs?*

In the Dutch Wadden Sea, spawning of *M. balthica* takes place at the end of March or at the beginning of April, 1–2 months earlier than the spawning period of *C. edule* and *M. edulis* (Honkoop and Van der Meer, 1998). As far as we know, *M. balthica* is the earliest spawning bivalve in this area. Two possible advantages of early spawning are (1) to avoid food competition during the early planktonic larval stages (later in spring, after the spawning of other bivalve species, the competition for food is intense), and (2) to avoid high predation pressure of zooplankton-eating predators, which are abundant later in spring (for example fish larvae), and benthic predators such as shrimps and crabs, whose abundance on the flats rapidly increases in the course of spring (Beukema, 1991, 1992).

Early spawning has some disadvantages too: the relatively low water temperature in early spring makes larval growth slower than later in the year. Development to the first feeding stage (D-stage) (Kraeuter et al., 1982) will indeed last longer in *M. balthica* than in *M. edulis* and *C. edule* (rough estimations: 4 days at 12.5ºC in *M. balthica*, and 2 and 1.5 day at 15ºC in *M. edulis* and *C. edule*, respectively, from own observations). Because of this longer non-feeding stage, more energy will be needed in *M. balthica* than in the other two species to reach the first-feeding stage. A second disadvantage might be low food availability to the larvae. Benthic algae (mainly diatoms) are already available in early March, but planktonic food (mainly flagellates) not until the end of March or early April, with peak values around mid-April or even in May (Cadée and Hegeman, 1979; Cadée, 1986). Due to their large size, most individual algal cells and colonies are probably not suitable food for the larvae. Thus despite the absence of food competition, the availability of suitable food items can be low in early April. Therefore, early spawning has to go with a large parental food supply to the eggs.

The main energy sources in adult bivalves are thought to be carbohydrates among which glycogen is the most important (Bayne, 1976; Navarro et al.,

1989). In most bivalve larvae, lipids are used as energy source (Helm et al., 1973, 1991; Holland and Spencer, 1973; Bayne, 1976; Chu and Webb, 1984; Gallager et al., 1986; Whyte et al., 1992). In many bivalve species, the survival to the first feeding stage is positively correlated with the egg lipid content. Thus, the fitness of the larvae depends on the lipid content of the eggs.

In *M. balthica*, the lipid content (expressed as a percentage of total ash-free dry mass) of eggs was indeed higher than in the eggs of the later spawning *M. edulis* and *C. edule* (∼1.8 and 2.8 times higher, respectively; Fig. 2). If the differences in egg size between the species are also taken into account (Table 2), the between-species differences are even larger. With an amount of $5.9 \times 10^{-3}$ J $egg^{-1}$, *M. balthica* produces relatively energy-rich, and thus expensive, eggs compared to the other species ($1.97 \times 10^{-3}$ J $egg^{-1}$ and $1.78 \times 10^{-3}$ J $egg^{-1}$ in *C. edule* and *M. edulis*, respectively). The energy per egg of *M. edulis* ($1.78 \times 10^{-3}$ J $egg^{-1}$) is within the range of values found by Bayne et al. (1978) in eggs obtained from experimentally stressed *M. edulis*.

Although the energy content per egg is higher in *M. balthica*, this does not necessarily mean that the energy content of the total clutch of eggs is always higher too. Under the same experimental conditions, *C. edule* produced about 10 times as many eggs as *M. balthica* (Honkoop and Van der Meer, 1998). This means that, although *C. edule* eggs contain 7 times less lipid (0.078/0.011, see Table 2) and 3 times less energy, the total investment per female could be higher than in *M. balthica*.

In conclusion, the costs of early spawning and the slow larval development until the D-stage of *M. balthica* larvae appear to be paid for by the production of large eggs containing a large amount of nutrients with a high caloric value (i.e. lipids).

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