Bivalve reproduction in the Wadden Sea

Effects of winter conditions on reproductive effort and recruitment

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RIJKSUNIVERSITEIT GRONINGEN

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Pieter Johannes Cornelis Honkoop

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te Goes

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Contents

Summary

Year-to-year variability in bivalve recruitment can be very high. Differences in recruit numbers of two to three orders of magnitude are not uncommon. Which causal factors are involved in determining recruit numbers is, until now, unknown as are the possible mechanisms. However, there are some environmental factors which are known to be correlated with recruit numbers, *e.g.* winter character and predation pressure. High numbers of recruits have been observed particularly after cold winters, whereas these numbers were generally low after mild winters. Possible explanations for this phenomenon are, (1) predators (juvenile shrimps or shore crabs) appear later in spring and in lower numbers on tidal flats after cold than after mild winters. During the period of appearance of these predators, the average size the bivalve spat has attained is larger after a cold than after a mild winter and higher proportions of spat are too large to be suitable as food for most predator individuals, and (2) loss of adult-bivalve body mass is lower during a cold winter than during a mild winter. Therefore, more reserves are available to develop gonads and gametes, resulting in a better reproductive output, *i.e.* more and / or larger eggs.

In the present thesis, effects of winter water temperature on reproductive output (egg numbers and egg sizes) were studied in particular. Ambient temperature affects the energy balance of an organism. The outcome of a positive balance (*i.e.* energy intake is higher than energy expenditure) is expressed in mass gain and can be used for growth of somatic and / or gonadal tissue. On the one hand, metabolic energy demands are higher at high temperatures than at low temperatures. On the other hand, food intake will increase at higher temperatures and possibly (partly) compensate for the higher energy demands. The net effect of these processes on changes of total body mass is unknown. One aim of this study was to prove that the magnitude of the usual seasonal mass loss in winter is causally related to temperature and food availability. Therefore, replicate groups of three in the Dutch Wadden Sea common bivalve species, the Baltic tellin *Macoma balthica*, the cockle *Cerastoderma edule* and the mussel *Mytilus edulis*, were subjected to one of two different temperature regimes (one resembling a cold to normal winter and the other a mild winter) and to one of two immersion regimes (*i.e.* feeding time) (one immersion time of

100 % and one of 57 %). Because temperature and immersion time treatments were combined (split-plot design), four replicate treatments were applied, *viz*. cold-tidal, cold-subtidal, mild-tidal, and mild-subtidal.

Changes in body mass were studied during winter until the spawning season in spring. Mass loss was more pronounced (or mass gain was slower) during periods of higher temperatures than during periods of lower temperatures. This resulted in higher values of body mass just prior to spawning at the regimes of lower than of the higher temperatures. In addition, mass gain was higher at the subtidal (long daily feeding periods) than at the tidal (short daily feeding periods) level. Both factors resulted in differences in body mass just prior to spawning between the four different groups. The highest body mass values were observed at the cold-subtidal treatment, the lowest at the mild-tidal treatment, and intermediate values at the cold-tidal and mild-subtidal treatments.

The most important aim of the present study was to estimate the consequences of winter temperature and immersion time on the reproductive output (*i.e.* egg size and egg number) of the three studied bivalve species. Therefore, groups of individuals subjected to the treatments described above were forced to spawn. The egg diameter was measured and the numbers of spawned eggs per female were counted. No differences between egg size between the four groups of *M. balthica* were observed, whereas eggs of *C. edule* were larger at lower water temperatures and eggs of *M. edulis* were larger at the subtidal level. These differences between the species can be explained by the differences of timing of their gametogenesis, which is finished before the winter in *M. balthica*, starts shortly before spawning in *C. edule*, or lasts until spawning in *M. edulis*.

Egg numbers per female (fecundity) differed dramatically between groups. Roughly ten times more eggs were produced by *M. balthica* and *C. edule* after a simulated cold winter than after a simulated mild winter. Effect of immersion time were less pronounced, but generally more eggs were produced by subtidal than by tidal *M. balthica* and *C. edule*. No data are available for *M. edulis*. In *M. balthica* and to a lesser extent also in *C. edule*, a strong positive relation was observed between adult body mass and fecundity. Thus, the temperature and feeding regimes primarily have affected body masses and as a consequence reproductive output (either or both as egg size and egg numbers).

Measurements in field-collected individuals of *M. balthica* confirmed the above experimentally collected evidence. After the cold winter of

1995/1996, the fecundity was much higher than after the mild winter of 1994/1995 and the fecundity was higher at lower than at the higher intertidal levels. Egg sizes differed in the field groups too, eggs were smaller at higher tidal levels. The highly significant positive relationship between the body mass just prior to spawning and fecundity was similar in the field and the experimental groups. Therefore, the two relationships could be combined to a common regression equation, predicting egg numbers from body mass just prior to spawning. Below a body mass of 5.6 mg Ash-Free Dry Mass per cubic shell length no eggs were produced, whereas about 7700 eggs were produced per extra mg AFDM of a *M. balthica* female with a standard shell length of 15 mm.

Using the above relationship between body mass and spawned egg numbers, together with the estimated caloric content and ash-free dry mass of individual eggs, it was possible to calculate the caloric content of the total clutch of eggs of individual females with different body mass values. Because the eggs of *M. balthica* proved to be very energy rich (due to the high lipid content), the investment in energetic term could be considerable in this species. Above a body mass of 5.6 mg per cm^3 shell length, 55 % of all extra mass was egg mass. This means (due to the relative high energy content of the eggs) that about 60 % of the energy of the extra mass was present in egg mass. Depending on its mass (5.6 - 14 mg AFDM per cm^3), a female *M. balthica* spawned 0 - 33 % of its total body mass as egg material. The early spawning *M. balthica*, (end of March or the beginning of April) produced larger energy richer eggs than the later spawning species *C. edule* and *M. edulis* (spawning only in May or June).

The final aim of this study was to estimate the effect of winter temperatures on recruitment and year-class strength via the mechanism of temperature-influenced variability in egg production. *M. balthica* was used as the test-species. For each year since 1972, data are available on number of adult individuals per m^2 just prior to spawning, their age and body mass per cm^3 shell length, and also the resulting number of recruits per m^2 in summer. These long-term data series were available for Balgzand, a tidal flat area in the southwestern part of the Dutch Wadden Sea. Using the relationship between body mass and of numbers spawned egg, the total egg production per m^2 was reconstructed for each of the years 1973 - 1996. Variance in winter temperature explained 68 % of the variance in fecundity and 23 % of variance in total egg production. The relationship between winter temperatures and annual recruitment was somewhat closer, as 37 %

of the variance in recruitment was explained by year-to-year variability in winter temperatures. However, only 7 % of recruitment variance was explained by temperature-influenced annual variability in egg production. This suggests that another mechanism, also related to winter temperature, was involved in determining recruit numbers. Although no experimental data are available, it is probable that predation by juvenile shrimp was an important factor in determining final recruit numbers. Another factor may have been density-dependent mortality of larvae during their pelagic life stage or during the period of settlement.

To summarise, winter temperature as well as immersion time influenced adult individual body mass. The body mass just prior to spawning was, at least in *M. balthica*, closely related to individual egg production and to total egg production per square meter. However, only a minor part of the variation in recruit densities could be explained via this mechanism.

CHAPTER ONE

Introductie

Recruitment bij schelpdieren

Populatiegrootte bij schelpdieren

Bij schelpdieren wordt een grote jaar-op-jaar variatie in aantallen adulten waargenomen. Het aantal dieren in een populatie wordt bepaald door vier processen: geboorte, sterfte, immigratie en emigratie. Op hun beurt worden deze processen weer beïnvloed door factoren die al dan niet afhankelijk zijn van de dichtheid van de dieren in de populatie. Voorbeelden van dichtheids-onafhankelijke factoren zijn temperatuur of neerslag. Een voorbeeld van een dichtheids-afhankelijke factor is de hoeveelheid voedsel per individu (Royama 1992). Is de totale hoeveelheid voedsel constant, maar zijn er veel individuen dan is de hoeveelheid voedsel per dier klein, zijn er weinig dieren, dan is de hoeveelheid voedsel per individu groot. De voedselhoeveelheid per individu zal invloed kunnen hebben op onder meer de sterfte.

Bij één van de meest voorkomende schelpdiersoorten in de Waddenzee, het nonnetje *Macoma balthica*, is de sterfte van de dieren van één jaar en ouder vrijwel constant (Van der Meer 1997) en kan dus geen oorzaak zijn van de variatie in populatiegrootte. Regulatie vindt dus waarschijnlijk plaats voordat de dieren één jaar oud zijn. Een factor waarin wel een duidelijke jaar-op-jaar variatie is waargenomen is de recruitment. De in dit proefschrift gehanteerde definitie van dit begrip is de volgende: recruitment is het aantal broedjes (juvenielen, nulde-jaarsklasse) van een bepaalde soort per vierkante meter dat aan het eind van de eerste zomer na de paaiperiode van die soort (doorgaans in het voorjaar) achterblijft op een zeef met een maaswijdte van 1 mm. Aantallen broedjes kunnen het ene jaar 100 tot 1000 maal groter of kleiner zijn dan in het andere jaar (voor literatuur zie de introductie van hoofdstuk 6). Dit betekent dat er in een bepaald jaar een enorme voorraad aan (juveniele) schelpdieren kan zijn terwijl er in andere jaren bijna geen voorraad is.

De hoeveelheid schelpdierbroedjes in een populatie is waarschijnlijk onafhankelijk van de dichtheid adulten in die populatie, met andere woorden, een stock-recruitment relatie ontbreekt. Dit is aangetoond voor in

ieder geval twee soorten, de kokkel *C. edule* (Van der Meer 1997, Hancock 1973) en het nonnetje *M. balthica* (Van der Meer 1997). Een al dan niet onderkend feit in deze studies is dat de biologische populatie (een groep van individuen van eenzelfde soort die met elkaar reproduceren) groter kan zijn dan de bestudeerde populatie: vaak wordt (om praktische redenen) maar een deel van de biologische populatie bestudeerd. Een consequentie kan dan zijn dat de adulte stock niet veel meer te maken heeft met de recruits die in het onderzoeksgebied terecht komen.

De periode tot het moment waarop de recruitment gemeten wordt kan ruwweg ingedeeld worden in twee perioden, de pre-settlement- en de post-settlement periode. De mortaliteit van mariene ongewervelden is in de periode tussen settlement en recruitment min of meer constant (zie voor literatuur Hunt & Scheibling 1997), er bestaat in de tijd een duidelijke correlatie tussen aantallen post-settlers en aantallen broedjes. Dit is recentelijk ook voor *M. balthica* aangetoond (Beukema *et al.* 1998). Dit betekent dat aantallen recruits, en dus ook de aantallen van de oudere jaarklassen, bepaald worden voor of tijdens de settlement.

Tweekleppige schelpdieren (bivalven), die in of op de zeebodem leven, zijn vanaf een bepaalde leeftijd immobiel en bewegen zich in principe alleen over korte afstanden. Dus migratie van oudere individuen speelt bij deze soorten een vrij onbelangrijke rol. Migratie speelt echter wel een belangrijke rol bij jonge individuen. De belangrijkste fase in het leven van schelpdieren wat betreft migratie, is de larvale fase. Een groot deel van de bivalven produceert vrijzwemmende (pelagische) planktotrofe larven (zie intermezzo 1). Een kenmerk van dit type larvale ontwikkeling is dat de dispersie, het uitwaaieren van larven vanuit de plaats waar zij 'geproduceerd' zijn, erg groot is (Levin *et al.* 1987). Een kleiner deel van de schelpdiersoorten produceert lecithotrofe larven (zie intermezzo 1). Deze larven zijn minder mobiel en migratie speelt alleen een rol op kleine schaal en voornamelijk binnen de populatie.

Een tweede periode waarin migratie belangrijk kan zijn is de periode na settlement. De dieren hebben de metamorfose van een pelagische- naar een bentische vorm ondergaan en leven nu in of op de bodem. Zij worden dan post-larven of juvenielen genoemd. In deze periode kan ook nog migratie plaatsvinden. Juveniele *M. balthica* kunnen zich tot een leeftijd van enkele maanden (totdat zij een schelplengte van zo'n 9 mm bereikt hebben) verplaatsen met behulp van een slijmdraadje (Beukema & De Vlas 1989).

INTRODUCTION

Zwevend door het water aan zo'n draadje, kunnen juvenielen zich over grote afstanden verplaatsen en terechtkomen in een heel andere omgeving.

Intermezzo 1

Larvale ontwikkeling bij schelpdieren

Verschillende typen van larvale ontwikkeling kunnen onderscheiden worden. Allereerst is er de lecithotrofe ontwikkeling. Bij deze vorm is het voedsel, dat nodig is gedurende de larvale periode (de periode vanaf de bevruchting tot en met de metamorfose van larve naar juveniel), afkomstig uit het ei. In het algemeen zijn dit relatief grote eieren waarin veel energierijke voedingsstoffen zijn opgeslagen. Lecithotrofe larven hebben meestal geen ontwikkeling in de waterkolom (pelagische ontwikkeling) maar ontwikkelen zich binnen het ouderdier (directe ontwikkeling) of op de bodem (benthische ontwikkeling). Naast de lecithotrofe ontwikkeling is er de (veel voorkomende) planktotrofe ontwikkeling. Bij deze vorm wordt een deel van het benodigde voedsel opgenomen uit het water (phytoplankton). Het overgrote deel van de planktotrofe larven ontwikkelt zich voor een kortere of langere tijd in de waterkolom (pelagische fase). Eieren van soorten met deze vorm van larvale ontwikkeling zijn in het algemeen kleiner dan eieren bij soorten met lecithotrofe ontwikkeling en bevatten minder voedsel (Thorson 1946, Ockelmann 1962, Mileikovsky 1971, Vance 1973a, b). De kosten voor de aanmaak van een lecithotroof ei zijn hoog vergeleken met de aanmaak van een planktotroof ei. Omdat er altijd maar een beperkte hoeveelheid energie voor de reproductie beschikbaar is, zullen er per vrouwtje bij soorten met lecithotrofe eieren relatief weinig en bij soorten met planktotrofe eieren relatief veel eieren geproduceerd kunnen worden (Smith & Fretwell 1974).

Het meest voorkomende type larvale ontwikkeling bij schelpdieren in de Waddenzee is de planktotrofe pelagische ontwikkeling. Voorbeelden hiervan zijn *M. balthica*, *C. edule* en *M. edulis*. Een soort met een voor de Waddenzee uitzonderlijk type larvale ontwikkeling is *Abra tenuis*. Vrouwtjes van deze soort leggen maximaal 1800 (relatief grote, 140 µm) eieren in pakketjes op het sediment (één pakket per vrouwtje), waaruit na drie weken miniatuur volwassenen tevoorschijn komen (Gibbs 1984). Dit is een voorbeeld van een lecithotrofe directe niet-pelagische ontwikkeling.

CHAPTER ONE

Oorzaken van variatie in recruitment

Waardoor de variatie in aantallen recruits veroorzaakt wordt, is nog niet opgelost. Het is mogelijk dat de aantallen gepaaide eieren variëren, de kwaliteit van de eieren niet constant is waardoor er geen of slechte bevruchting optreed of de mortaliteit in de larvale fase variabel is. Bij elk van deze factoren kan men zich ook weer afvragen waardoor variatie veroorzaakt wordt.

Uit langjarige studies blijkt dat de broedval van enkele schelpdiersoorten vooral goed is na een koude winter en slecht na een zachte winter. Soorten waarbij dit is beschreven zijn de mossel *Mytilus edulis* (Beukema 1982, 1992a, Jensen & Jensen 1985, McGrorty *et al*. 1990, Young *et al*. 1996), de kokkel *Cerastoderma edule* (Kristensen 1957, Hancock 1973, Beukema 1982, 1992a, Möller & Rosenberg 1983, Jensen & Jensen 1985, Yankson 1986, Ducrotoy *et al*. 1991, Young *et al*. 1996), de strandgaper *Mya arenaria* (Beukema 1982, 1992a, Möller & Rosenberg 1983, Jensen & Jensen 1985), en het nonnetje *Macoma balthica* (Beukema 1982, 1992a, Jensen & Jensen 1985).

Temperatuur beïnvloedt het energiebudget van een koudbloedig schelpdier. Bij hogere temperaturen zijn de uitgaven aan metabolische kosten hoger dan bij lagere temperaturen. Als die extra uitgaven niet gecompenseerd worden door extra inkomsten, hetzij door een hoger voedselaanbod, hetzij door een verhoogde opname efficiëntie, worden de kosten betaald uit aanwezige voorraden. Dit betekent dat er bij hogere temperaturen meer energie nodig is voor de processen die nodig zijn om in leven te blijven. Er is dan in principe minder energie beschikbaar voor de reproductie met als gevolg een verlaagde reproductieve output; een verlaagde eiproductie (fecunditeit) en/of een verlaagde eikwaliteit. Mogelijk heeft de verlaagde reproductieve output nadelige gevolgen op de recruitment.

In dit proefschrift worden effecten van temperatuur, en dan speciaal van wintertemperatuur (zie intermezzo 2), op het voortplantingssucces van *Cerastoderma edule*, *Mytilus edulis* en *Macoma balthica* benadrukt. De drie soorten vertonen verschillen in timing van gametogenese (zie hoofdstuk 3) en de mogelijkheid bestaat dat zij verschillend reageren als hun energiebudgetten beïnvloed worden. Omdat de hoeveelheid opgenomen voedsel beperkt is, is de hoeveelheid energie die gebruikt wordt om te reproduceren ook beperkt. Zoals in intermezzo I is opgemerkt, zijn er

verschillen tussen schelpdiersoorten voor wat betreft eigrootte en eiaantal. De ene soort produceert grote eieren waaruit na korte tijd goed ontwikkelde zelfstandige jongen geboren worden en de andere soort produceert duizenden kleine eitjes met relatief weinig voedsel waaruit, na bevruchting, embryo's komen die na kortere of langere tijd afhankelijk zijn van extern voedsel. Bij een gelijke reproductieve output (het product van eiaantal maal de eigrootte) produceert een soort dus weinig grote of veel kleine eieren (Smith & Fretwell 1974). Omdat de voedselsituatie per dier jaar-op-jaar niet altijd gelijk is, is er dus niet ieder jaar evenveel energie voor reproductie beschikbaar. Men kan zich afvragen of er binnen een soort ook verschillen in reproductieve output gevonden kunnen worden. Met andere woorden, worden er bij een laag voedselaanbod per dier minder en / of kleinere eieren geproduceerd dan bij een hoog voedselaanbod?

De eiproduktie van *M. balthica*, *C. edule* en *M. edulis* zal dan ook speciale aandacht krijgen: wordt de reproductieve output (het produkt van eiaantal en eigrootte) kleiner bij een hogere energie-uitgave (hogere temperatuur) vóór het paaien of blijft de output gelijk en worden de hogere energie-uitgaven betaald door meer niet-reproductief weefsel als brandstof te gebruiken? Als de reproductieve output inderdaad kleiner wordt, gaat dit dan ten koste van de energie-inhoud per ei (worden de eieren kleiner en / of energiearmer) of wordt het aantal per vrouwtje gepaaide eieren (fecunditeit) lager? Vervolgens kan bekeken worden of er effecten zijn op de recruitment.

Naast waarnemingen over de invloed van temperatuur aan de kostenkant worden ook resultaten beschreven van experimenteel onderzoek aan de inkomstenkant. De vraag is of voedselbeschikbaarheid effect kan hebben op de reproductieve output en als dit zo is, wordt dan de fecunditeit en / of de eikwaliteit (grootte of samenstelling) beïnvloed? In onze experimenten is de voedselbeschikbaarheid niet gekoppeld aan de temperatuur. Dat wil zeggen dat het voedselaanbod bij verschillende temperaturen gelijk is. In het veld zijn temperatuur en voedsel niet van elkaar los te koppelen, temperatuur en voedsel zijn met elkaar gecorreleerd. Tijdens koude winters is er vaak veel instraling van zonlicht (weinig bewolking) en komt de algenbloei eerder op gang dan tijdens warme winters met veel bewolking.

De gemiddelde jaartemperaturen vertonen de laatste decennia de neiging tot stijgen (wat op een klimaatsverandering zou duiden). Hieraan

zijn mogelijk maatschappelijke belangen verbonden (o.m. voor de commerciële schelpdiervisserij). Een belangrijk deel van dit onderzoek werd dan ook gefinancierd door het Nationaal Onderzoek Programma I (NOP I) 'Mondiale Luchtverontreiniging en Klimaatverandering'. In dit grote project zijn verschillende aspecten van wereldwijde klimaatsverandering bestudeerd. Het in dit proefschrift beschreven onderzoek is ten dele uitgevoerd binnen thema C 'De gevolgen' en subthema 'De Waddenzee'. Het NOP project was getiteld: 'Wintertemperatuur en reproductief succes van bivalven op zandplaten in west Europa' (projectnummer: 851053).

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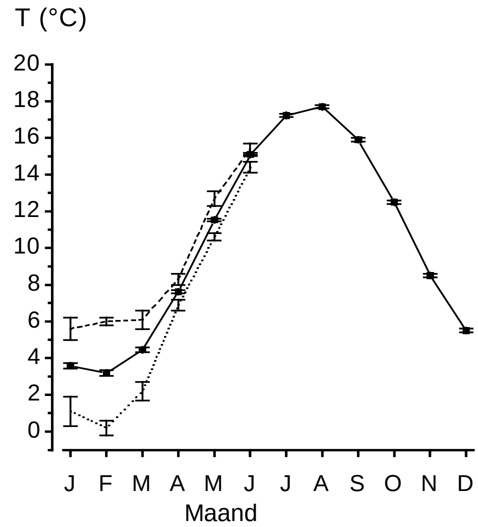
Intermezzo 2

Temperatuur

Temperatuur is een voor de biologie belangrijke karakteristiek van zeewater. In het algemeen geldt dat de temperatuur in tropische zeeën het hoogst is, 28 - 30 °C. Door de in de richting van de polen steeds verder afnemende instraling van de zon wordt de watertemperatuur geleidelijk lager, tot ongeveer -1.9 °C in de poolzeeën. Plaatselijk kunnen zeer grote afwijkingen van de gemiddelde zeewatertemperatuur optreden, voornamelijk veroorzaakt door seizoensvariatie in instraling, het effect van zeestromingen en de nabijheid van land. De seizoensvariatie in watertemperatuur in tropische en polaire zeeën is relatief klein, ongeveer 2 °C. Het verschil tussen winter- en zomerwaarden in gematigde streken kan echter wel zo'n 10 - 20 °C bedragen (Tchernia 1980). Als voorbeeld is in figuur 1.1 het langjarig maandgemiddelde van de temperatuur van het water van het Marsdiep weergegeven. Het Marsdiep is het zeegat tussen Texel en Den Helder en verzorgt de in- en uitstroom van zeewater naar en van het meest zuidwestelijke deel van de Waddenzee. Naast de seizoensvariatie in temperatuur in gematigde streken is er ook nog een jaar-op-jaar variatie die voornamelijk tot uitdrukking komt in het verschil in wintertemperatuur. De verschillen in de gemiddelde watertemperatuur van warme- en koude winters bedraagt 5 tot 6 °C. Als illustratie zijn in figuur 1.1 de gemiddelde maandtemperaturen (januari - juni) van het Marsdiep van de zachte winters van 1974, 1988, 1989, 1990 en 1995 en de gemiddelde maandtemperaturen (januari - juni) van de strenge winters van 1979, 1985, 1986, 1987 en 1996 vermeld. Het zijn de aanpassingen van

enkele schelpdiersoorten aan deze jaar-op-jaar verschillen in wintertemperatuur die in dit proefschrift beschreven worden.



Figuur 1.1. Watertemperaturen gemeten in het Marsdiep. De vermelde waarden zijn langjarige gemiddelden (± SE) van de maandgemiddelden. De ononderbroken lijn geeft het gemiddelde weer van de hele periode 1861 - heden, de streepjeslijn geeft het gemiddelde weer in een aantal recente zachte winters (1974, 1988, 1989, 1990 en 1995) en de fijne stippellijn geeft het gemiddelde weer in een aantal recente koude winters (1979, 1985, 1986, 1987 en 1996).
Figure 1.1. Long-term montly averages of water temperatures (± SE) of Marsdiep, the major Wadden Sea tidal inlet near Den Helder. The solid line represents the long-term average during the entire period 1861-present, the dashed line the average temperature of some recent mild winters (1974, 1988, 1989, 1990 and 1995), and the dotted line the average water temperature of some recent cold winters (1979, 1985, 1986, 1987 en 1996). (data from Van der Hoeven 1982 and by courtesy of H. van Aken.)

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Samenvatting van de hoofdstukken

Effecten van temperatuur en immersietijd op de gewichten van ouderdieren

Ouderdieren die tijdens de gametogenese stress hebben geleden (bijvoorbeeld in de vorm van voedselgebrek, ongunstige saliniteit of een te hoge of te lage temperatuur) vertonen niet alleen zelf een slechte 'conditie', maar hebben bovendien een slechtere reproductieve output dan controledieren die onder zo optimaal mogelijke omstandigheden zijn

gehouden (Bayne 1972, Bayne *et al.* 1975, 1978). Omdat de 'conditie' van de ouderdieren dus van belang is voor hun reproductief succes, worden de gewichtsveranderingen van de adulten van *Cerastoderma edule*, *Mytilus edulis* en *Macoma balthica* beschreven (**hoofdstuk 2**) tijdens een experiment waarin voedselaanbod en temperatuur gemanipuleerd werd tijdens de periode voorafgaand aan het paaiseizoen in het voorjaar. Speciale aandacht wordt geschonken aan de effecten van temperatuur en immersietijd. Er is gekozen voor twee temperaturen, één hoge temperatuur waarvan de gemiddelde waarde vergelijkbaar is met de gemiddelde temperatuur van een normale tot zachte winter (3.6 - 6.2 °C) en één lage temperatuur waarvan de gemiddelde temperatuur vergelijkbaar is met de gemiddelde temperatuur van een normale tot koude winter (1.2 - 3.6 °C). Bij elk temperatuurregime werden twee getijde-niveaus toegepast, één met een immersietijd van 100 % ('subtidal') en één met een immersietijd van 57 % ('tidal'). Er waren dus vier behandelingen, koud-subtidal (Cs), koud-tidal (Ct), warm-subtidal (Ms) en warm-tidal (Mt).

De resultaten komen er in het kort op neer dat bij alle soorten de gewichtsafname tijdens de winter geringer was bij een lagere temperatuur. Door de lagere metabolische activiteit bij een lagere temperatuur is minder energie nodig om levensprocessen gaande te houden. Omdat er tijdens de wintermaanden vrijwel geen voedsel beschikbaar is zal de benodigde energie uit reservestoffen moeten komen. Dit heeft een gewichtsafname tot gevolg en deze is bij lagere temperaturen dus kleiner dan bij hogere temperaturen. Effecten van immersietijd waren pas merkbaar toen er weer voedsel beschikbaar kwam, in de tweede helft van maart. Het bleek dat de groei bij een lagere temperatuur eerder begon en dat vlak voor het paaiseizoen het gemiddelde lichaamsgewicht bij een standaard schelplengte van 1 cm (de Body Mass Index, BMI) het hoogst was bij de groepen die gehouden waren bij een lage temperatuur en een immersietijd van 100 % (Cs). De groepen die gehouden waren bij een hoge temperatuur en bij een korte immersietijd (Mt) hadden de laagste BMI waarden op het moment van paaien, terwijl de groepen die gehouden waren bij een lage temperatuur gecombineerd met een korte immersietijd (Ct) of een hoge temperatuur gecombineerd met een lange immersietijd (Ms), BMI-waarden hadden die tussen de twee uitersten in lagen. Als aanvulling op de experimentele gegevens is ook bij populaties van *C. edule* en *M. balthica* in het veld het gewichtsverloop beschreven tijdens twee winters met een verschillend karakter; een zachte en een koude winter. Vergeleken met de

gewichtsveranderingen in de experimenten vermagerden de velddieren sneller, maar het effect van getijde-hoogte en temperatuur was in het algemeen hetzelfde; hoe langer de immersietijd en hoe kouder de winter, hoe hoger het gewicht vlak voor het paaien.

Effecten van temperatuur en immersietijd op de reproductieve output

Welke gevolgen kunnen de verschillende experimentele behandelingen nu hebben op de reproductie? Proberen de schelpdieren ten koste van hun eigen lichaam te reproduceren en op deze manier ongeacht hun conditie een maximum aan gameten te produceren? Worden er minder en / of kleinere eieren geproduceerd (zoals dat bij de mossel is waargenomen door Bayne *et al.* 1978), of worden er concessies gedaan aan de kwaliteit van de eieren (bijvoorbeeld minder hoogwaardige reservestoffen per ei)? In **hoofdstuk 3** wordt de vraag betreffende de fecunditeit en eigrootte aan de orde gesteld. Onder ongunstige omstandigheden, dus een hogere wintertemperatuur en een kortere overstromingsduur, werden er door *M. balthica* en *C. edule* minder eieren geproduceerd dan onder gunstiger condities (een lagere wintertemperatuur en een langere immersietijd). Omdat het tijdens de in dit proefschrift beschreven experimenten nooit gelukt is om mosselen kompleet leeg te laten paaien, kan er geen uitspraak worden gedaan over de eiproductie van deze soort. Maar het is al bekend dat deze lager is bij dieren in een slechte conditie (Bayne *et al.* 1975). Daarom kan gesteld worden dat de fecunditeit bij de drie bestudeerde soorten lager is na een periode van relatief slechte omstandigheden.

Verschillen in gemiddelde eigrootte tussen de experimentele groepen werden alleen geconstateerd bij *M. edulis* en *C. edule*. Bij *M. edulis* was de immersietijd positief gecorreleerd met de eigrootte en bij *C. edule* was de temperatuur negatief gecorreleerd met de eigrootte. Bij *M. balthica* bleken temperatuur noch immersietijd effect op de eigrootte te hebben. De gevonden verschillen tussen *M. balthica* enerzijds en *M. edulis* en *C. edule* anderzijds kunnen verklaard worden door verschillen in timing van de gametogenese van deze soorten. De aanleg van gonaden begint bij *M. balthica* al vroeg in de zomer en is volgens sommige auteurs al voltooid aan het begin van de winter (Caddy 1967, Lammens 1967, Chambers & Milne 1975, Bachelet 1986, Bonsdorff & Wenne 1989). Waarschijnlijk wordt de eigrootte bij deze soort al vroeg tijdens de gametogenese bepaald, dus al aan het eind van de zomer of in de herfst. Dit blijkt ook uit het feit dat eigrootte bij *M. balthica* beter correleert met de BMI in Augustus

voorafgaand aan het paaiseizoen dan met de BMI direct vóór het paaien (zie hoofdstuk 4). Gametogenese bij de andere twee soorten begint later en wordt pas voltooid vlak voor het paaien (kokkel: Boyden 1971, Newell & Bayne 1980, Ivell 1981, Iglesias & Navarro 1991 en mossel: Kautsky 1982, Sprung 1983, Bayne 1984) en dus is de eigrootte wel te beïnvloeden tijdens de winter en het voorjaar.

Bij het nonnetje *M. balthica* bleek er een significante positieve relatie te bestaan tussen de aantallen gepaaide eieren en de BMI vlak vóór het paaien. In **hoofdstuk 4** wordt deze in de experimenten gevonden relatie ondersteund met veldgegevens. De vraagstelling was: zijn de in de experimenten gevonden effecten van temperatuur en immersietijd op de aantallen en grootte van de eieren terug te vinden in een veldsituatie? In dit hoofdstuk zijn de resultaten beschreven die verkregen zijn in het paaiseizoen na de zachte winter van 1995 en de koude winter van 1996.

Op het Balgzand zijn groepen nonnetjes verzameld van drie verschillende getijde-hoogten; één plaats vlak onder de dijk met een korte overstromingsduur, één plaats op een lager wad met een lange overstromingsduur en één plaats met een gemiddelde getijde-hoogte en een gemiddelde immersietijd. Anders dan bij de experimenten, bleek de eigrootte tussen de populaties in het veld wel degelijk te verschillen en was er een negatieve correlatie met de hoogte van de monsterpunten; hoe hoger de adulte dieren zaten (hoe korter de immersieduur) hoe kleiner de eieren waren. Dat er dergelijke verschillen zijn in eigrootte, die echter niet te initiëren zijn in experimenten gedurende de winter, is ook een aanwijzing dat eigrootte al vóór de winter is vastgelegd.

De resultaten betreffende aantallen gepaaide eieren kwamen overeen met de verwachtingen. Na de koude winter van 1996 waren deze aantallen bij de drie veldgroepen significant hoger dan na de zachte winter van 1995. Het effect van getijde-hoogte was na de zachte winter ook volgens verwachting: getijde-hoogte was negatief gecorreleerd met gepaaide aantallen eieren. Na de koude winter van 1996 was het beeld weliswaar iets anders (de eiproduktie was het hoogst bij het hoogst gelegen monsterpunt), maar de gepaaide eiaantallen waren wel significant positief gecorreleerd met de BMI-waarde direct vóór het paaien (net als in de experimenten).

INTRODUCTION

Energetische aspecten van de reproductie bij M. balthica

Wat kost het een nonnetje om te reproduceren? Wordt de beschikbare energie in de voortplanting gestopt of ook in de groei van lichaamsweefsels? Deze vragen worden in **hoofdstuk 5** beantwoord. Met de in hoofdstuk 4 voor het nonnetje beschreven relatie tussen BMI en het gepaaide aantal eieren is het mogelijk om voor elke BMI-waarde de gepaaide eiaantallen uit te rekenen. Door de berekende eiaantallen te vermenigvuldigen met het gemiddelde gewicht per ei, kan uitgerekend worden hoeveel procent van de totale individuele lichaamsmassa vlak voor het paaien bestaat uit eimateriaal. Als vervolgens ook nog rekening wordt gehouden met de biochemische samenstelling van ei- en van niet-ei materiaal, is het mogelijk om de energie-inhoud van de eimassa ten opzichte van de rest van de lichaamsmassa uit te rekenen. Het blijkt dat eieren van *M. balthica* energetisch duur zijn vergeleken met de eieren van *M. edulis* en *C. edule*. Ten eerste omdat het vetgehalte hoger is, zo'n 32.5 % van de as-vrij droge massa bestaat uit 'duur' vet (in *M. edulis* en *C. edule* respectievelijk 20 % en 11 %), en ten tweede omdat de eieren groter zijn en dus meer voedingsstoffen bevatten (eieren van *M. edulis* en *C. edule* hebben een diameter van respectievelijk 73 µm en 78 µm, terwijl eieren van *M. balthica* een diameter van 106 µm hebben). Als de calorische waarde per ei wordt uitgerekend blijkt dat een ei van *M. balthica* een veel hogere energie-inhoud heeft ($5.9 * 10^{-3}$ J) dan een ei van *C. edule* ($1.97 * 10^{-3}$ J) of *M. edulis* ($1.78 * 10^{-3}$ J).

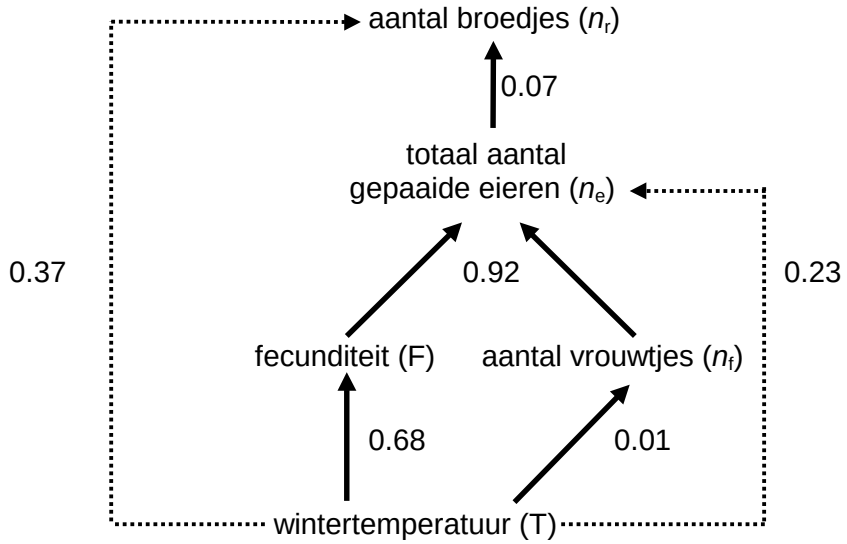
Uit de gegevens van hoofdstuk 4 bleek dat *M. balthica* beneden een BMI-waarde van 5.6 geen eieren meer produceert. Men kan het lichaamsgewicht bij deze BMI-drempelwaarde beschouwen als de minimale massa die nodig is om een aantal noodzakelijke processen om in leven te blijven in gang te houden. Als er meer voedsel (= energie) beschikbaar is, kan dit voor verschillende doeleinden gebruikt worden, bijvoorbeeld voor lichaams- of schelpgroei of voortplanting. Als alle energie in de voortplanting gestoken zou worden zou dus alle massa boven de minimale massa in eimateriaal of gonaden aanwezig moeten zijn. Maar het bleek dat 'maar' 55 % van deze boven-minimale massa terug was te vinden in de eieren. Een deel (hoe groot dit deel is, is echter niet bekend) van de overgebleven 45 % is echter noodzakelijk voor de aanmaak, het onderhoud en de opslag van gameten en is dus in feite ook noodzakelijk voor de reproductie. Met andere woorden, de werkelijke investering in

reproductieve massa was meer dan 55 %. Het resterende deel van de 45 % niet-ei materiaal wordt waarschijnlijk gebruikt voor somatische groei.

Broedval van Macoma balthica

Sinds 1973 is voor *M. balthica* in augustus van ieder jaar op het Balgzand het aantal nulde-jaars individuen per m² bepaald (broedjes), de recruitment. In **hoofdstuk 6** wordt beschreven wat de reproductieve inspanning van *M. balthica* uiteindelijk oplevert aan nakomelingen. Wordt de grotere reproductieve output na een koude winter ook gevolgd door een betere recruitment? Met andere woorden, heeft een lage temperatuur een positief effect op de recruitment en komt dit tot stand via een verhoogde eiproduktie?

Tijdens jaarlijkse voorjaarsbemonsteringen op 15 plaatsen op het Balgzand zijn, onder andere voor het nonnetje *M. balthica*, de schelplengten, de aantallen individuen per vierkante meter, de leeftijd en het individuele lichaamsgewicht bepaald. Met behulp van deze gegevens

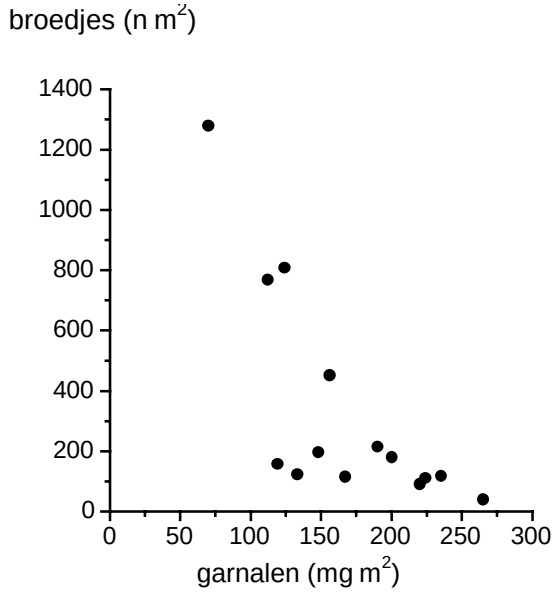


Figuur 1.2. Schematische weergave van de mogelijke stappen via welke de wintertemperatuur invloed heeft op het recruitment succes van *Macoma balthica*. De getallen geven het door de betreffende factor verklaarde deel van de variantie weer, dus wintertemperatuur verklaart bijvoorbeeld 68% van de in fecunditeit waargenomen variatie.

Figure 1.2. Schematic presentation of the possible mechanism via which winter temperatures (T) can influence the number of recruits per m² (n_r). The numbers in the schedule are the explained parts of the variances caused by a certain factor. F = fecundity of a female *M. balthica*, n_f = number of reproducing females per square meter, n_e = total number of spawned eggs per square meter.

en met behulp van de in hoofdstuk 4 beschreven relatie tussen BMI en gepaaide eiaantallen was het mogelijk om voor ieder jaar sinds 1970 achteraf de gepaaide hoeveelheid eieren per vierkante meter te reconstrueren. De jaar-op-jaar variatie in eiproduktie was groot en bleek duidelijk gerelateerd te zijn aan de wintertemperatuur; hoe kouder de voorafgaande winter, hoe hoger de aantallen gepaaide eieren. Winter temperatuur bleek tevens een significant effect te hebben op de recruitment (na een koude winter was de recruitment beter), 37% van de variatie in recruitment kon worden verklaard door variatie in wintertemperatuur.

Ondanks de significante correlatie tussen winter temperatuur en gepaaide individuele eiaantallen ($R^2 = 0.68$), bleek dat uiteindelijk maar zo'n 7 % van de totale variatie in recruitment verklaard kon worden door variatie in eiaantallen. Een schema, met hierin vermeld de in hoofdstuk 6 uitgerekende correlatiecoëfficiënten, wordt getoond in figuur 1.2. Het effect



Figuur 1.3. Relatie tussen de garnalenbiomassa (as-vrij droog gewicht per m²) in het voorjaar (mei / juni) en de recruitment van *M. balthica* in de zomer van hetzelfde jaar (naar

Beukema *et al*. 1998).

Figure 1.3. Relationship between shrimp biomass (ash-free dry mass per square meter) in spring (May / June) and *M. balthica* recruitment in subsequent summer (after Beukema *et al.* 1998).

van wintertemperatuur op de recruitment blijkt dus maar voor een klein deel veroorzaakt te worden door variatie in eiaantallen. Dit betekent dat er andere factoren in het spel zijn die het temperatuureffect moeten verklaren. Een mogelijke verklaring is de predatie op broedjes door bijvoorbeeld kleine krabben en kleine garnalen. De dichtheid van deze predatoren en het moment waarop zij op de wadplaten actief zijn blijkt duidelijk gecorreleerd te zijn met de wintertemperatuur. Na een koude winter zijn er minder en komen zij later op de wadplaten dan na een warme winter (Beukema 1991 1992b). Dat er een duidelijke negatieve correlatie ($R^2 = 0.61$, $P < 0.001$) bestaat tussen de biomassa van garnalen in het voorjaar en de recruitment van *M. balthica* broedjes in de erop volgende zomer wordt getoond in figuur 1.3 (naar Beukema *et al*. 1998).

Conclusies

In deze paragraaf worden in het kort de belangrijkste conclusies opgesomd:

Tijdens een koude winter is het gewichtsverlies van adulte schelpdieren minder dan tijdens een zachte winter. Daardoor is na een koude winter het lichaamsgewicht direct vóór het paaien hoger.

Een langere immersietijd geeft langer de gelegenheid om te fourageren. Dit heeft tot gevolg dat groei, mits er voedsel aanwezig is, vroeger in het voorjaar begint en dat de groeisnelheid hoger is dan bij een kortere immersietijd.

Er blijkt een positieve relatie te bestaan tussen lichaamsgewicht, uitgedrukt in BMI-eenheden en gepaaide eiaantallen.

Eigrootte is alleen te manipuleren tijdens die periode van de gametogenese waarin de eigrootte bepaald wordt. Is de eigrootte eenmaal bepaald, dan verandert hij ook niet meer. Dit in tegenstelling tot het aantal gepaaide eieren.

Ondanks het feit dat winter temperatuur een duidelijk effect heeft op zowel de individuele- en totale eiproduktie als de recruitment van *M. balthica*, is een positieve relatie tussen de aantallen geproduceerde eieren en de eruit opgegroeide broedjes vrijwel afwezig. De voor het succes

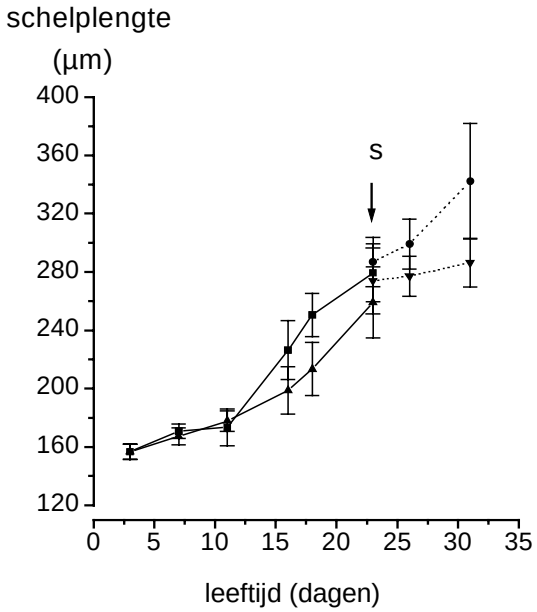
van de recruitment beslissende processen spelen zich af tussen het paaien en de broedval.

Hoe nu verder?

De afwezigheid van de relatie tussen het totale aantal gepaaide eieren en de recruitment (**hoofdstuk 6**) betekent dat er een negatieve relatie bestaat tussen eiproduktie en de overleving van ei tot recruit. Immers, hoe meer eieren er worden gepaaid, hoe lager de gemiddelde overlevingskans per ei. Dit betekent dat er gedurende de periode van paaien tot recruitment dichtheidsafhankelijke mortaliteit optreedt en dat dan de uiteindelijke jaarklassterkte (populatiegrootte) gereguleerd wordt. De perioden waarin de hoogste larvale mortaliteit optreedt zijn in het algemeen de periode direct na het paaien (Gosselin & Qian 1997), waarbij de mortaliteit soms wel 50 % per dag is (Stoner 1990), en de periode rond settlement (Gosselin & Qian 1997, Hunt & Scheibling 1997). De correlatie tussen de aantallen individuen in vroege larvale stadia en de aantallen broedjes is in het algemeen zeer laag, niet alleen bij marine evertebraten maar ook bij vissen. De correlatie wordt beter, maar blijft laag, naarmate de larven ouder worden (Peterman *et al*. 1988, Bradford 1992). Pas na settlement, als de perioden van hoge mortaliteit voorbij zijn, wordt de correlatie tussen aantallen postlarven en broedjes veel beter (Hunt & Scheibling 1997). Hetzelfde lijkt ook op te treden bij *Macoma balthica*; post-settlement mortaliteit lijkt onafhankelijk te zijn van de postlarven-dichtheid na settlement (Beukema *et al*. 1998) en variatie in recruitment lijkt dan ook gereguleerd te worden in de pelagische fase en tijdens de settlement.

Ondanks het feit dat er steeds meer aanwijzingen zijn dat de pelagische fase een zeer belangrijke periode is in de populatieregulering, wordt aan deze fase nog zeer weinig onderzoek gedaan. Voornamelijk omdat er veel praktische moeilijkheden zijn, zal dit soort onderzoek erg moeizaam en frustrerend zijn en het is dan ook de vraag of het mogelijk is om door middel van veldwerk binnen afzienbare tijd een tipje van de sluier op te lichten over de populatieregulerende factoren in de pelagische fase. Het moet dan ook gevreesd worden, dat in een tijd waarin liefst op voorhand al een sterke verwachting van conclusies uit toekomstig onderzoek aanwezig moet zijn, geen plaats zal zijn voor zulk (onzeker) fundamenteel onderzoek.

Een andere manier om regulerende processen in de pelagische fase te onderzoeken is het experimenteren met larven in het laboratorium. Voor het verkrijgen van larven is de natuurlijke bron, het zoöplankton in zee, echter ongeschikt omdat de oorsprong en leeftijd van de daarin aanwezige larven niet bekend zijn en omdat de eerste larvale stadia van de verschillende soorten (bijna) niet te onderscheiden zijn. De beste manier is dan ook om larven te kweken vanaf het eistadium. Ervaring met de kweek van bivalven is echter alleen opgedaan bij commercieel aantrekkelijke soorten. Omdat *M. balthica* geen commercieel interessante soort is, zijn kweekresultaten met deze soort nog nooit in de literatuur verschenen. Zelfs basale gegevens als larvale ontwikkeling en groeisnelheid in de pelagische fase zijn niet bekend. Omdat de noodzaak tot kweken onderkend is, hebben wij in het voorjaar van 1998 besloten om kweekexperimenten op te starten om de praktische mogelijkheden voor het op laboratoriumschaal kweken van larven van *M. balthica* te onderzoeken. De resultaten zijn veelbelovend: ondanks een hoge mortaliteit is het mogelijk gebleken om per ouderpaar enige honderden larven tot ruim na de settlement op te kweken.

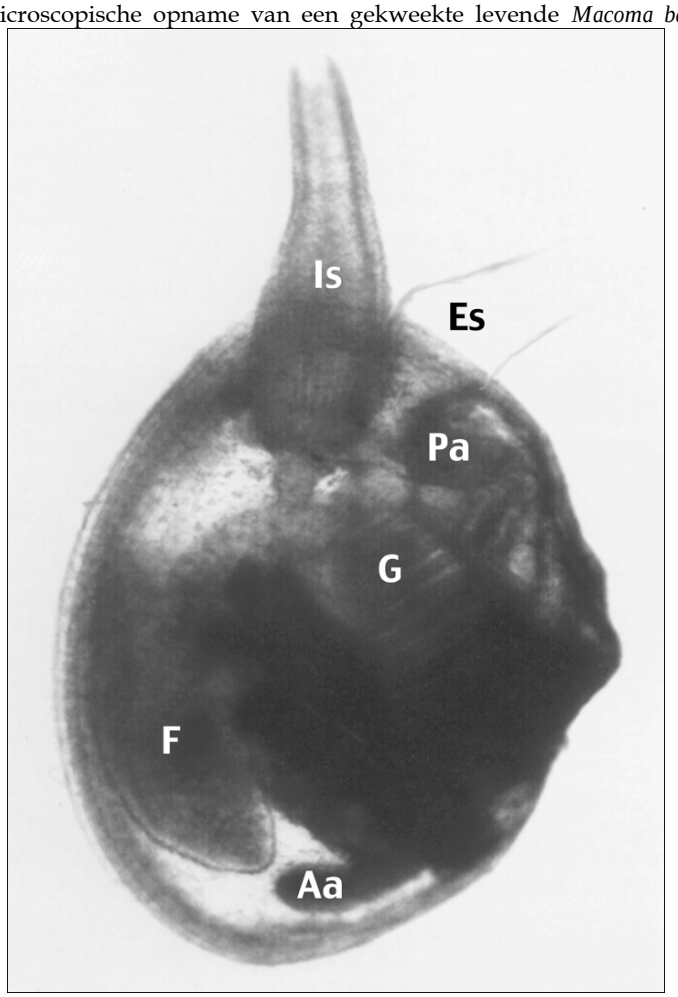


Figuur 1.4. Schelpgroei (gemiddelde ± standaardafwijking) van gekweekte larven van

Macoma balthica in de periode tot 31 dagen na de bevruchting. S geeft het moment aan waarop de larven zijn overgebracht naar een opstelling waarin de metamorfose (= settlement) plaatsvindt.

Figure 1.4. Shell growth (mean ± SD) of reared larvae of *M. balthica* during the period from fertilisation to an age of 31 days. S refers to the age at which the larvae were ready to settle and were transfered to a settlement set-up.

Figuur 1.5. Microscopische opname van een gekweekte levende *Macoma balthica* op een



leeftijd van 70 dagen en met een schelplengte van 1.4 mm. Op de foto zijn onder andere de volgende organen zichtbaar: voet (F), instroomsiphon (Is), uitstroomsiphon (Es), kieuwen (G), voorste - (Aa) en achterste (Pa) sluitspier.

Figure 1.5. Microscopic photograph of a live reared juvenile of *M. balthica* at an age of 70 days and a shell length of 1.4 mm. The following organs can be recognised, foot (F), inhalant - (Is) and exhalant siphon (Es), gills (G), Anterior - (Aa), and posterior (Pa) adductor muscle.

Hoewel de kweekmethode nog geoptimaliseerd dient te worden is het met ingang van het voortplantingsseizoen van 1999 al mogelijk om te experimenteren met larven van *M. balthica*. Ter illustratie van de larvenkweek is in figuur 1.4 de schelpgroei van twee groepen larven

weergegeven tot een leeftijd van 31 dagen en in figuur 1.5 is een foto te zien van een gekweekte *M. balthica* met een leeftijd van 70 dagen.

Als vervolg op de in dit proefschrift beschreven resultaten zou het op deze wijze mogelijk zijn om de belangrijkste factoren te bepalen die verantwoordelijk zijn voor de (dichtheidsafhankelijke) mortaliteit gedurende de pelagische fase. Een andere mogelijke toepassing van de gevonden kweekmethode is het onderzoek naar temperatuurtoleranties van larven waarvan de ouderdieren uit verschillende populaties afkomstig zijn. Dit onderzoek zou mogelijk een antwoord kunnen geven op de vraag waarom *M. balthica* niet verder zuidelijk voorkomt dan de monding van de Gironde (Frankrijk) en niet noordelijker dan de Witte Zee. Tevens lijkt het mogelijk de vraag te beantwoorden wat er met onze *M. balthica* populatie gebeurt als de gemiddelde watertemperatuur stijgt; wordt onze populatie vervangen door zuidelijke *M. balthica* of kunnen de huidige populaties zich handhaven? Kortom, de mogelijkheid van het kweken van larven van (niet commercieel interessante) bivalven opent een scala aan onderzoeksmogelijkheden, waarmee intrigerende vragen beantwoord kunnen worden.

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CHAPTER TWO

Loss of body mass in winter in three intertidal bivalve species: an experimental and observational study of the interacting effects between water temperature, feeding time and feeding behaviour

Abstract

At temperate latitudes, mass of the soft parts of bivalve molluscs generally declines during winter. Long-term field data collected in the western part of the Dutch Wadden Sea indicate that losses are more substantial during mild than during cold winters. Moreover, food supply appears to be involved. We tried to find experimental evidence to prove that the correlative relationships observed in the field are based on cause-effect relationships. To this end, three common intertidal bivalves, the common cockle *Cerastoderma edule*, the common mussel *Mytilus edulis*, and the Baltic tellin *Macoma balthica* were subjected to manipulated water temperatures and immersion (feeding) times during the first half of two years (the end of December till spawning). Water temperatures were kept either at outdoor values or were lowered by a few degrees. In all three species, higher water temperatures and shorter daily feeding periods resulted in faster body-mass declines in winter and slower subsequent growth in spring than lower temperatures and unrestricted feeding times.

Introduction

Annual body-mass cycles have been observed in animals as well as in plants (Gwinner 1986). In all these cycles, two main periods can be distinguished, a period of gain of body mass and a period of stagnant or loss of body mass. Such cycles of mass gain and mass loss are well documented in several species of bivalves living in coastal areas in temperate climates, *e.g. Macoma balthica* (L.) (Lammens 1967, Beukema and De Bruin 1977, Pekkarinen 1983, Beukema and Desprez 1986, Bonsdorff and Wenne 1989, Harvey and Vincent 1990, Zwarts 1991), *Cerastoderma edule* (L.) (Kristensen 1957, Beukema 1974, Newell and Bayne 1980, Ivell 1981, Zwarts 1991) and *Mytilus edulis* (L.) (De Zwaan and Zandee 1972,

Dare and Edwards 1975, Barkati and Ahmed 1990, Zwarts and Wanink 1993).

The pattern of changes of body mass often appears to be connected with food availability. In the Wadden Sea, the main growing season for intertidal benthic organisms is spring and early summer (March - July) (Beukema 1974), a period when concentrations of phytoplankton are maximal (Cadée and Hegeman 1977, Cadée 1987, Kamermans 1992). In the autumn and winter season, bivalves generally lose high proportions of the mass of their soft parts.

Two factors appear to enhance rates of mass loss in autumn and winter: low food supply (causing low food intake rates) and high temperatures (causing high energy demands). The evidence for an influence of low food availability on rate of mass loss in the autumn - winter season is rather anecdotal. Beukema and Desprez (1986) observed that, along the French Atlantic coast, a period of mass gain of *M. balthica* in the autumn season coincided with relatively high phytoplankton concentrations, indicating that growth can occur in other seasons than the usual growing season if sufficient food is available. In several bivalves in the Wadden Sea, Beukema and Cadée (1996) observed unusually low mass losses in a winter (1990/1991) with abnormally high phytoplankton concentrations in the overlying sea water.

The influence of temperatures on bivalve mass loss in winter has been studied more systematically. In four bivalve species, *Mya arenaria* (L.), *Scrobicularia plana* (Da Costa), *C. edule* and *M. balthica*, Zwarts (1991) found a consistent relationship between temperature in a number of winter months and the changes in body mass during these months. The lower the temperature during a certain winter period, the smaller the mass loss. In *M. balthica* this negative relationship was also found for comparison between entire winter seasons. Body masses at a given shell length measured prior to spawning (in March) were higher after cold than after mild winters (Beukema 1992).

Low body masses at the end of winter may reduce subsequent survival (Beukema, unpubl. obs.) as well as reproductive output (Honkoop and Van der Meer 1997, 1998). According to Bayne *et al*. (1978) starved *M. edulis* produce fewer and smaller eggs which in turn yield larvae with higher proportions of deformations (Bayne 1972) and lower growth rates (Bayne *et al*. 1975). If reproducing individuals of the European flat oyster, *Ostrea edulis* (L.), were kept under food stress, low-quality eggs were

produced (low neutral-lipid content) and subsequent survival of larvae was low (Helm *et al*. 1973). Due to reduced amounts of stored nutrients, the larvae from smaller eggs of *Mercenaria mercenaria* (L.) and *Argopecten irradians* (Lamarck), had a lower survival than larvae hatched from larger eggs (Kraeuter *et al*. 1982). Such processes could explain the frequently observed recruitment failure after mild winters and also the recruitment successes after cold winters in bivalves (Beukema 1982, 1992, Möller and Rosenberg 1983).

The aim of the present study is to present experimental evidence that both temperature and food affect body mass losses during winter. To this end, groups of three species of bivalves, *M. balthica*, *C. edule* and *M. edulis*, were kept simultaneously during several months at two different winter temperatures and two different food regimes. Field observations on body mass changes in winter supplemented the experimental work.

Materials and Methods

Field observations

To follow mass changes and gametogenesis in the field, samples of 25 - 35 specimens were regularly taken from tidal-flat populations of *Macoma balthica* and *Cerastoderma edule* between the end of October 1994 until their spawning period in 1995. *M. balthica* were sampled from three sites on Balgzand (Fig. 2.1A), each of which had populations characterised by different body mass values at the same shell length. *M. balthica* with a relatively high body mass were found low in the intertidal area (A: transect 8 of Beukema 1988, at 52 ?57 ?5.9 ? N, 4 ?50 ?10.6 ? E). *M. balthica* with an intermediate body mass were found close to one of the gullies (B: square B of Beukema 1988, at 52 ?55 ?26.7 ? N, 4 ?49 ?1.3 ? E). *M. balthica* with a relatively low body mass were found at a high intertidal site with short immersion times (C: transect 2 of Beukema 1988, at 52 ?54 ?13.9 ? N, 4 ?50 ? 34.4 ? E).

C. edule of different body mass values were also sampled at three different stations. These sites were located along a transect in the Mok (Fig. 2.1B), a small bay at the southeastern part of Texel (48 ?52 ?41.0 ? N, 10 ?52 ? 52.3 ? E). At cockle station D, close to low-tide level (LTL), *C. edule* had a relatively high body mass. *C. edule* from the second station, (E, located between LTL and the shore) had an intermediate body mass. *C. edule* from

the third station (F, close to the shore) had a low body mass. Abiotic characteristics of the six sampling stations are summarised in Table 2.1.

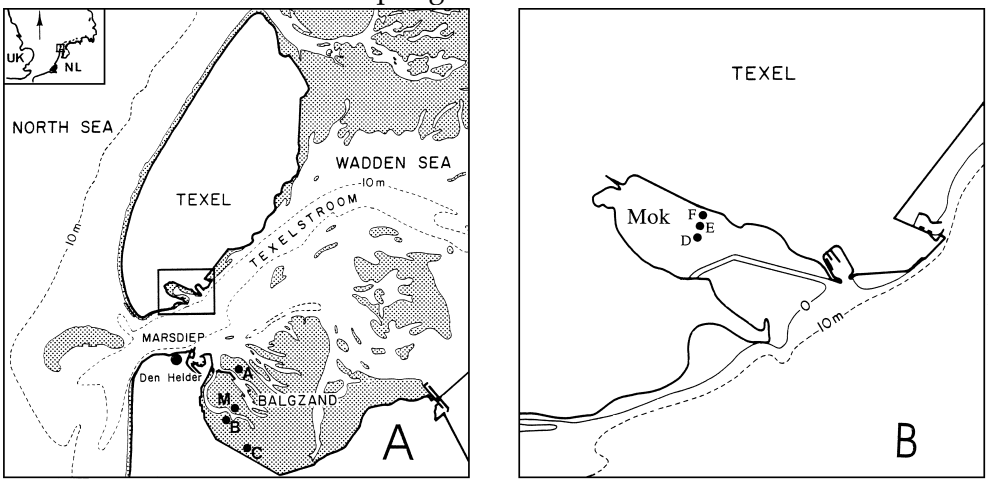


Figure 2.1. Map of the southwesternmost part of the Dutch Wadden Sea, showing (A) the Balgzand *Macoma balthica*-sampling stations (A, B, and C) and *Mytilus edulis*-sampling station (M), and (B) Mok with the *Cerastoderma edule*-sampling stations (D, E and F)

Observations were made from the autumn 1994 until the start of the spawning season in spring 1995. In *M. balthica*, gametogenesis takes place in the second half of the year and continues until January when mature gametes are present (Caddy 1967, Gilbert 1978, Harvey and Vincent 1989, Lammens 1967). Between the end of December and the end of March (prior to the spawning period) we sampled the field populations once a month. In *C. edule* gametogenesis takes place between February and spawning which takes place mostly in May (Boyden 1971, Iglesias and Navarro 1991, Ivell 1981, Newell and Bayne 1980). During some months before gametogenesis

Table 2.1. Abiotic characterisation of the sampling stations. Stations A, B and C are on Balgzand, stations D, E and F are Mok stations.

Station	Sediment fraction < 50 µm (%)	Median grain size (µm)	Height compared to MTL (cm)	Immersion period (% of tidal cycle)
A	1.3	182.4	-20	58
B	1.3	179.8	0	53
C	7.6	114.8	+30	43
D	24.5	157.1	-75	83
E	14.4	184.7	-70	80
F	16.2	190.6	-40	65

(the end of October until the end of January), we sampled *C. edule* once a month, but during gametogenesis (from January until spawning), fortnightly.

M. balthica were collected at low tide by removing the top layer of the sediment and collecting the animals of 14 - 17 mm shell length. *C. edule* of 28 - 32 mm shell length were sampled in the same way. Because the body mass of *M. balthica* can be affected by infection with the trematode parasite *Parvatrema affinis* (Zwarts 1991), all animals were checked and only uninfested animals (25 specimens) were used to study changes in body mass.

Within a few hours after collection, the animals were shortly immersed in boiling water until their shell opened. Parasitised *M. balthica* were not analysed but they were replaced by healthy individuals to ensure that the sample size remained constant. The soft parts were completely removed from the shells and placed individually in porcelain cups and dried for 4 days at 60 °C 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. Weighing was to the nearest 0.1 mg (W1). Subsequently the dried flesh was incinerated for 4 hours at 580 °C, cooled to room temperature, weighted again (W2) and the ash-free dry mass (AFDM) was determined (W1 - W2). The body mass index (BMI) is defined as the AFDM (mg)/shell length^3 (cm^3).

Long-term data on body mass of *M. balthica* are available from a sampling series on Balgzand. During the years 1970 - 1995 annually (in March) and during 1980 - 1995 twice a year (in March and August), *M. balthica* were sampled at 15 stations. Their body masses were determined in the way described above. For more details see Beukema (1992).

Experiments

Design

Two replicate plots were used for each of two temperature levels. Each of these four plots was divided into two sub-plots, one tidal and one subtidal. This 'split-plot' design (Cochran and Cox 1957) was statistically analysed with the appropriate ANOVA procedures in SYSTAT (Wilkinson 1990). The effect of temperature (1 df) was tested using the plots within each temperature (2 df) as the error term. For the effects of tidal regime (1 df) and the interaction between temperature and tidal regime (1 df), the tidal regime by plots within each temperature (2 df) was used as the error term. Because of the low power of the experimental set up (only one replicate per treatment), significance levels were set to $\alpha = 0.1$.

Practical set up

The plots of the set up consisted of 4 double-walled and isolated basins (l x b x h = 265 x 77 x 61 cm). Each basin was divided into 2 compartments (sub-plots) (Fig. 2.2A). The compartment (175 l) at the inlet side was used to adjust the water temperature by either heating or cooling. It was separated from the rest of the basin by a wooden partition. Sea water pumped out of the Wadden Sea entered the inlet compartment at a rate of 6 or 8 l min^{-1} (in 1994 and 1995, respectively). Either cooled, heated, or untreated, it flowed into the second compartment, over the total width of the partition. The second compartment was longitudinally divided into 2 parts with different heights ('subtidal' and 'tidal'), each with an area of 0.65 m^2 (Fig. 2.2A).

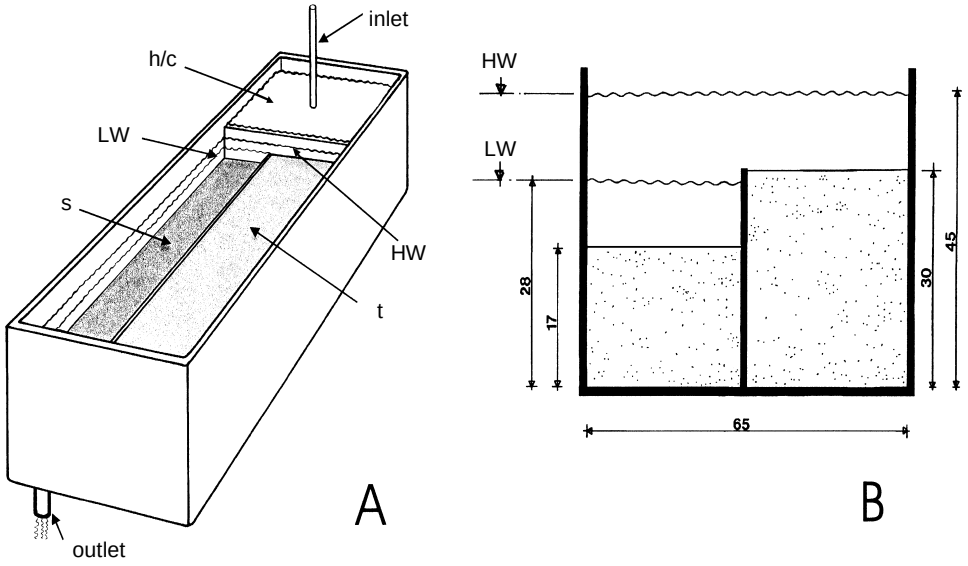


Figure 2.2. (A) Schematic drawing of one of four identical basins used for a simulation of a tidal (t) and a subtidal sand flat (s). Water temperature could be manipulated by heating or cooling the sea water in the heating / cooling compartment (h/c). Water level was regulated manually by removing a part of the overflow. (B) Cross section of the flat compartment. Dimensions are in cm. HW = high water level, LW = low water level. For more details see text.

In both parts the sediment was sand without organic material (dune sand) with a median grain size of 291 µm. The depth of the sediment layers was 17 and 30 cm in the subtidal area and the tidal area, respectively (Fig. 2.2B). To avoid temperature peaks (due to radiation of the sun) and growth of macro algae, the basins were covered with a 4-cm layer of opaque isolating material.

Tidal regime

The water level, and thus the time available for pelagic feeding, was manipulated manually by changing the height of the outlet overflow. During the day (08.00 h - 17.00 h), except during the weekends, the water level was lowered to 11 cm above the subtidal area (Low Water Level), thus exposing the higher ('tidal') level. Once per week the water level was also kept low during the night. Thus, the simulated low-water level was maintained at 72 hours a week, draining the tidal level for 43 % of the time.

The water level during the remaining part of the week (96 hours) was kept 17 cm above LWL, resulting in submersion of both levels.

Temperature regime

The starting point of the temperature regime was the water temperature of the nearby Marsdiep tidal inlet. To simulate a cold winter, the water of two basins was cooled by 2.5 ?C. The water of the other two basins remained unchanged, simulating a relatively mild winter (all winters during the period of these measurement happened to be mild). During days at which the temperature of the inflowing water happened to be around freezing point, cooling was terminated and the water of the two other basins was heated to maintain temperature differences of about 2.5 °C. The water temperatures maintained in our set up in 1994 and 1995 and the water temperature of the Marsdiep inlet measured in the same period are shown in Figure 2.3.

Animals

M. balthica in the size class of 14 - 17 mm shell length were collected in December at Balgzand on the above mentioned station B. The animals were randomly divided into 8 groups of 350 animals each and they were scattered over the parts of the sediment that was not occupied by mussel boxes. They buried rapidly. *C. edule* used in the first period (3 January 1994 until spawning) originated from station E and in the second period (22

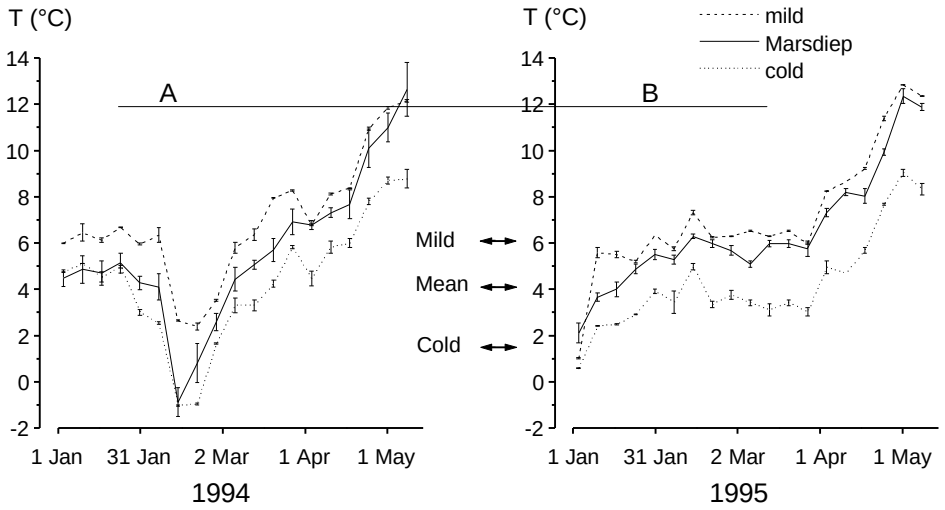


Figure 2.3. Water temperatures (weekly means of duplicates ± SD in °C) of two different temperature treatments, simulating a relative cold (means of 3.0 and 3.1 °C in the Jan. - March periods in 1994 and 1995, respectively) and mild winter (means of 5.6 and 5.7 °C in the Jan. - March period in 1994 and 1995, respectively), compared with the water temperatures of the Marsdiep inlet (means of 3.9 and 5.1 °C in the Jan. - March period in 1994 and 1995, respectively), measured in the same period during two years of experiments, (A) in 1994 and (B) in 1995. In addition, the mean levels of water temperature during the first three months of the year are indicated for three types of winters (arrows between Figs A and B): cold (mean of the years 1979, 1985, 1986, and 1987), mild (mean of the years 1974, 1988, 1989, and 1990), and normal (mean of the other 18 winters of the 1970 - 1995 period).

December 1994 until spawning) from station D. After *M. balthica* had buried into the sediment, 480 cockles were allowed to bury themselves into the same parts of the sediment of the eight compartments as *M. balthica*. *Mytilus edulis* in the size class 45 - 55 mm were collected on a wild mussel plot at Balgzand (M at 52 ? 55 ?49.9 ? N, 4 ? 49 ?31.7 ? E) and placed in the set up at 3 January 1994 and 22 December 1994 for the two experimental periods respectively (however in this species only results from the second period are presented). Eight groups of 450 animals, one group per plot and per tidal level, were placed in polyethylene boxes with perforated sides and bottom and covered with a net to prevent escape of the animals. The boxes were placed on the sediment near the outlets.

Sampling of animals

Once a month, 10 animals per plot and per tidal level were sampled randomly and used for the determination of their BMI. In the first winter, application of the different treatments started at 3 January 1994. In the second winter period, the first BMI sample was taken somewhat earlier, 22 December 1994, though the application of treatments started 1 January. Spawning of *M. balthica* takes place at a water temperature of 7 - 14 ?C (Caddy 1967, De Wilde 1975, De Wilde and Berghuis 1976, Gilbert 1978, Harvey and Vincent 1989). In early 1994 a water temperature of 9 - 10 ?C was reached in the second half of April. Therefore, our last BMI sample before the probable spawning of *M. balthica* was at 21 April. In 1995 the critical water temperature was reached somewhat earlier. In 1995 our last BMI sample before spawning of *M. balthica* was taken at 18 April. Spawning by *C. edule* takes place at the end of May and in June (Boyden 1971, Seed and Brown 1977, Ivell 1981) and spawning by *M. edulis* takes place in this same period (Pipe 1985, 1987, Zwarts and Wanink 1993). Our last BMI samples in 1994 were taken at 16 May (only *C. edule*) and in 1995 at 15 May (*C. edule* and *M. edulis*).

Results

Field observations

Body mass changes in Macoma balthica

Body mass values decreased during the 1994/1995 winter at all three stations (Fig. 2.4A). At the start of the observations, the BMI values at the three sampling stations differed as expected: A > B > C. This ranking was maintained throughout the period of observation, November - March. Minimal values were found in February or March. These minimal values amounted to 62, 65, and 73 % of the initial values in autumn at the station A, B, and C, respectively. Note that the sharpest decline (38 %) was observed at the station with the highest initial value (A) and the smallest decline (27 %) at the station with the lowest initial value (C).

Both initial value and rate of decline determine the final body mass values in late winter. The long-term data (1980 - 1995) on seasonal (August - March) mass loss showed similar differences between stations. Where summer values of BMI were high (most of Balgzand), proportions left in late winter were relatively low (60 - 66 % on average), whereas in the

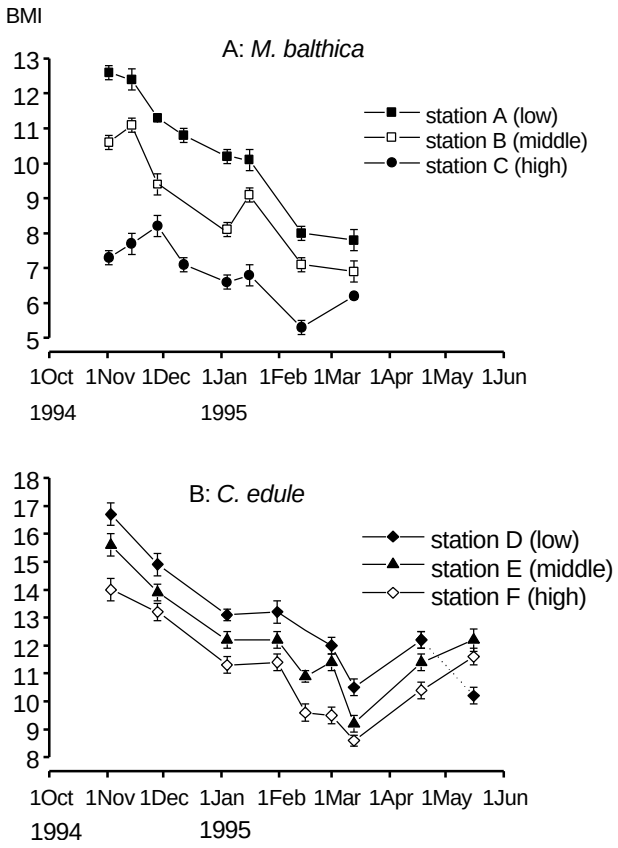


Figure 2.4. Changes in body mass of (A) *Macoma balthica* and (B) *Cerastoderma edule* at three stations during the period 2 November 1994 till a date just before spawning in 1995, expressed as changes in BMI (mg Ash Free Dry Mass / shell length^3 (cm^3) (mean ± SE, n = 25).

restricted coastal area with low summer values, the proportions left in March amounted 70 - 82 % of the summer values. As a consequence, regional differences are generally smaller in late winter than in summer.

Long-term observations in M. balthica

The results obtained from the long-term (1970 - 1995) data series on Balgzand indicated a negative relationship between winter temperature and mean body mass at the end of winter (Fig. 2.5A). This correlation was

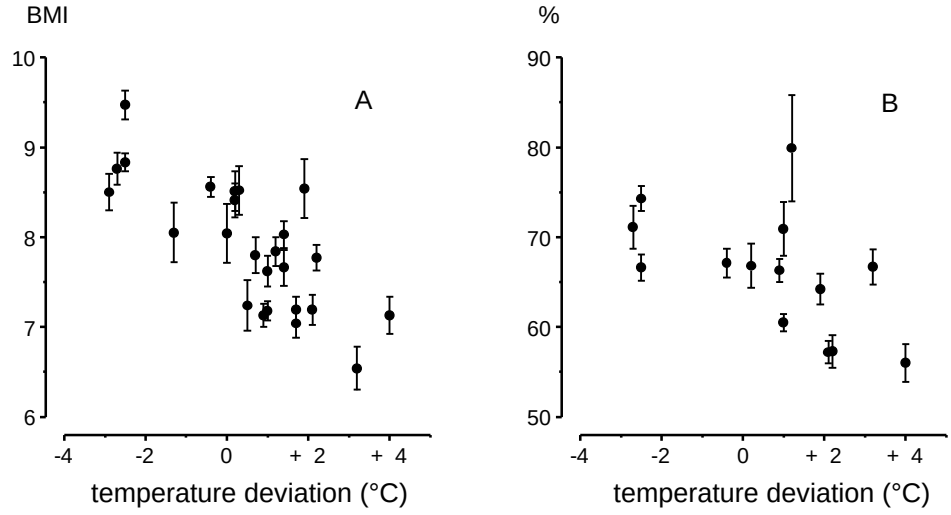


Figure 2.5. Relationships between over-winter temperatures (plotted in °C as the deviation in each year from the long-term average for the Jan. - March period) and (A) Mean BMI of adult *Macoma balthica* in March in each of the years 1970 - 1995 (excl. 1991); and (B) proportion of mass of soft parts left in March (% of preceding August value) in each of the years 1981 - 1995 (excl. 1990 - 1991). BMI is quotient M / L^3 (M = Ash Free Dry Mass in mg, L = shell length in cm). Percentage mass remaining = 100 * BMI March / BMI preceding August. Standard errors, reflecting the place-to-place variability, ranged from 0.10 to 0.33 for the 25 years in (A), and from 1 to 3 (6 in the one outlying point) of March / August values for the 14 years in (B).

highly significant for the annual averages of the 15 stations (R = -0.63, n = 25, $P < 0.001$). Correlations with winter temperature were also negative at all 15 individual stations and these correlations were statistically significant ($P < 0.05$, 1-tailed) at 13 out of the 15 stations.

The incidence of lower BMI values after mild than after cold winters points to more serious mass loss as average winter temperatures get higher. However, body mass in March is not only dependent on the amount of mass lost during winter, but also on the body mass before the start of winter. Therefore, the percentages of the August body mass still present by the following March were also plotted as a function of winter temperature (Fig. 2.5B). The proportions of initial body mass left in March were also significantly correlated with temperature deviations (R = -0.54, n = 14, $P < 0.05$). Thus, *M. balthica* body mass prior to spawning was higher

as pre-winter values were higher and water temperatures in winter were lower.

A multiple regression analysis of the long-term data of BMI data in August in year n and March in year n + 1 (14 pairs, see Fig. 2.5B) showed the best fit to be:

$$BMI_{March} = 4.9 + 0.27 * BMI_{August} - 0.29 * \text{winter temperature deviation.}$$

Thus, for each degree the winter temperature was higher the BMI in March was on average 0.29 mg cm^{-3} lower. As cold and mild winters differ by more than 6°C, resulting BMI values differ by about 2 mg cm^{-3}, about 25 %. Initial BMI (range 9.8 - 13.8) values had a minor (up to about 1 mg cm^{-3}), but statistically significant effect. The above equation explained 80 % of the variance of the BMI values observed in March. The standard errors attached to the three coefficients were 1.2, 0.10 and 0.06, respectively. Thus all coefficients differed significantly from 0 ($P < 0.02$ for the August BMI values and $P < 0.001$ for the temperature coefficient).

Body mass changes in Cerastoderma edule

At all three sampling stations, BMI values declined during the November 1994 - March 1995 period (Fig. 2.4B). The initial body mass of *C. edule* at station D, the station with the lowest position in the intertidal area, was relatively high, whereas the body mass at F, the highest station, was low. Animals from the intermediate station E, had an intermediate BMI. The differences between the groups remained similar throughout the observation period. Rates of decline appeared to be independent of initial body mass. The declines were followed by an increase in body mass prior to spawning, taking place in the second half of May. Animals at station D spawned earlier than animals at station E and F. Spawning at station D was completed before 16 May, resulting in a reduced body mass at that time.

Unfortunately, data from long-term series cannot be used in *C. edule*, because this species shows in the Wadden Sea a very low survival after cold winters (Beukema 1985, 1990).

Experiments

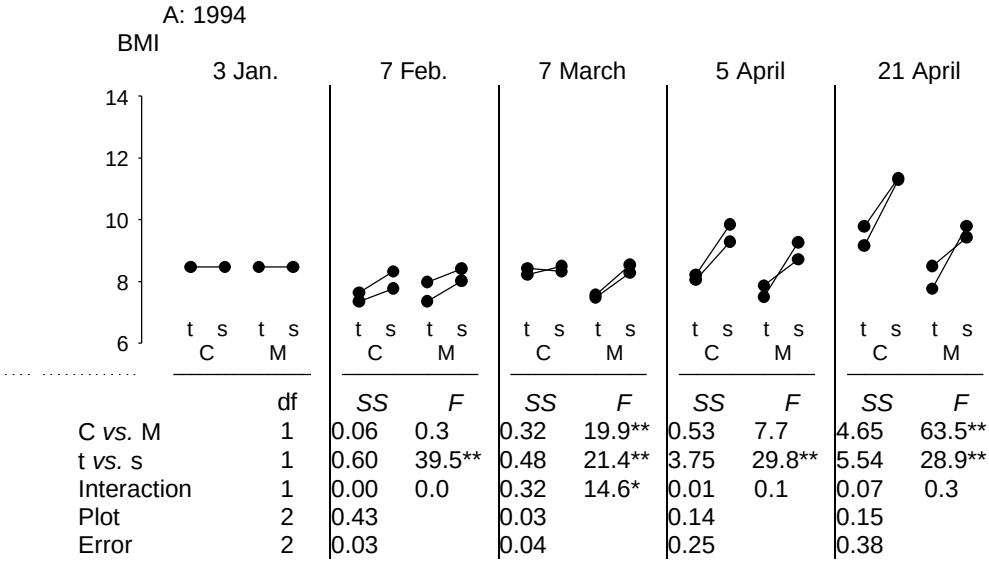
Water temperatures

The mean water temperatures during the three winter months (Jan., Feb. and March) were similar in the two winter periods during which the experiments were performed (Fig. 2.3). In early 1994, the higher water temperature was on average 5.6 °C and the lower one 3.0 °C. In early 1995, these temperatures were 5.7 °C and 3.1 °C, respectively. The intended difference of 2.5 °C was thus realised rather closely in both years. Because the set up was placed outdoors and was fed with Marsdiep water, the temperature of the inflowing water fluctuated in parallel with the water temperature of the Marsdiep inlet (Fig. 2.3). The mean Marsdiep water temperature of the first three months of the year was 4.1 °C during most of the years of the 1970 - 1995 period (except for the four mildest and four coldest years). The mean water temperatures in the four mildest winters (1974, 1988, 1989 and 1990) and in the four coldest winters of this period (1979, 1985, 1986 and 1987) were 6.1 and 1.5 °C, respectively. Thus the two temperature regimes during our simulated winters were similar to mild (5.6 and 5.7 *versus* 6.1 °C) and cold to normal winters (3.0 and 3.1 *versus* 1.5 to 4.1 °C), respectively.

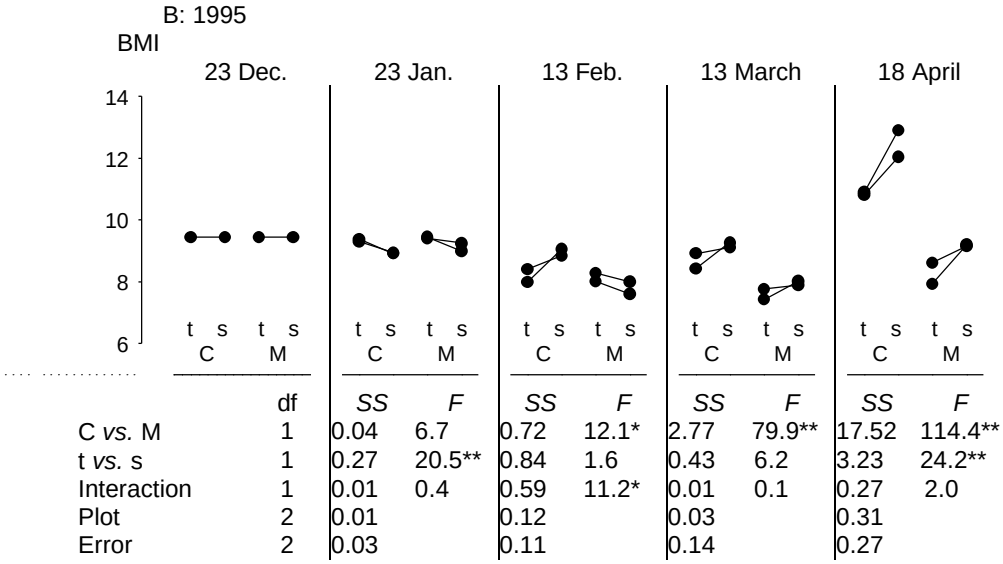
Effects of water temperature

During the periods of mass loss, similar temperature effects in all three species, in the two years, and in the two immersion treatments were observed. Losses of body mass were largest and the resulting mass smallest at the higher of the two water temperatures. At intermediate days, comparisons of the groups kept at the same tidal level showed a consistent difference between the two temperature regimes. If a significant temperature effect was observed at any sampling day, the BMI of animals kept at the higher temperature was lower than BMI at the lower temperature (Figs 2.6 - 2.8).

In *M. balthica* the periods of mass loss lasted until 7 March and 13 March in 1994 and 1995, respectively (Fig. 2.6A and 2.6B). Significant temperature effects ($F = 19.86$, $P < 0.05$ in 1994 and $F = 79.9$, $P < 0.01$ in 1995) were observed in both years at the end of this period. During the period between the onset of growth and start of spawning, the animals at the lower temperature stayed significantly heavier than those at higher temperatures (Fig. 2.6).



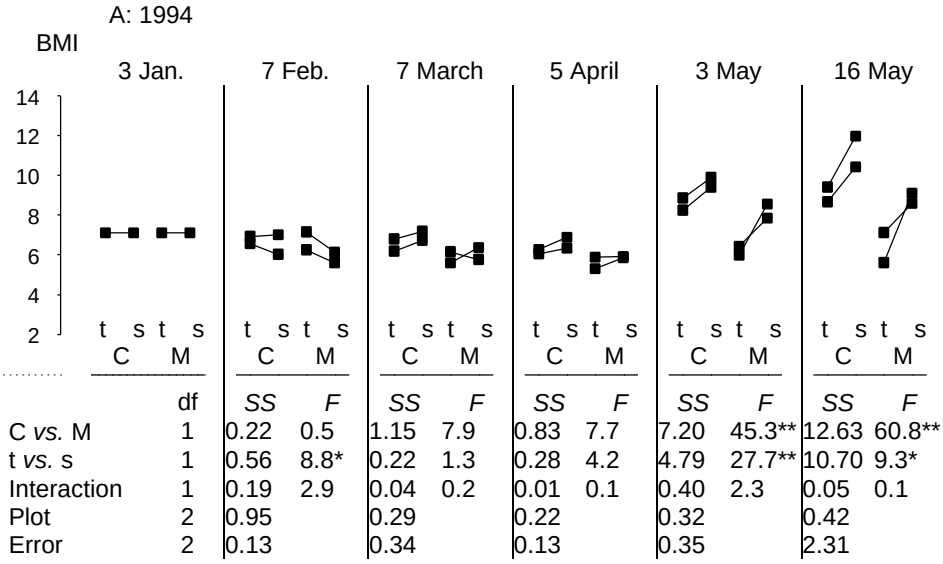
	df	3 Jan. *SS*	3 Jan. *F*	7 Feb. *SS*	7 Feb. *F*	7 March *SS*	7 March *F*	5 April *SS*	5 April *F*	21 April *SS*	21 April *F*
C *vs.* M	1	0.06	0.3	0.32	19.9**	0.53	7.7	4.65	63.5**		
t *vs.* s	1	0.60	39.5**	0.48	21.4**	3.75	29.8**	5.54	28.9**		
Interaction	1	0.00	0.0	0.32	14.6*	0.01	0.1	0.07	0.3		
Plot	2	0.43		0.03		0.14		0.15			
Error	2	0.03		0.04		0.25		0.38			



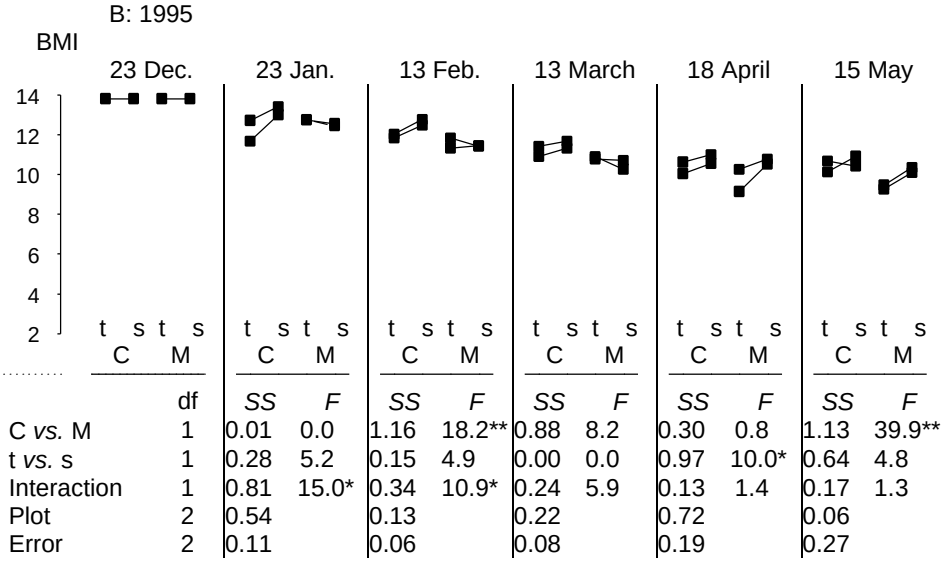
	df	23 Dec. *SS*	23 Dec. *F*	23 Jan. *SS*	23 Jan. *F*	13 Feb. *SS*	13 Feb. *F*	13 March *SS*	13 March *F*	18 April *SS*	18 April *F*
C *vs.* M	1	0.04	6.7	0.72	12.1*	2.77	79.9**	17.52	114.4**		
t *vs.* s	1	0.27	20.5**	0.84	1.6	0.43	6.2	3.23	24.2**		
Interaction	1	0.01	0.4	0.59	11.2*	0.01	0.1	0.27	2.0		
Plot	2	0.01		0.12		0.03		0.31			
Error	2	0.03		0.11		0.14		0.27			

Figure 2.6. Changes of BMI of *Macoma balthica* in the (A) 1994 and (B) 1995 experimental winter period. Mean BMI values of 10 animals per plot per treatment are plotted. Each line connects the BMI value of the tidal and subtidal group for each plot. For each sampling day the temperature (C = cold, M = mild) and the immersion treatment (t = tidal, s = subtidal) are indicated. The Y-axis shows the BMI values expressed as the quotient M / L^3 (M = Ash Free Dry Mass in mg, L = shell length in cm). Below the results of each date the ANOVA results

are given per sampling day. Significance levels are given as ** if $P < 0.05$ and * if $P < 0.10$.



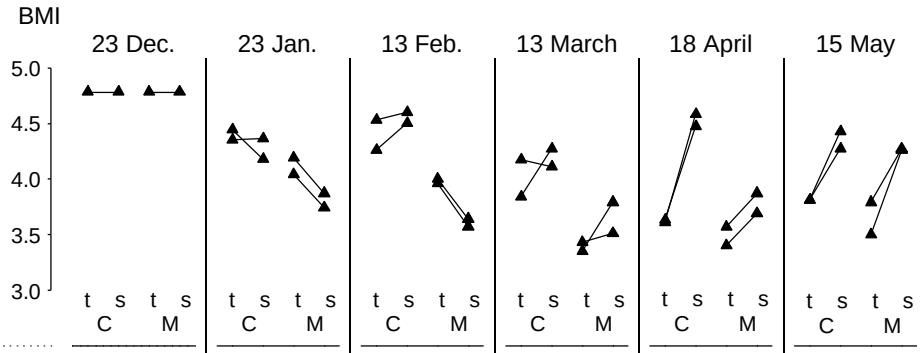
	df	3 Jan. SS	3 Jan. F	7 Feb. SS	7 Feb. F	7 March SS	7 March F	5 April SS	5 April F	3 May SS	3 May F
C *vs.* M	1	0.22	0.5	1.15	7.9	0.83	7.7	7.20	45.3**	12.63	60.8**
t *vs.* s	1	0.56	8.8*	0.22	1.3	0.28	4.2	4.79	27.7**	10.70	9.3*
Interaction	1	0.19	2.9	0.04	0.2	0.01	0.1	0.40	2.3	0.05	0.1
Plot	2	0.95		0.29		0.22		0.32		0.42	
Error	2	0.13		0.34		0.13		0.35		2.31	



	df	23 Dec. SS	23 Dec. F	23 Jan. SS	23 Jan. F	13 Feb. SS	13 Feb. F	13 March SS	13 March F	18 April SS	18 April F
C *vs.* M	1	0.01	0.0	1.16	18.2**	0.88	8.2	0.30	0.8	1.13	39.9**
t *vs.* s	1	0.28	5.2	0.15	4.9	0.00	0.0	0.97	10.0*	0.64	4.8
Interaction	1	0.81	15.0*	0.34	10.9*	0.24	5.9	0.13	1.4	0.17	1.3
Plot	2	0.54		0.13		0.22		0.72		0.06	
Error	2	0.11		0.06		0.08		0.19		0.27	

Figure 2.7. Changes of BMI of *Cerastoderma edule* in the (A) 1994 and (B) 1995 experimental winter period. Mean BMI values of 10 animals per plot per treatment are plotted. Each line connects the BMI value of the tidal and subtidal group for each plot. For each sampling day the temperature (C = cold, M = mild) and the immersion treatment (t = tidal, s = subtidal) are indicated. The Y-axis shows the BMI values expressed as the quotient M / L^3 (M = Ash Free

Dry Mass in mg, L = shell length in cm). Below the results of each date the ANOVA results are given per sampling day. Significance levels are given as ** if $P < 0.05$ and * if $P < 0.10$.



	df	23 Dec. *SS*	23 Dec. *F*	23 Jan. *SS*	23 Jan. *F*	13 Feb. *SS*	13 Feb. *F*	13 March *SS*	13 March *F*	18 April *SS*	18 April *F*	15 May *SS*	15 May *F*
C *vs.* M	1	0.28	25.7**	0.93	49.7**	0.67	77.4**	0.28	153.1**	0.03	2.2		
t *vs.* s	1	0.10	10.3*	0.02	6.5	0.10	2.1	0.89	84.4**	0.67	51.8**		
Interaction	1	0.02	1.9	0.14	37.7**	0.00	0.1	0.11	10.7*	0.00	0.2		
Plot	2	0.02		0.04		0.02		0.00		0.03			
Error	2	0.18		0.01		0.09		0.02		0.03			

Figure 2.8. Changes of BMI of *Mytilus edulis* in the 1995 experimental winter period. Mean BMI values of 10 animals per plot per treatment are plotted. Each line connects the BMI value of the tidal and subtidal group for each plot. For each sampling day the temperature (C = cold, M = mild) and the immersion treatment (t = tidal, s = subtidal) are indicated. The Y-axis shows the BMI values expressed as the quotient M / L^3 (M = Ash Free Dry Mass in mg, L = shell length in cm). Below the results of each date the ANOVA results are given per sampling day. Significance levels are given as ** if $P < 0.05$ and * if $P < 0.10$.

In *C. edule* the pattern of change of body mass was in 1994 (Fig. 2.7A) similar to the patterns observed in *M. balthica*, though the increase of body mass started somewhat later (after 5 April). Although the expected trends were present (lower BMI values at the higher temperature), temperature effects were not statistically significant during all of the period of mass loss in this species. Differences connected with temperature treatment started to become statistically significant as soon as growth started, from the beginning of April on until spawning (Fig. 2.7A).

The pattern obtained in *C. edule* in 1995 (Fig. 2.7B) differed from that of 1994 (Fig. 2.7A). Due to different sampling sites in 1994 and 1995, the initial BMI values in the two years differed substantially (7.1 in 1994, 13.8 in 1995). Subsequent mass losses were in 1995 greater than in 1994 and lasted almost until spawning (*viz.* till 18 April 1995). As in 1994, the 'cold'

treatment resulted generally in higher masses than the 'mild' treatment, significantly so at 13 February and 15 May.
In *Mytilus edulis* (Fig. 2.8), the expected trend was also present. From 23 January on till the end of the period of mass loss (13 March), the temperature effect was significant at all sampling dates. This was also true at 18 April. Due to the relatively rapid growth of the mild-subtidal group and the retarded growth of the cold-subtidal group, the temperature effect became relatively less important after this date (Fig. 2.8). As in the natural situation, growth started in the beginning of spring (between mid March and mid April).

Effects of immersion time

Throughout the whole experimental period significant tidal-treatment effects were observed, although not at each sampling date. Though BMI values from the end of the period of mass loss on until spawning of the subtidal groups were invariably higher than in the tidal groups, this was not consistently so during the foregoing period of gradual mass loss.

Examples of aberrant differences are shown in Fig. 2.6B in the 1995 data series in *M. balthica* with significantly higher BMI values in the tidal group on 23 January and also (in the mild group) on 13 February (with an almost significant interaction term). In *C. edule* such higher BMI values of the tidal groups were observed on 7 February 1994 (Fig. 2.7A) and on 23 January and 13 February 1995 (Fig. 2.7B) at the higher temperature (with almost significant interaction terms). In *M. edulis* the tidal groups showed almost significant higher BMI values on 23 January (Fig. 2.8) and at the higher temperature on 13 February (with a significant interaction term).

Discussion

Body mass changes, winter temperature and tidal level

On the tidal flats in the Dutch Wadden Sea, significant correlations have been observed between winter temperatures and loss of body mass of four bivalve species. Zwarts (1991) reports significant relationships between temperature and loss rates during several winter periods in *Mya arenaria*, *Cerastoderma edule* and *Scrobicularia plana*. In *Macoma balthica* a positive relationship has been observed between the mean winter temperature of the entire winter and mass loss between summer and late winter (Beukema 1992, this paper: Fig 2.5B). As a consequence, the resulting BMI values in

March were generally much lower after mild than after cold winters (Fig. 2.5A).
Because relatively high body masses after cold winters are positively correlated with subsequent recruitment (Beukema 1992), over-winter losses are an important life-history phenomenon. So far, experimental evidence was lacking that the correlations between winter temperature and mass loss are causal. Evidence for such causality was found in our experiments. Some general patterns caused by temperature could be observed in the successive periods of loss and gain of body mass. During the initial period all species tended to lose mass. At the higher water temperature loss rates were always higher than at the lower temperature. Because the rate of loss was higher and the period of loss lasted generally longer, body mass values at the end of the period of loss of body mass were lower at the higher than at the lower temperature. Results to be published in a subsequent paper indicate that the losses of body mass in *M. balthica* were mainly caused by a decreasing gonadal mass, *i. e.* gametes were resorbed and used as energy source. The following period, with a gain of body mass, started earlier and as a consequence the body mass just prior to spawning was higher at the lower than at the higher water temperature.

In both years, differences in body mass (as caused by temperature differences) were significant from the beginning of March on and were maximal just prior to spawning. This can be explained by the fact that differences in energy needed at the relatively low temperatures in the beginning of the year are smaller than the differences in energy needed at the relatively high water temperatures prior to spawning. In other words, energy needed for maintenance and growth increases exponentially with a rise in temperature. This has been demonstrated for *Mytilus edulis* in which energy demands were measured as seasonal changes in O_2 consumption. In early spring a non-linear increase in O_2 consumption was observed (Bayne *et al*. 1976, 1977, De Vooys 1976).

Energy from ingested food is used in the first place for maintenance, the remainder is available for growth, [the so-called κ -rule of Kooijman (1993)]. This rule can be used to explain the patterns of changes in body mass observed. Because maintenance energy is lower at the lower temperature, more energy will be available for growth, assuming an equal food supply at the two temperatures, than at the higher temperature. As a consequence, a relatively early growth start from a relatively high

remaining body mass is possible in the groups kept at a low water temperature.

Not only temperature will affect rates of loss and gain of body mass, also immersion time will be important in these processes. Long immersion periods allow long feeding times. This will be favourable at high food densities, usually starting in March (Cadée and Hegeman 1977). Indeed during the period from the beginning of growth on until spawning, the subtidal groups showed consistently higher body mass values than the tidal groups. However, in the preceding period of loss of body mass, in all species and in both years (except in *M. balthica* in 1994, Fig. 2.6A), an inverse immersion-time effect was observed, with subtidal groups showing temporarily lower BMI values than tidal groups. This was more clearly so in *C. edule* (Fig. 2.7) and *M. edulis* (Fig. 2.8) than in *M. balthica* (Fig. 2.6). Thus temporarily energy balances were more negative in the groups with prolonged feeding opportunities. Maybe, extra foraging efforts at unprofitably low food densities resulted in extra mass loss. An alternative hypothesis might be that during the winter period the air temperatures are generally lower than the water temperatures (De Wilde and Berghuis 1979) resulting in a stronger seasonal decrease of metabolic rates in the tidal groups than in the subtidal groups during the daily the low-water periods. Because *M. balthica* was buried deep in the sediment, a possible effect of lower air temperatures on body mass would be smaller than in the shallow buried *C. edule* and the epifaunal *M. edulis*. However, as the set up was covered almost completely by a thick isolating layer, the cooling effects of the wind will have been slight.

There are some differences between the courses of mass loss in 1994 (Figs 2.6A and 2.7A) and 1995 (Figs 2.6B and 2.7B): in early 1995 declines were more substantial than in early 1994. This may be related to either or both of two factors: (1) initial BMI values were higher in 1995 than in 1994 in both *M. balthica* and *C. edule* and (2) food supply will have been higher 1994 (water renewal at a rate of 8 l min^{-1}) than in 1995 (6 l min^{-1}).

Because the water temperatures of the 'cold' treatment were lower, and the immersion time of the subtidal groups was longer than in the tidal-flat field situation, growth of *M. balthica* under experimental conditions started earlier than in the field populations (Fig. 2.6 *vs.* Fig. 2.4A). Especially the start of the growth of the cold subtidal groups was relatively early and was followed by the growth start in the cold tidal and

mild subtidal groups. In both years, growth of the tidal groups kept at higher water temperatures started in early April, as in the field situation.
The patterns of change in body mass in *C. edule* (Fig. 2.7A and 2.7B) were similar to the patterns observed in *M. balthica*, with one remarkable difference. Although the trend was already present, significant effects of temperature and immersion time were observed later in time, appearing only when growth started. Body mass changes in *M. edulis* in 1995 (Fig. 2.8) were in accordance with the patterns found in *C. edule* (Fig. 2.7B) and *M. balthica* (Fig. 2.6B), and temperature effects were significant almost from the beginning of the experiments onwards. Again, subtidal groups initially lost more mass than tidal groups, but gained mass more rapidly once growth had started.

Differences between places and species

Initial BMI values of groups in field observations were different. Such differences can be related to differences in daily submersion periods (Beukema *et al.* 1977). Populations living high in the intertidal zone experiencing short immersion showed the lower initial autumn BMI values. The greatest distance between the sampling stations for *M. balthica* and *C. edule* were only 5 km and 130 m, respectively, and similar differences in body mass at this spatial scale have been reported by other authors. Kristensen (1957) reported differences in *C. edule*, and although there were a few exceptions to this rule, the cockles at the lower tidal levels had a higher body mass. By means of transplantation experiments, De Montaudouin (1996) confirmed that growth rates and masses in *C. edule* are higher at lower tidal levels and showed that such tidal-level related differences are largely phenotypical. Tidal-level effects in *M. balthica* have been reported by Harvey and Vincent (1989, 1990, 1991) and Beukema *et al.* (1977) and their results confirm our observations: shorter immersion results in lower body mass values.

The exact pattern of loss of body mass differed between the two species (Fig. 2.4). In *C. edule* decreases of body mass were similar at the three places: the lines in Fig. 2.4B run parallel. In *M. balthica* however, the decrease differed between places. Animals from the lower stations, A and B, showed a similar and relatively fast decrease of body mass (62 and 65 % of initial body mass were left prior to spawning, respectively) while animals from the highest station C showed a relatively low decrease in

body mass (84 % of initial body mass was still present prior to spawning). Such place-to-place differences are consistent with results obtained from the long-term data series of Balgzand.
The differences in the course of mass loss between *C. edule* and *M. balthica* can be explained by their different ways of feeding. *C. edule* is an obligate suspension feeder. Hardly any food is available in the water column during late autumn and winter (Cadée 1978, Beukema and Cadée 1996). Therefore, *C. edule* has to depend entirely on energy stores to survive the winter. Because local differences in water and air temperature are small on the scale of observation, the rates of decrease of body mass were similar in all groups.

M. balthica is a facultative filter feeder (Brafield and Newell 1961, Hummel 1985, Ólafsson 1986), although its morphology is that of a deposit feeder (Yonge 1949, Gilbert 1977). *M. balthica* can feed at the sediment surface if food in the water column is absent (Hummel 1985, Kamermans 1992, Lin and Hines 1994). The relatively heavy *M. balthica* from the lower tidal levels A and B (Fig. 2.4A) can rely on their own body stores to survive the winter months. *M. balthica* with a low initial BMI need to feed during the winter, but in absence of food in the water column, they have to feed at the sediment surface. Because at high tidal flats growth of benthic diatoms already starts in January (Cadée and Hegeman 1977), food is available at station C on the sediment surface in relatively high amounts during the winter period.

Conclusions

Temperature and immersion time influence body mass changes in winter and spring, but the timing of their effects is different. Effects of food availability were most pronounced during the growing period whereas temperature affected body mass continuously. *Cerastoderma edule* appears to be less sensitive to differences in temperature than *Macoma balthica* and *Mytilus edulis*.

Small-scale spatial differences in body mass in *C. edule* and *M. balthica* were mainly caused by differences in food supply at the different tidal levels. The decrease in body mass in autumn and winter was due to lack of food during this period. But, if it was morphologically and ecologically possible (*i.e.* if deposit feeding was possible), and if it was necessary to eat during the winter, the decrease of body mass could be

slowed down. If deposit feeding was not possible, the decrease of body mass was equal at all tidal levels.

Effects of immersion time or food supply were demonstrated in our experiments. The decrease in body mass from the tidal groups was higher than from the subtidal groups. This effect was strengthened by higher water temperatures. At higher water temperatures the decrease in body mass lasted longer, due to the higher metabolic costs, than at the lower water temperatures. As a consequence, the loss of body mass at lower water temperatures was less than at higher water temperatures.

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CHAPTER THREE

Experimentally induced effects of water temperature and immersion time on reproductive output of bivalves in the Wadden Sea

Abstract

Results of experiments are reported on the effects of water temperature and immersion time in winter on egg size and egg numbers in three intertidally living bivalves in the Dutch Wadden Sea, the Baltic tellin *Macoma balthica*, the common cockle *Cerastoderma edule* and the common mussel *Mytilus edulis*. *M. balthica* (14 - 17 mm shell length) produced large eggs (diameter of 107 µm) in relatively small numbers (20 000 - 70 000) in early spring. Later in spring, *C. edule* (28 - 33 mm shell length) produced smaller eggs (77 µm, excluding the surrounding jelly layer) in tenfold larger numbers (200 000 - 700 000). *M. edulis* (45 - 55 mm shell length) spawned even smaller eggs (72 µm) in high (but not easily assessed) numbers over a more extended period. In *M. balthica* egg size was not affected by winter temperatures or immersion time. Effects of winter - spring temperatures and immersion time on egg size could be demonstrated in *C. edule*. Smaller eggs were produced at the higher temperatures. Effects of immersion time were non-consistent: at lower water temperatures larger, but at higher temperatures smaller eggs were produced by animals kept at longer immersion times. In *M. edulis*, no temperature effects were observed. However, a longer immersion time resulted in larger eggs. In *M. balthica* as well as in *C. edule* significantly more eggs were produced at the lower temperature. Immersion time effects were most pronounced at the lower temperature, where more eggs were produced at the subtidal level than at the tidal level. At the higher water temperature differences between egg numbers produced at the two tidal levels were small. Just prior to spawning, egg numbers were strongly positively related to body mass at a certain shell length.

CHAPTER THREE

Introduction

In a number of species of bivalves strong year-to-year variability in recruitment has been reported. Examples of long-term series of recruitment success include the common cockle *Cerastoderma edule* (L.) (Kristensen 1957: 8 years, Beukema 1982: 13 years, Möller and Rosenberg 1983: 6 years, Ducrotoy *et al.* 1991: 6 years), the gaper clam *Mya arenaria* (L.) (Beukema 1982: 13 years, Möller and Rosenberg 1983: 6 years), the Baltic tellin *Macoma balthica* (L.) (Beukema 1982: 13 years), *Abra alba* (Wood) (Dekker and Beukema 1993: 19 years), the peppery furrow shell *Scrobicularia plana* (da Costa) (Essink *et al.* 1991: 20 years) and the common mussel *Mytilus edulis* (L.) (Beukema 1982: 13 years, McGrorty *et al*. 1990: 7 years). All of these studies show the same characteristics, *i..e.* that variation in recruitment can be quite large with year-to-year differences up to 100 or 1000 times not being uncommon.

Fluctuations in recruitment can been explained by:

1 differences in stock size of adults. Potentially a large stock can produce more eggs,
2 the average reproductive output per individual parent. For example, differences in reproductive output (measured as gonadal mass per female) between bivalve populations have been reported for the giant scallop *Placopecten magellanicus* (Gmelin) (MacDonald and Thompson 1985, Barber *et al*. 1988), in *M. balthica* (Harvey and Vincent 1989, 1991, Harvey *et al*. 1993), and in *M. edulis* (Bayne and Worrall 1980, Sprung 1983). So far, little attention has been paid to individual reproductive output in terms of egg size and egg numbers (fecundity per female), and
3 survival of larvae and juveniles. At high adult densities of *C. edule* larval mortality is high due to inhalation of larvae during foraging (André and Rosenberg 1991, André *et al.* 1993). Other factors influencing larval survival are the viability of the eggs and larvae (Bayne *et al.* 1975, 1978, Gallager and Mann 1986, Laing and Millican 1986), the availability of suitable substratum to settle (Kristensen 1957), *e.g.*, disturbance of sediment by high adult densities (Flach 1996), and predation on postlarvae after settlement by juvenile shrimps *Crangon crangon* (L.) and shorecrabs *Carcinus maenas* (L.) (Jensen and Jensen 1985, Beukema 1991, 1992b).

REPRODUCTIVE OUTPUT OF BIVALVE SPECIES

Reproductive output in egg producing animals consists of two components, egg volume and egg number. An organism can produce either large numbers of small eggs or small numbers of large eggs [for a model see: Smith and Fretwell (1974); for an empirical study in bivalves see: Bayne (1984) and Boyden (1971)]. Two main factors appear to be involved in causing differences in reproductive output,

1 food availability. In *M. balthica* (Harvey and Vincent 1989, 1991) and the ribbed mussel *Geukensia demissa* (Dillwyn) (Borrero 1987), better feeding conditions at lower than at higher intertidal levels cause a higher reproductive output at the lower levels. In the giant scallop reproductive output is higher in shallow and food-rich waters, than in deeper water with a relatively low food supply (MacDonald and Thompson 1985, Barber *et al*. 1988), and
2 water temperature. Several authors report that successful recruitment in bivalves is the rule after severe winters (Möller and Rosenberg 1983, Yankson 1986, Beukema 1992a). A relatively low loss of parental body mass during cold winters (Zwarts 1991, Beukema 1992a, Honkoop and Beukema 1997) probably allows adults to produce more and larger eggs.

In the present paper, effects on egg sizes and egg numbers in bivalves are reported of water temperature during winter and of immersion time (*i.e.* feeding time). To this end, three bivalve species that play an important role in the ecosystem of the Dutch Wadden Sea, *C. edule*, *M. balthica* and *M. edulis*, were kept during several months in large basins at two different temperatures and at two different regimes of immersion time. In *M. balthica* gametogenesis starts already soon after spawning and is finished early in winter (Caddy 1967, Lammens 1967, Chambers and Milne 1975, Bonsdorff and Wenne 1989), although some authors reported a longer lasting gametogenesis (De Wilde and Berghuis 1976, Gilbert 1978, Madsen and Jensen 1987, Harvey and Vincent 1989). In contrast to *M. balthica*, gametogenesis in *C. edule* (Boyden 1971, Iglesias and Navarro 1991, Ivell 1981, Newell and Bayne 1980) and *M. edulis* (Seed and Brown 1977, Bayne *et al*. 1982, Kautsky 1982, Bayne 1984) takes place just prior to spawning. Therefore, groups of *M. balthica* were kept at different tidal levels not only during winter, but also during part of the autumn, to test whether or not egg size can be influenced during gametogenesis.

Materials and methods

One experiment (Experiment I) was performed to study the effects of water temperature and immersion time on egg size and egg numbers in *Macoma balthica*, *Cerastoderma edule* and *Mytilus edulis* and was performed twice, in winter - spring 1994 and winter - spring 1995. A second experiment (Experiment II) was developed to study the effects of immersion time on egg size and egg number of *M. balthica* and was performed during the period of 24 October 1994 - 10 April 1995. As details about the set up used in Experiment I were described earlier by Honkoop and Beukema (1997), the experimental outline will be discussed in short only.

Experiment I

Experimental design

To manipulate temperature as well as food supply, four plots were used. For each of the two temperature levels, two replicate plots were used. Each plot was divided into two sub-plots, one tidal and one subtidal. This is a 'split-plot' design (Cochran and Cox 1957) and was statistically analysed with the appropriate analysis of variance (ANOVA) procedures in SYSTAT (Wilkinson 1990). Because the variance in egg numbers within any group was proportional to the mean, data on egg numbers were log transformed. These log-transformed numbers were used to estimate temperature and immersion time effects.

Practical set up

Four double-walled and isolated basins (l x b x h = 265 x 77 x 61 cm) were used as experimental units (plots), each divided into two sub-plots representing one subtidal and one tidal level. Two plots were kept at low water temperatures and two at a higher water temperature.

Water temperature and immersion time

The outdoor set up was fed with fresh seawater from the nearby Marsdiep Wadden Sea inlet (Sal. = 27.4 ± 2.8 ‰, mean ± SD). To simulate a cold winter, the water of two plots was cooled by 2.5 °C, the water of the other plots remained unchanged (Honkoop and Beukema 1997). This treatment was maintained until the spawning period of *C. edule* in late May. The tidal

level was manually changed every day. On a weekly base, the tidal area was drained for 43 % of the time, corresponding to an intertidal height of about 20 cm below mean-tide level.

In the January - March periods in 1994 and 1995 the water temperatures in the experimental set up were similar. The lower water temperatures were on average 3.0 °C and 3.1 °C and the higher mean temperatures were 5.6 °C and 5.7 °C in 1994 and 1995, respectively. Because our set up was placed outdoors, and fed with Marsdiep inlet water, the temperature fluctuations paralleled the natural fluctuations. Water temperatures were close to those during a normal winter for the lower water temperatures and to a mild winter for the higher water temperatures.

Experiment II

Experimental design

To see whether egg size and egg numbers of *M. balthica* could be influenced during the period of gametogenesis, an experiment was performed in which immersion time was manipulated during the autumn and winter months. The experimental period was divided into two parts. The first part began at the start of the experiment (24 October 1994) and ended 16 January 1995, when the second period started which lasted until spawning (April 1995).

During each period, experimental units were placed either subtidally (S) or tidally (T). At the beginning of this experiment, eight units were placed at the subtidal level and eight at the tidal level. At the beginning of the second period (16 January 1995), four experimental units from each tidal level were changed to the other tidal level. The tidal level of the other units remained unchanged. Thus four treatments were performed (Fig. 3.1), four units experienced a subtidal level throughout the whole period (SS), four units an intertidal level throughout the whole period (TT), four units a subtidal level during the first and a tidal level during the second period (ST), and four units a tidal level during the first and at a subtidal level during the second period (TS). The 4 x 4 units were spatially arranged according to a 'Latin square' design (Cochran and Cox 1957), with the only difference that two basins were used each with eight units (Fig. 3.1).

ANOVA procedures were performed with the appropriate procedures in SYSTAT (Wilkinson 1990). The between-treatment effects (3 df) was divided in a first-period effect (1 df), a second-period effect (1 df), and an interaction effect (1 df). The between-column effect (3 df) was divided in a between-basin effect (1 df), and a between-column-within-basin effect (2 df).

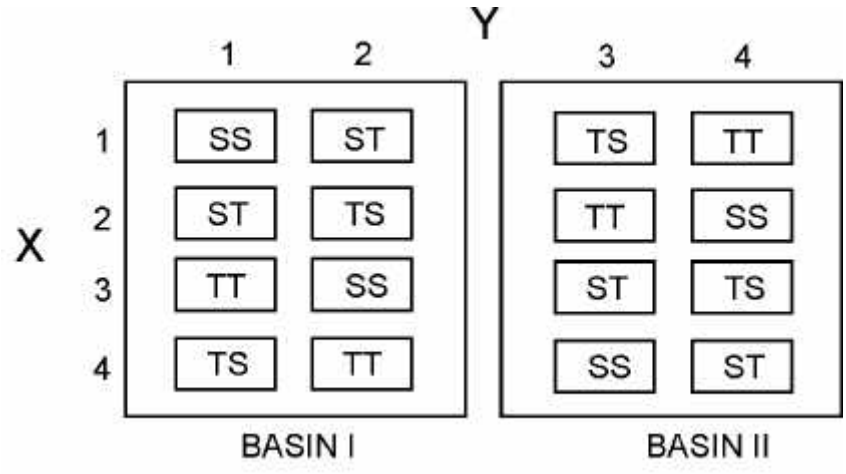


Figure 3.1. Schematic drawing of the experimental set up used in Experiment II. Treatments are noted per sub-unit. The first character refers to the treatment during the period 24 October 1994 - 16 January 1995, the second character refers to the treatment during the second period, 16 January 1995 - April 1995. S = subtidal and T = tidal treatment.

Practical set up

Two large wooden experimental units (l x b x h = 2.5 x 2.5 x 0.5 m) were placed outdoors and fed with fresh Marsdiep inlet seawater at a rate of 6l min^{-1}. In each basin eight units (l x b x h = 60 x 40 x 20 cm) were placed, filled with dune sand with a median grain size of 291 µm. Water level was changed as described for Experiment I. Each row and column contained one sub-unit per treatment.

Animal treatment

Collecting the animals

Animals used in Experiment I were collected in the field at the end of December. *M. balthica* with a shell length of 14 - 17 mm and *M. edulis* with a shell length of 45 - 55 mm were collected at Balgzand. *C. edule* with a shell length of 28 - 32 mm were collected at the Mok (Texel). At each sub-plot, 350 *M. balthica*, 480 *C. edule* and 450 *M. edulis* were placed, from which about 200 animals were left to perform spawning experiments (the others were used to follow mass changes, see Honkoop and Beukema 1997).
M. balthica with a shell length of 14 - 17 mm used in Experiment II were collected in the beginning of October 1994 at Balgzand. In each sub-unit 170 animals were allowed to burrow in, from which about 100 animals were left to perform spawning experiments.

Spawning initiation

In order to collect eggs, groups of animals were forced to spawn in the laboratory. The experimental initiation of spawning in *M. balthica* started when the Marsdiep inlet water temperature reached a critical level of 9 - 10 ºC. At this temperature, under natural conditions, spawning takes place (Caddy 1967, De Wilde 1975, De Wilde and Berghuis 1976, Gilbert 1978, Harvey and Vincent 1989). Spawning initiation in *C. edule* and *M. edulis* started later, at the end of May, when under natural conditions spawning occurs (Boyden 1971, Seed and Brown 1977, Ivell 1981, Pipe 1985, 1987, Zwarts and Wanink 1993). Because critical temperature levels were reached somewhat earlier at the higher water temperature than at the lower temperature, the groups kept at the higher water temperature were forced to spawn 1 - 2 weeks before the groups kept at the lower temperature.

The day before the animals were forced to spawn, appropriate numbers of about 100 animals were collected from the sub-plots and stored overnight at 4 ºC. The next morning *M. balthica* and *C. edule* were individually placed in 100 ml beakers filled with aerated sand-filtered fresh seawater with a temperature of 12 ºC for *M. balthica* and 15 ºC for *C. edule*. Thirty minutes after this temperature shock, the water was changed with fresh aerated seawater. In most cases, 10 min after this first refreshment, spawning started, mostly by males. Each 30 min the water was changed until no further animals started spawning. All remaining females which

had not yet spawned were stored at 4 °C. Experiments were repeated until all collected animals were used at least once or until sufficient material per group was collected to estimate egg sizes and numbers.

The attempts to initiate spawning in *M. edulis* were different from the attempts to force *M. balthica* and *C. edule*. It was found that a mere temperature shock failed. Therefore, according to the method described by Bayne (1965), *M. edulis* were injected with 2 ml of 0.5 *M* KCl in the mantle cavity prior to a temperature shock. After injection, the animals were stored dry for 30 min after which they were placed individually in 250 ml beakers filled with 200 ml of aerated seawater with a temperature of 15 °C. The seawater was changed after the same time interval as in *M. balthica* and *C. edule*. Sometimes spawning started within 10 min after they were placed in water for the first time, but mostly spawning began 45 - 60 min later.

All females that had spawned were numbered, stored each night at 4 °C, and each day a temperature shock was performed in order to complete spawning and completely empty the gonads. The shells of *M. balthica* were opened 2 - 3 weeks after the first spawning and gonads were checked. *C. edule* mostly died within 3 - 4 days after the first temperature shock. After they had died, their shells were opened and gonads were checked. Only egg counts of animals that had released all of their eggs were used to estimate egg numbers. Sufficient material to perform a statistical analysis on egg numbers of Experiment I was collected in both years (1994 and 1995) for *M. balthica*, but only in 1995 for *C. edule*. In 1994, many *C. edule* died (possibly due to their relatively low body mass) before their gonads were completely empty and therefore only egg sizes could be determined that year.

Measurements

Egg diameter and egg numbers

Freshly spawned eggs are multiform because they were tightly clumped together within the gonads. Within 30 min after the release of the eggs, the shape of most of the eggs changed to a round or only slightly aspherical shape. From each spawning female, 30 min after spawning started, a few hundreds of eggs were removed with a capillary pipette, placed on flat glass microscope slides and photographed. The photographed eggs were quantitatively returned to the clutch from which were removed.

From the eggs of each female, two slides were made, using ISO 100 colour slides, with a Zeiss M-35 camera fitted to a Zeiss stereo microscope at a magnification of 63. After developing the film, the slides were projected on a transparent screen with the slide projector placed on a fixed distance. Opposite the projector and at the backside of the screen the longest and shortest axis of 30 sharply focused eggs per female were measured with a Mitutoyo CD-15D digital calliper to the nearest 0.01 mm. The calliper was connected to a computer which stored the data immediately (using a Mitutoyo DP-1 HS digimatic Mini Processor as interface). Egg size was defined as its diameter represented by the mean of the longest and shortest axis. Due to the transparency of the jelly layer around *C. edule* eggs, it was not possible to measure its thickness exactly, therefore only the diameter of the egg without its jelly layer was measured.

After a female had finished spawning, mostly within an hour after spawning began, all spawned eggs were removed with a Finn pipette and filled to a known volume, 40 % formalin was added to a final concentration of 4 % and stored until counting. After stirring, a known aliquot, containing 100 - 200 eggs, was placed on a grid and eggs were counted under a stereo microscope. For each female this was repeated at least five times. From these counts the number of eggs spawned by each female could be calculated.

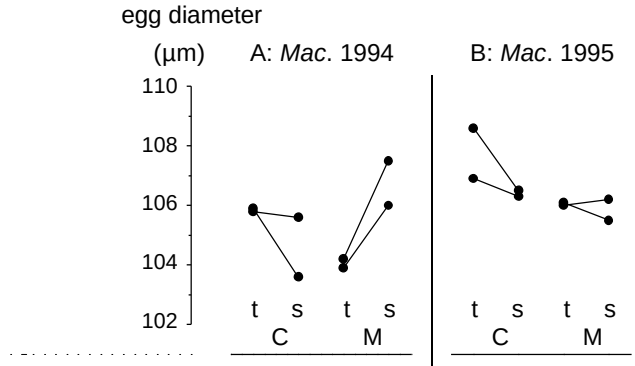
Body mass index (BMI)

In Experiment I the BMI just prior to spawning was measured (Honkoop and Beukema 1997). At the beginning of Experiment II, a sample of 25 animals was taken and at the beginning of the second period and again prior to spawning, samples of 10 animals were collected to determine BMI. Within a few hours after collection the animals were briefly immersed in boiling water until their shell opened. Soft parts were removed from the shells and placed individually in porcelain cups and dried for 4 days at 60 °C 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. Weighing was to the nearest 0.1 mg (W1). Subsequently the flesh was incinerated for 4 h at 580 °C, 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 (mg) / shell length^3 (cm^3).

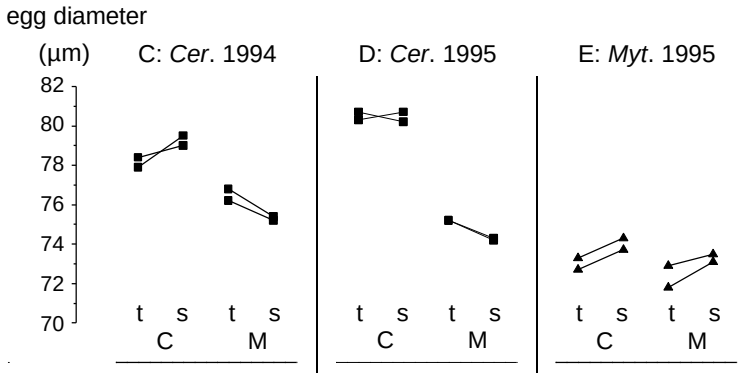
Results

Interspecific differences in timing of spawning, egg size and egg numbers

In 1994 as well as in 1995 *Macoma balthica* spawned in the first half of April at a water temperature of 9 - 12 °C. In both years, spawning in *Cerastoderma edule* took place later in spring when the water temperature was higher, 12 - 15 °C. For *M. balthica* as well as for *C. edule*, experimental spawning started somewhat later than natural spawning (except in Experiment II) (pers. obs.). The spawning period in *Mytilus edulis* was very prolonged. In February, attempts to make *M. edulis* spawn succeeded as well as in April to June. However, the spawning never resulted in empty gonads. Therefore, total egg numbers could not be determined in this species.



	df	*SS*	*F*	*SS*	*F*
C *vs*. M	1	0.08	0.1	2.40	4.8
t *vs*. s	1	1.06	1.4	1.13	3.0
Interaction	1	7.81	10.3*	0.68	1.8
Plot	2	1.60		1.00	
Error	2	1.52		0.76	



	df	C: *Cer*. 1994 *SS*	C: *Cer*. 1994 *F*	D: *Cer*. 1995 *SS*	D: *Cer*. 1995 *F*	E: *Myt*. 1995 *SS*	E: *Myt*. 1995 *F*
C *vs*. M	1	16.14	173.3**	65.88	122984**	0.98	2.1
t *vs*. s	1	0.00	0.0	0.54	7.3	1.77	25.4**
Interaction	1	2.78	21.3**	0.42	5.7	0.01	0.1
Plot	2	0.19		0.00		0.91	
Error	2	0.26		0.15		0.14	

Figure 3.2. Mean diameter (µm) of freshly released unfertilised eggs of *Macoma balthica* in (A) 1994 and (B) 1995, of *Cerastoderma edule* in (C) 1994 and (D) 1995, and of *Mytilus edulis* in (E) 1995. Until spawning, the animals were kept at two different water temperatures, cold (C) and mild (M) and at two different tidal levels, tidal (t) and subtidal (s). Below each part of the figure, ANOVA results are given. Significance levels are shown as ** if $P < 0.05$, and * if $P < 0.10$. Diameters of *C. edule* are exclusive of the jelly layer.

In both years, the diameter of the eggs of *M. balthica* was larger than in the other two species, *i.e.* ~ 107 µm (Figs 3.2A and B), whereas the diameter of the eggs of *C. edule* was ~ 77 µm (Figs 3.2C and D), and the diameter of *M. edulis* eggs was even smaller, ~ 72 µm (Fig. 3.2E). Differences in diameter imply much larger differences in egg volume. The volume ratio is *M. edulis* : *C. edule* : *M. balthica* = 1: 1 .2 : 3.3. The jelly layer around the eggs of *M. balthica* and *M. edulis* is very thin, < 1 µm, whereas the jelly layer around the eggs of *C. edule* is very thick, ~ 30 µm. Thus the total egg diameter in *C. edule* was about 140 µm.

The numbers of eggs produced by *C. edule* were about ten times larger than in *M. balthica* (Fig. 3.3). The groups of *C. edule* and *M. balthica* kept at low temperatures at the subtidal level produced on average 700 000 and 80 000 eggs per female, respectively. Some individual females produced many more eggs. Numbers up to 1.7 million and 135 thousand eggs were counted for individual *C. edule* and *M. balthica*, respectively. As

mentioned before, total egg numbers could not be quantified in *M. edulis*, but rough counts suggested about 1 - 2 million eggs per female per spawning.

Effects of temperature and immersion time on egg diameters

In Experiment I, *M. balthica* egg diameter did not vary significantly with temperatures and immersion times during the three months prior to spawning (Figs 3.2A and B). In 1994 a large (although non-significant) interaction term was found, but this effect was not found again in 1995. The longer treatment periods of Experiment II did not produce any significant difference in mean egg size between the different immersion regimes (Table 3.1). Thus, no tidal-level effects on egg size could be demonstrated in *M. balthica* (Table 3.2).

In contrast to *M. balthica*, *C. edule* showed clear temperature effects on egg diameter. In both years, eggs were significantly larger after a cold treatment than after a period with mild temperatures (Figs 3.2C and D). Differences between temperature treatments were smaller in 1994 (cold, ~ 79 µm *vs*. mild, ~ 76 µm) than in 1995 (cold, ~ 81 µm *vs*. mild, ~ 75 µm). As a consequence, differences in egg volume between cold and mild were more pronounced, at the higher water temperature eggs were 11% and 21% smaller (in 1994 and 1995, respectively). In 1994 a significant interaction term was found (Fig. 3.2C), indicating an opposite immersion-time effect at the two temperatures (at the lower temperature, the subtidal group had

Table 3.1. Differences in means of body mass index BMI (mg AFDM / cm^3), egg size, and egg numbers produced by *Macoma balthica* with a standardised shell length of 15 mm after different immersion regimes during the period 24 October 1994 - 16 January 1995 and 16 January 1994 - April 1995 (Experiment II). SS = subtidal during the entire period, TT=tidal during the entire period, ST = subtidal during the first and tidal during the second period, TS = tidal during the first and subtidal during the second period. For each group the mean value ± SD of four replicates is given.

Treatment	BMI 16 Jan.	BMI prior to spawning	Egg size (µm)	Egg number
SS	10.1 ± 0.3	12.2 ± 1.6	105.7 ± 1.0	54954 ± 6480
TS	10.2 ± 0.1	12.1 ± 0.4	105.4 ± 0.8	34292 ± 6442
TT	10.4 ± 0.3	9.8 ± 0.7	104.1 ± 1.4	25721 ± 11456
ST	9.7 ± 0.9	10.7 ± 0.9	105.3 ± 0.9	30793 ± 2240

Table 3.2. Analysis of variance of immersion time effects during the period 24 October 1994 - 16 January 1995 (first period) and the period 16 January 1994 - April 1995 (second period) on egg size of *M. balthica* (Experiment II), and estimates of the effects basin, effects of row (X), effects of columns nested within a basin (Y{basin}), and interaction between the first and second period (interaction).

Source of variation	*SS*	df	*MS*	*F*-ratio	*P*
Blocks					
X	5.395	3	1.798	1.841	0.240
Basin	1.000	1	1.000	1.024	0.351
Y{basin}	0.445	2	0.222	0.228	0.803
Treatments					
First period	2.250	1	2.250	2.304	0.180
Second period	2.560	1	2.560	2.621	0.157
Interaction	0.810	1	0.810	0.829	0.398
Error	5.860	6	0.977		

larger eggs than the tidal groups whereas at the higher water temperature the tidal groups had somewhat larger egg than the subtidal groups). In 1995, similar differences between tidal and subtidal groups were observed as in 1994, though no significant interaction and immersion time effects were found (Fig. 3.2D).

In *M. edulis* only a significant effect of immersion time was found, the subtidal groups produced the larger eggs at both temperatures. However, differences in egg size between tidal and subtidal groups were small, amounting to 1 µm only (Fig. 3.2E).Effects of temperature and immersion time on egg numbers

Two to three weeks after the first spawning, all *M. balthica* were killed to check their gonadal stage. It was found that gonads of 40 - 50 % of all spawned *M. balthica* were completely empty. Most *C. edule* died within 3 - 4 days after their first spawning. Also in this species only 40 - 50 % (in 1994 even a higher percentage) had spawned completely empty before they died. In the following, only the numbers found in completely spawned individuals will be used.

Before data analysis, produced egg numbers were standardised to a female of 15 mm shell length for *M. balthica* and of 30 mm for a *C. edule*. The following conversion factor was used (Honkoop and Van der Meer 1997):

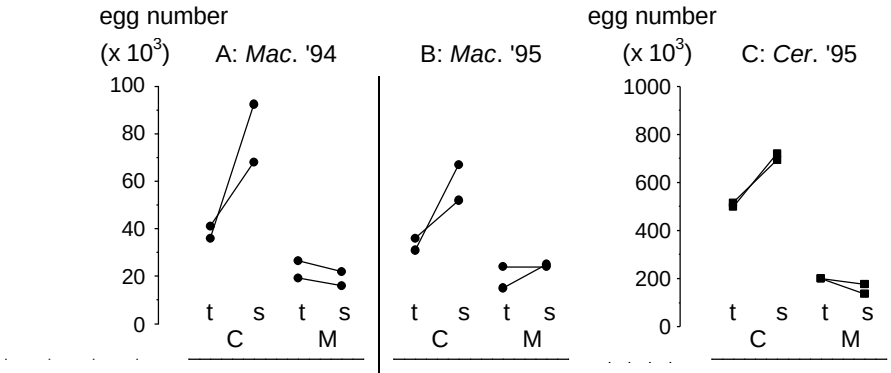
$$n_a = n_b * (a / b)^3$$

where n_a = egg number produced at standardised shell length
n_b = observed egg number produced at shell length b
a = standardised shell length (15 mm and 30 mm for *M. balthica* and *C. edule*, respectively)
b = actual length of spawned female.

This standardisation has no effect on the outcome of the statistical analysis because shell length did not differ within experiments. Standardisation only facilitates comparison between experiments of average egg numbers.

Observed relationships between standardised egg number and the factors temperature and immersion time were similar in *M. balthica* and *C. edule* (Fig. 3.3). Significantly more eggs were produced at lower than at higher water temperatures. At lower water temperatures always more eggs were produced at the subtidal than at the tidal level. In contrast, at the higher water temperatures differences in egg numbers were always small and inconsistent: in *M. balthica* in 1994 (Fig. 3.3A) and *C. edule* in 1995 (Fig. 3.3C), slightly more eggs were produced at the tidal level than at the subtidal level (resulting in a significant interaction term), but this was not so in *M. balthica* in 1995 (Fig 3.3B).

Manipulation of the tidal level also during a part of the autumn of 1994 (Experiment II) confirmed the results found in the cold treatment of Experiment I, that egg numbers at the subtidal level were significantly higher than at the tidal level (Tables 3.1 and 3.3). Largest differences were



	df	A: *SS*	A: *F*	B: *SS*	B: *F*	C: *SS*	C: *F*
C *vs*. M	1	0.45	28.7**	0.18	20.5**	0.65	209.0**
t *vs*. s	1	0.01	1.2	0.08	13.6*	0.00	0.4
Interaction	1	0.15	16.2**	0.01	2.3	0.05	11.4*
Plot	2	0.03		0.02		0.01	
Error	2	0.02		0.01		0.01	

Figure 3.3. Numbers of egg produced by a standardised *Macoma balthica* with a shell length of 15 mm in (A) 1994 and (B) 1995 and egg numbers produced by a standardised *Cerastoderma edule* with a shell length of 30 mm in (C) 1995. The animals were kept during the winter (and spring) months until spawning at two different water-temperature regimes, cold (C) and mild (M) and two different tidal levels, tidal (t) and subtidal (s). The calculated egg numbers per sub plot are given and per plot connected by lines. At the bottom of each part of the graphs, an analysis of variance is given. Significance levels are indicated as ** if $P < 0.05$, and * if $P < 0.10$. Note the differences in scale between *M. balthica* (A and B) and *C. edule* (C).

found between the SS (subtidal during the entire period) and TT (tidal during the entire period) groups, from which the first group produced most eggs. Intermediate numbers of eggs were produced in the TS and ST groups. Differences arising during the first period (24 October - 16 January) were smaller than those related to the second period (16 January - 10 April). There was no significant interaction effect between the two periods.

Body mass related differences in egg number

In Experiment II, all *M. balthica* groups lost similar proportions of their body mass during the first period. The BMI at the beginning was 12.1 mg cm^{-3}. The BMI at the end of the first period was about 10.0 mg cm^{-3} in all groups (Table 3.1) and did not differ significantly between the four

Table 3.3. Analysis of variance of immersion time effects during the period 24 October 1994 - 16 January 1995 (first period) and the period 16 January 1994 - April 1995 (second period) on log10(egg number) of *Macoma balthica* (Experiment II), and estimates of the effects of basin, effects of row (X), effects of columns within a basin (Y{basin}), and interaction between the first and second period (interaction).

Source of variation	*SS*	df	*MS*	*F*-ratio	*P*
Blocks					
X	0.041	3	0.014	0.911	0.489
Basin	0.008	1	0.008	0.502	0.505
Y{basin}	0.004	2	0.002	0.140	0.872
Treatments					
First period	0.110	1	0.110	7.305	0.035
Second period	0.166	1	0.166	10.986	0.016
Interaction	0.011	1	0.011	0.735	0.424
Error	0.091	6	0.015		

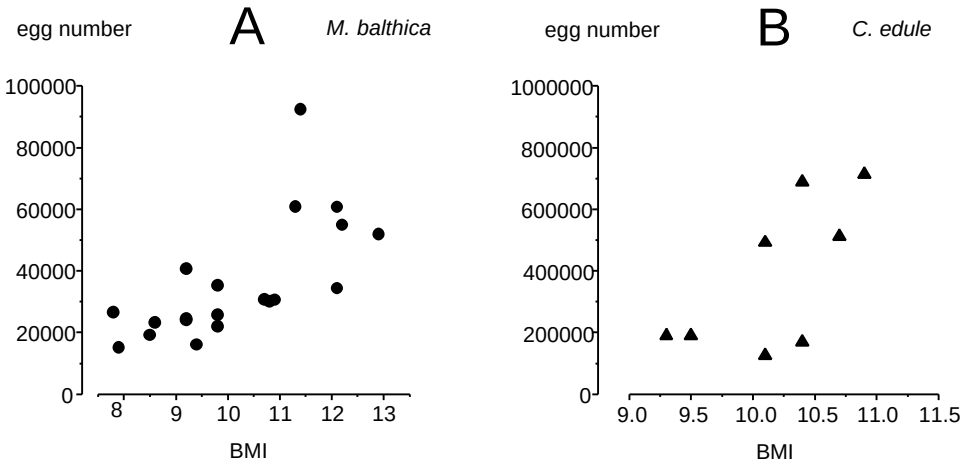


Figure 3.4. Mean egg numbers produced by groups of (A) *Macoma balthica* with a standardised shell length of 15 mm and (B) *Cerastoderma edule,* after manipulation of winter water temperature and immersion time, in relation with BMI just prior to spawning. BMI = M / l^3 in which M = ash free dry mass in mg and l = shell length in cm.

groups. Differences arose during the second period. At the end of this period only the groups kept at the subtidal level gained mass, whereas the body mass values of the groups kept at the tidal level had hardly changed.

Thus, significant differences ($P < 0.01$) were only observed between groups with differences in treatment during the second period (*i..e.*, SS and TS differed significantly from TT and ST).

Body mass values just prior to spawning in the groups of *M. balthica* (Experiment I in 1994 and 1995 and Experiment II) and *C. edule* (Experiment I in 1995) together with their numbers of produced eggs at standardised shell length (15 mm and 30 mm for *M. balthica* and *C. edule*, respectively) were used to show that a positive correlation existed between the BMI values just prior to spawning and egg numbers. However, this correlation was significant only in *M. balthica* (F-test, $P < 0.01$). In *C. edule* an almost significant correlation (F-test, $P = 0.06$) was found (Fig. 3.4). Causes of differences in BMI were discussed in more detail in Honkoop and Beukema (1997).

Discussion

Effects of immersion time and temperature on egg size

In *Cerastoderma edule* winter temperature clearly affected mean egg size whereas such effects were absent in *Macoma balthica* (Fig. 3.2). This difference can be explained on the basis of differences in timing of gametogenesis. Although some authors report a long lasting gametogenesis in *M. balthica* (De Wilde and Berghuis 1976, Gilbert 1978, Madsen and Jensen 1987, Harvey and Vincent 1989) with a relatively low percentage mature females in the beginning of the winter, our results are in consonance with the results presented by other authors (Caddy 1967, Lammens 1967, Chambers and Milne 1975, Bonsdorff and Wenne 1989) suggesting a morphologically finished gametogenesis, thus a high percentage of mature gametes present, in early winter. In *M. balthica*, egg size during the winter period could not be influenced either by temperature or by immersion time, suggesting that gametogenesis is morphologically finished before the winter, maybe even before the start of our Experiment II, in October. In *C. edule* gametogenesis takes place between February and spawning, mostly in May (Boyden 1971, Iglesias and Navarro 1991, Ivell 1981, Newell and Bayne 1980). Manipulation of immersion time and water temperatures during the winter period resulted in smaller egg sizes at higher temperatures.

Because significant differences in egg sizes of Balgzand *M. balthica* populations have been observed (Honkoop and Van der Meer 1997) it was

tested whether or not manipulation of egg size was also possible in *M. balthica*. Therefore, Experiment II was developed in which immersion time was manipulated already in autumn. Presumably because the experiment started too late in the period of gametogenesis (24 October), no differences in egg size between treatments were observed (Tables 3.1 and 3.2).

In *Mytilus edulis* gametogenesis starts in autumn (Sprung 1983) and continues, depending on the water temperature (Bayne 1984) and the availability of food, until spawning in late spring or early summer. According to Kautsky (1982), food abundance is the primary controlling factor in gonad growth and, according to Bayne (1984), gonadal development is delayed or ceased below a water temperature of 7 °C. The present results, including only (slight) effects of immersion (feeding) time and no temperature effects on egg size (Fig. 3.2E), point to the prevalence of feeding conditions as determinant of egg size, corroborating views of Seed and Brown (1977), Bayne *et al*. (1982), Kautsky, (1982); and Sprung (1983).

As some authors suggest, the survival and growth rate of bivalve larvae from smaller eggs is lower than from larger eggs (Bayne 1972, Bayne *et al*. 1975, Kraeuter *et al*. 1982), possibly as a consequence of the lower amount of stored nutrients. The main energy source for bivalve larvae are lipids, especially poly-unsaturated fatty acids (Helm *et al*. 1973, 1991, Holland and Spencer 1973, Bayne 1976, Chu and Webb 1984, Gallager *et al*. 1986, Whyte *et al*. 1992). The proportion reaching the D-stage, at which the first larval shell is developed, was positively correlated with the initial lipid content of the eggs (Helm *et al*. 1973, Gallager and Mann 1986). Own observations showed that the lipid content of the eggs in each of the three species was slightly lower at the higher than at the lower water temperatures (whereas immersion time had no effects) (Honkoop *et al*. 1999). Combined with the smaller eggs at the higher water temperatures (except for *M. balthica*), it is concluded that the viability of the eggs will be lower at the higher water temperature.

Effects of immersion time and temperature on egg numbers

Effects of immersion time and water temperature on egg numbers were more consistent and were more dramatic than on egg size in both *C. edule* and *M. balthica* (Fig. 3.3). The underlying gametogenesis mechanisms may be different in the two species. Because gametogenesis in *M. balthica* is

probably finished before the experimental treatments started (in the beginning of January), it is reasonable to suppose that *M. balthica* egg numbers were maximal at the beginning of the experiments. Therefore, lower-than-maximal egg numbers were probably a consequence of resorption of gametes to survive periods of food scarcity. Lack of food prior to the spawning period may be an important factor stimulating resorption. Resorption of gametes during gametogenesis, at relatively high water temperatures and in the absence of food, has been reported in the bivalves *M. edulis* (Bayne *et al*. 1978, 1982, Pipe 1987) and *Mya arenaria* (Coe and Turner 1938), but not yet in *M. balthica*, although in this species similar processes were reported in the period immediately after spawning (Caddy 1967, Gilbert 1978). Though no data appear to be available, resorption processes are plausible also in *M. balthica*. Because metabolic demands are higher at higher water temperatures, more eggs will have been resorbed at higher than at lower water temperatures. At the subtidal level, a higher proportion of the eggs was resorbed at the higher than at the lower water temperature, resulting in almost equal numbers at the two tidal levels at the higher water temperature (Fig. 3.3). Probably, this larger resorption was caused by higher foraging activities in the absence of food at the subtidal level during the first week of the year.

In *C. edule* gametogenesis starts in spring as soon as food becomes available (Newell and Bayne 1980, Iglesias and Navarro 1991). Due to the lower energy demands and the lower mass loss during the first months of the manipulations at lower as compared to higher temperatures (Honkoop and Beukema 1997), more energy from ingested food is available for gonadal development at the lower temperature resulting in higher egg numbers at the lower than at the higher water temperature. As a consequence of the longer foraging periods at the subtidal level one would expect that more eggs were produced in the groups kept at the subtidal level than at the tidal level. However, this proved to be true only at the lower temperatures. At the higher water temperatures more mass was lost at the subtidal than at the tidal level (Honkoop and Beukema 1997). Therefore, ingested food will have been used to compensate for the extra mass loss at the subtidal level, rather than to produce eggs. As a consequence, less food is available for gametogenesis, resulting in the lowest fecundity in the groups kept at the higher water temperature at the subtidal level (Fig. 3.3C).

Total body mass consists of both somatic and gonadal (including gametes) tissue. Therefore, it is reasonable to suppose that body mass prior to spawning is correlated with numbers of eggs produced. Indeed, in *M. balthica* a significant correlation was found (Fig. 3.4A), indicating that water temperature and immersion time can influence fecundity via body mass changes. As observed in the giant scallop *Placopecten magellanicus* by MacDonald and Thompson (1985), somatic mass appears to be similar at the various conditions and the variation in total body mass is almost entirely due to differences in egg numbers. In *C. edule* the correlation between body mass and egg numbers was also positive (Fig. 3.4B), but it was not statistically significant, probably as a consequence of the low number of observations.

Inter-species differences in reproductive strategies

To compare the reproductive strategies of the three species investigated in this paper, the relevant features of their life history are assembled in Fig. 3.5. Seasonal body-mass cycles in the three studied species show a general pattern in gain and loss of body mass (Fig. 3.5A). In spring and (early) summer animals are growing. This annual period is followed by a period of loss of body mass which lasts until the next growing season. In the Wadden Sea, *M. balthica* generally reaches its maximum body mass in early June (Beukema and De Bruin 1977, Beukema *et al*. 1985, Zwarts 1991). Maximum body mass in *C. edule* (Zwarts 1991) and *M. edulis* (Zwarts and Wanink 1993) is reached somewhat later.

Two strategies of utilising energy to build up gonadal tissue have been observed (Boyden 1971, Bayne 1984). To build up their gonads, the first group of species uses energy from nutrients stored during the growing season. The second group uses energy directly from ingested food and stored nutrients are used only to survive periods of food scarcity. *M. balthica* belongs to the first group, gametogenesis starting after the growing season has been completed (Caddy 1967, Lammens 1967, Chambers and Milne 1975, Bonsdorff and Wenne 1989) and ending well before the start of the new growing season. *C. edule* belongs to the second group, gametogenesis taking place during the early part of the growing season

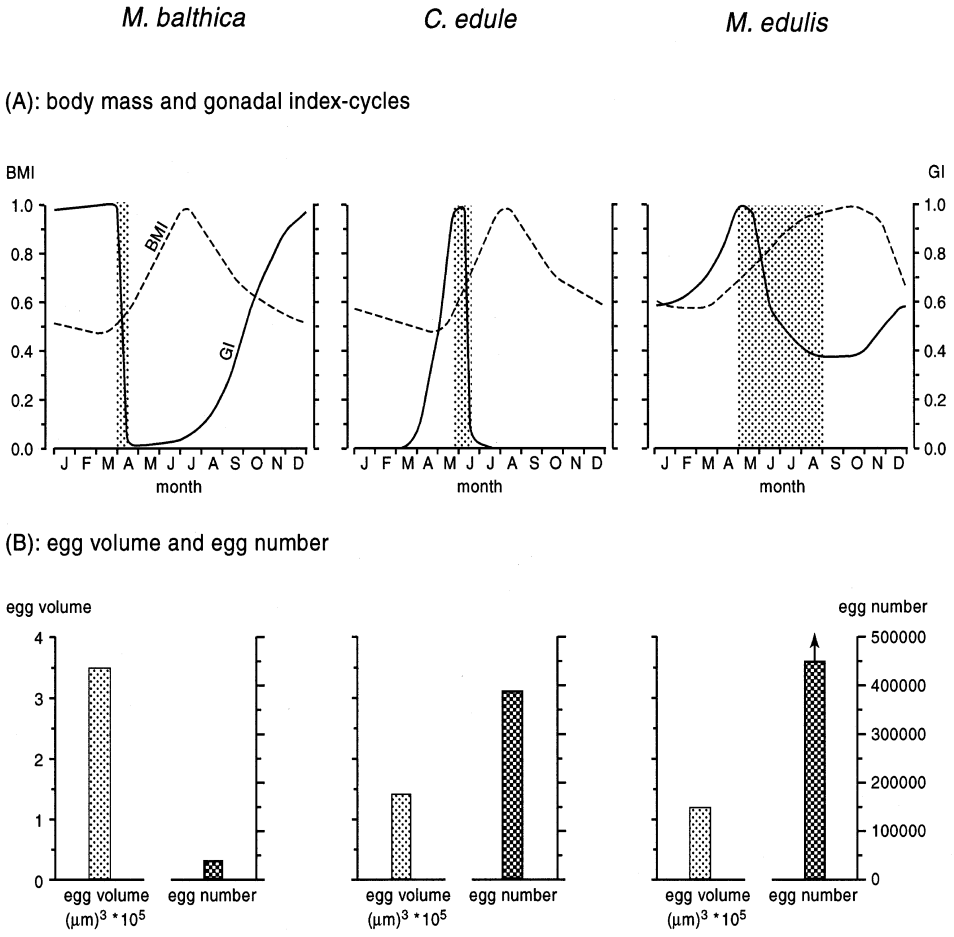


Figure 3.5. Graphical performance of annual course in (A) body mass (broken lines) and gonadal index-cycles (solid lines), and (B) egg sizes and numbers in *Macoma balthica*, *Cerastoderma edule*, and *Mytilus edulis*. Maximum BMI and maximum GI (maximal maturation of gametes) are set to 1. The shaded areas refer to the spawning periods. Relevant literature used for Figs A and B can be found in the text. Data on egg volumes of the three species and egg numbers of *C. edule* and *M. balthica* can be found in the present paper. Data on egg numbers of *M. edulis* are from Bayne *et al.* (1975), and refers to incompletely spawned females. Therefore, the real egg numbers should be much higher in this species.

just prior to spawning. (Newell and Bayne 1980, Hummel and Bogaards 1989, Iglesias and Navarro 1991). *M. edulis* apparently can use both energy sources; gametogenesis starts in autumn at the expense of stored nutrients, is delayed below a certain temperature in winter (Bayne 1984), and is finished during the early part of the new growing season, thus using also ingested food as an energy source (Kautsky 1982, Bayne 1984) (Fig. 3.5A).

Timing of spawning in *M. balthica* is different from that of *C. edule* and *M. edulis*. *M. balthica* spawns early in spring, whereas the two other species spawn at the end of spring or in early summer (under natural conditions as well as the present experiments). The production of relatively large eggs in small numbers (as compared to the small eggs and higher numbers of *C. edule* and *M. edulis*) (Fig. 3.5B) can be regarded as a reproductive adaptation of *M. balthica* to the relatively unstable and unpredictable environment and low water temperatures in which the egg are released. Larger eggs are supposed to survive a longer period of starvation (Miller *et al.* 1988) and because larval development is slower at lower temperatures (Mytilids: Bayne 1965, His *et al.* 1989, HrsBrenko and Calabrese 1969, Lough and Gonor 1971, Pechinek *et al.* 1990, other bivalves: His *et al.* 1989, Lutz *et al.* 1982), it is favourable for *M. balthica* to produce large and consequently few eggs (Smith and Fretwell 1974). Although mortality rates of larvae can be very high [in *M. edulis* 0.1 - 0.2 day^{-1} (Widdows 1991)], *C. edule* and *M. edulis* can afford to produce small eggs (and therefore have a high fecundity) because during late spring the algal concentrations in the water and the water temperatures are usually relatively high, resulting in a faster growth rate of larvae as compared to those of the early spawning *M. balthica*.

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CHAPTER FOUR

Reproductive output of *Macoma balthica* populations in relation to winter-temperature and intertidal-height mediated changes of body mass

Abstract

To study relationships between environmental conditions and reproductive output, numbers and sizes of eggs produced by the intertidal bivalve *Macoma balthica* were determined after the winters of 1995 and 1996 at three stations at different intertidal levels in the Dutch Wadden Sea. At all field stations significantly more (1.5 - 7 times) eggs were produced after the cold winter of 1996 than after the mild winter of 1995 when individual body masses were lower than in early 1996. At two stations the eggs were also significantly larger in 1996. In both years, eggs were larger at low than at high mud flats. Egg size was significantly positively correlated with adult body mass in the preceding summer (when gametogenesis takes place). Egg numbers, on the other hand, were only significantly positively correlated with the body mass just prior to spawning. Below a body mass of 5.6 mg ash-free dry mass per cm^3 (cubic shell length), *M. balthica* did not produce any eggs. Above this body mass, egg numbers increased by about 7700 per mg ash-free dry mass at a shell length of 15 mm.

Introduction

Animals first and foremost use energy from food intake for maintenance of both somatic and reproductive tissue. If a surplus of energy is available, it is used for growth and reproduction (Kooijman 1993). Thus, the reproductive output of animals will vary with changing environmental conditions, such as food availability (affecting energy gain) and temperature (affecting energy gain and energy expenditure).

The bivalve *Macoma balthica* (L.), a common species at all intertidal heights in the Dutch Wadden Sea, shows differences in growth rate at different intertidal levels (Beukema *et al.* 1977). *M. balthica* is a facultative filter feeder, which means that food can be collected from the water column as well as from the sediment surface (Brafield and Newell 1961,

Hummel 1985, Ólafsson 1986). Although benthic food densities are relatively high at high tidal levels (Cadée and Hegeman 1977), growth rate is low, probably as a consequence of the shorter immersion time, allowing only short daily periods of filter feeding. Similar observations were made in *M. balthica* populations on the Canadian Atlantic East coast (Harvey and Vincent 1990, 1991). The effects of food availability on growth of *M. balthica* have been described in several studies (Green 1973, Hummel 1985, Beukema and Desprez 1986), but so far reports on reproductive output (*i.e.* egg size and egg numbers) have only concerned egg size measured in dissected gonads and total reproductive-tissue mass (Harvey and Vincent 1989, 1991, Harvey *et al.* 1993). All studies, except that of Green (1973), point out that growth of both somatic and reproductive tissues is maximal at the lower intertidal levels. Therefore, reproductive output would be highest at low tidal levels (Harvey and Vincent 1991).

Growth of *M. balthica* is restricted to a range of water temperatures of 4 - 16 °C in spring (Beukema *et al.* 1985), with optimum growth at 10 °C (De Wilde 1975). In winter, masses decline and mass loss is more rapid at high than at low temperatures (Zwarts 1991, Honkoop and Beukema 1997). Effects of temperature on gonadal development are largely unknown. Some studies indicate that winter temperatures affect recruitment in the subsequent summer; with higher winter temperatures resulting in lower recruitment (Beukema 1982, 1992). In several species of intertidal bivalves, severe winters are also followed by high recruitment (Kristensen 1957, Beukema 1982, 1992, Möller and Rosenberg 1983, Jensen 1992). Such differences in recruitment may have been caused, at least partially, by lower fecundity of adult populations after mild than after cold winters.

The objectives of this study were to estimate the reproductive output (in terms of egg size and egg numbers) of *M. balthica* populations living at different intertidal levels in the Dutch Wadden Sea and to relate these outputs to immersion times and to water temperatures during winter. Size and numbers of released eggs were estimated at various field stations after two winters with different characters, the mild winter of 1994 - 1995 and the cold winter of 1995 - 1996. As shown in Honkoop and Beukema (1997), body mass of *M. balthica* prior to spawning can be influenced by manipulation of immersion time and water temperature during the preceding months. Thus, the body mass values of the various studied populations of *M. balthica* differed in a predictable way: high at low levels and in 1996, low at high levels and in 1995. Reproductive output could

thus be related to the consistently differing body mass values of adult *M. balthica*, both collected in the field and obtained from experimentally manipulated groups (Honkoop and Van der Meer 1997).

Materials and Methods

Macoma balthica

Field observations on egg numbers and egg sizes in *Macoma balthica* were made at three stations (station A: low, station B: intermediate, and station C: high) in the intertidal zone at Balgzand, a tidal-flat area in the westernmost part of the Dutch Wadden Sea. As in earlier studies, body mass values at a standard shell length tended to increase with decreasing tidal level. Exact locations and some environmental properties of the stations are described in an earlier paper (Honkoop and Beukema 1997).

At the end of March (in 1995 and 1996), immediately before spawning at a water temperature of 7 - 9°C, about 500 *M. balthica*, ranging from 13 - 18 mm, were collected at each of the three stations. Per site, the body mass index (BMI, mg cm^{-3}), defined as ash-free dry mass divided by cubic shell length, was determined directly after collection in 25 unparasitized individuals. BMI values were also determined in August 1994 and August 1995 as part of a long-term data series (J.J. Beukema and R. Dekker pers. comm.).

Spawning

Directly after collection in March, all specimens except those we used to determine the BMI, were placed in small buckets filled with sediment and allowed to burrow. The buckets were placed in a refrigerator at 4 °C. The next morning, 100 *M. balthica* from each station were individually placed in 100 cm^3 beakers filled with aerated seawater with a temperature of 12 °C. After 30 min this water was replaced by fresh aerated 12 °C seawater. Then, mostly within 10 min, spawning began; usually males first. Over four hours the water was changed every 45 min. After a few hours of this treatment no further specimens could be induced to spawn. Therefore, experiments were terminated after four hours and all *M. balthica* were replaced by fresh individuals and the procedure started again. This procedure was repeated until enough material had been collected to estimate egg size and egg numbers with sufficient precision.

Egg diameters and egg numbers

Freshly spawned eggs were multiform because the eggs in the gonads were tightly clumped together. Within 30 min after release, the shape of most of the eggs had changed to a round or only slightly aspherical shape. After 30 min, a few hundred of the eggs of each female were removed with a capillary pipette, placed on a flat microscopic slide and photographed twice. ISO 100 colour slides were used with a Zeiss M-35 camera fitted to a Zeiss stereo microscope at 63x magnification. The eggs were all returned to the remainder of the clutch from which they had been removed.

From the eggs of each female, two dia positive slides were made. These slides were projected on a transparent screen with the slide projector placed at a fixed distance. Opposite the projector and at the backside of the screen the longest and shortest axes of 30 sharply focused eggs per female were measured with a Mitutoyo CD-15D digital calliper to the nearest 0.01 mm. Using a Mitutoyo DP-1 HS digimatic Mini Processor as interface, the calliper was connected to a computer which stored all measurements directly. Egg size was defined as the mean of the lengths along the longest and shortest axes.

After the females finished spawning, generally within one hour after spawning had begun, all eggs were removed with a Finn pipette to a known volume, 40 % formalin was added to a final concentration of 4 % and the thus preserved eggs were stored until counting. After stirring, a known aliquot, containing 100 - 200 eggs, was placed on a grid and eggs were counted under a stereo microscope. For each female this was repeated at least 5 times. From these counts, the total number of eggs spawned by each female was calculated.

Each female that had spawned was numbered, and for a period of two to three weeks these females were forced to release all their eggs; they were stored each night at 4 °C and each morning the females were subjected to a temperature shock when they were placed in 12 °C seawater, after which the water was changed several times a day. After two to three weeks, the shells were opened and gonads were checked to see whether they were empty (or only a few residual eggs were left). Only the cumulative egg counts from (nearly) empty animals were used to calculate mean egg production.

Results

Spatial and temporal differences in body mass

Mean body mass values of the three groups of *Macoma balthica* as observed in March, just prior to spawning, are listed in Table 4.1 along with the mean values observed in the same populations in the preceding August. After the cold winter of 1995/1996, with a mean January - March water temperature of -0.1 °C (measured daily in the nearby Marsdiep inlet), the body mass at all three stations was higher and the mass loss smaller than after the mild winter of 1994 - 1995 which had a mean January - March water temperature of 5.1 °C. Two factors contributed to the higher body mass prior to spawning in 1996. The first was the higher initial body mass value (August value) and the second the lower loss of body mass during autumn and winter. During the mild 1994 - 1995 autumn-winter period, more (3.8 - 6.9 mg cm^{-3}) of the initial body mass was lost than during the cold 1995-1996 autumn-winter period (2.6 - 5.5 mg cm^{-3}).

The order of BMI values at the three stations was similar in August in the two years, which is consistent with the long-term observations (J.J. Beukema pers. comm.). After the winter of 1994 - 1995, BMI values were also highest at the station lowest in the intertidal area (station A), and lowest at the station highest in the intertidal area (station C). The BMI of

Table 4.1. Successive mean values of the body mass index (BMI) of *Macoma balthica* as observed at each of three field stations in August (*i.e.* eight months before spawning) and in March just prior to spawning in two years (1994/1995 and 1995/1996). Mass loss (mg cm^{-3}) is the proportion of mass loss between August and March. BMI (mg cm^{-3}) is ash-free dry mass divided by cubic shell length. Each mean value was determined from at least 25 specimens.

Station	Tidal level (cm) as to MTL	BMI August	BMI March	Loss in BMI (mg cm^{-3})
1994 - 1995				
A	-20	14.4	7.8	6.6
B	0	13.8	6.9	6.9
C	+30	10.0	6.2	3.8
Mean		12.7	7.0	5.8
1995 - 1996				
A	-20	16.1	10.6	5.5
B	0	15.3	9.9	5.4
C	+30	15.0	12.4	2.6
Mean		15.5	11.0	4.5

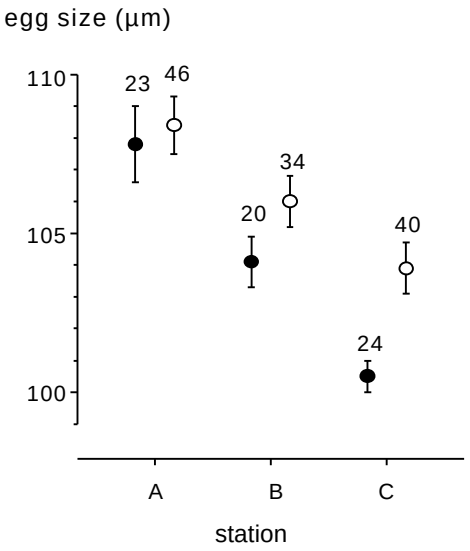

Figure 4.1. Diameters (mean ± SE in µm) of eggs produced by *Macoma balthica* at the three different Balgzand stations A (low), B (intermediate), and C (high). Mean egg size in 1995 and 1996 indicated by solid circles and open circles, respectively. Numbers above error bars indicate the number of females from each of which the diameter of 30 eggs was measured.

station B, at an intermediate intertidal level, was intermediate. Rankings after the cold winter of 1995 - 1996, however, were different, with an exceptionally high body mass value at station C, at the higher intertidal level. The other stations showed lower BMI values, of which the BMI at station A was again higher than that at station B.

The loss of body mass during the autumn - winter periods differed per station. Mass losses were relatively high at the two lower stations (A and B) and low at the higher tidal-level station (C).

Spatial and temporal differences in egg sizes

The mean size of the produced eggs differed between stations. In both years, the largest eggs were produced at station A, the lowest station. The smallest eggs were produced at station C, the station at the highest intertidal level. At the intermediate tidal level (station B), the eggs had an intermediate diameter (Fig. 4.1). These differences were highly significant (ANOVA, $F_{s\,[2,181]} = 21.8$, $P < 0.001$). The differences in size between the two

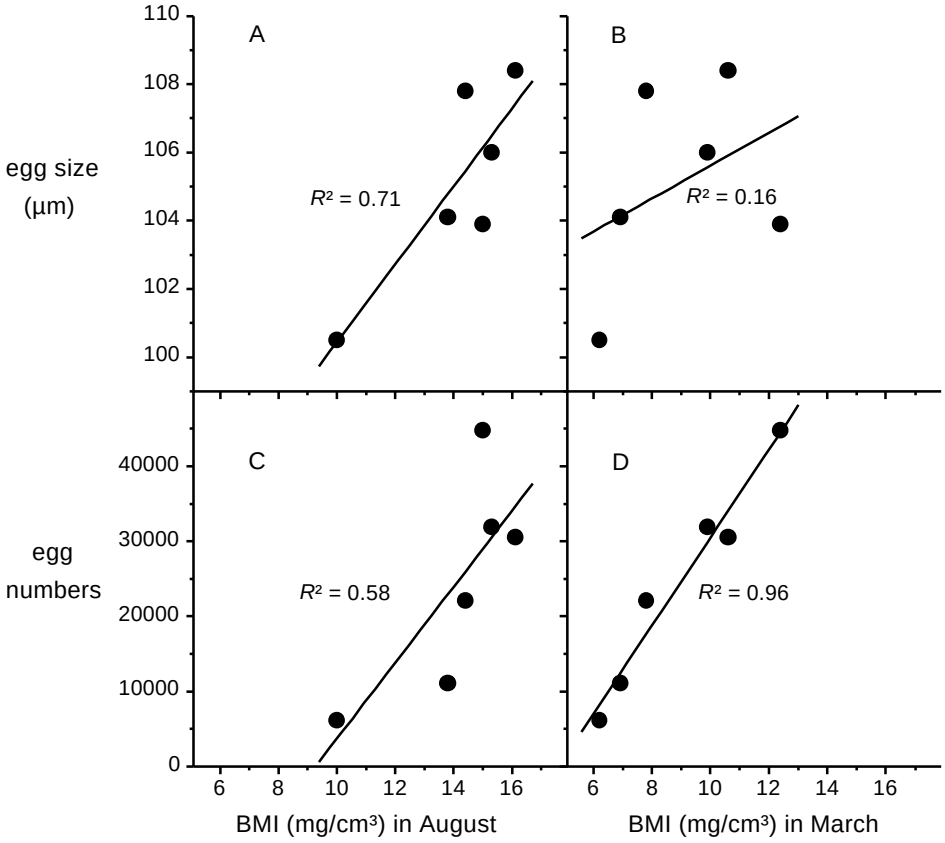

Figure 4.2. Relationship between egg size after spawning in April (in µm) and body mass index (BMI) in (A) the preceding August and (B) March just prior to spawning; and, the relationship between number of eggs produced in April and BMI in (C) the preceding August and (D) March just prior to spawning. Egg numbers are corrected for a *Macoma balthica* of standardised shell length (15 mm). BMI is ash-free dry mass divided by cubic shell length (mg cm^{-3}).

years were also significant (ANOVA, $F_{s\,[1,181]} = 6.5$, $P < 0.05$); eggs produced at stations B and C were larger after the cold winter of 1996 than after the mild winter of 1995. Interaction between site and year was not significant (ANOVA, $F_{s\,[2,181]} = 1.1$, $P > 0.1$). Interaction between site and year was not significant (ANOVA, $F_{s\,[2,181]} = 1.1$, $P > 0.1$).

The relationships between mean egg size and BMI values eight months prior to spawning (August) and just prior to spawning (March) are shown in Figs 4.2A and B. The regression between egg size and BMI was closer for the BMI values of the preceding August ($R^2 = 0.71$, $P < 0.05$; Fig. 4.2A) than for the BMI values observed just prior to spawning ($R^2 = 0.16$, $P > 0.1$; Fig. 4.2B).

Spatial and temporal differences in egg numbers

An allometric relationship between the number of eggs produced per individual female and the shell length of the female may be assumed, which after log-transformation looks in its most general form like this:

$$\log(n_{ijk}) = \{? + ?_i + ?_j + (??)_{ij}\} + \{? + ?_i + ?_j + (??)_{ij}\}*\log(l_{ijk}) + ?_{ijk} \qquad (1),$$

where $\sum_i ?_i = \sum_j ?_j = \sum_i ?_i = \sum_j ?_j = 0$,

$\sum_i (??)_{ij} = \sum_i (??)_{ij} = 0$ for each j,

$\sum_j (??)_{ij} = \sum_j (??)_{ij} = 0$ for each i,

and
- n_{ijk} = number of produced eggs at site i, in year j, of female k,
- $?$ = common intercept,
- $?_i$ = site deviation in intercept,
- $?_j$ = year deviation in intercept,
- $(??)_{ij}$ = interaction term,
- $?$ = common slope,
- $?_i$ = site deviation in slope,
- $?_j$ = year deviation in slope,
- $(??)_{ij}$ = interaction term,
- $?_{ijk}$ = error term, and
- l_{ijk} = shell length at site i, in year j, of female k.

The significance of the $?$, $?$, and $(??)$ terms was tested first. The so-called homogeneity-of-slopes test revealed that the terms were not significantly different from zero. Thus, the slopes of shell length *versus* egg production at the three sampling sites in the two years could be replaced by a common slope ✍ (Table 4.2), leading to the following simplified model:

Table 4.2. Homogeneity-of-slopes test of the relationship between egg production versus shell length among sites and years and test of the hypothesis that the common slope equals 3. n.s. means P>0.05.

Model	df	*SS*	*F*-ratio	*P*
Different slopes	64	4.064	1.37	n.s.
Common slopes	69	4.479	<1	n.s.
Slope = 3	70	4.499		

Table 4.3. Mean shell length of empty-spawned females, and the actual mean number of eggs produced and mean egg production recalculated for a standardised *Macoma balthica* of 15 mm shell length at each of the three sites (A, B, and C) in 1995 and in 1996 (mean ✍ SD). Numbers of empty-spawned females between brackets. The mean shell length was similar between sites and years ($P > 0.1$), except for the smaller value at station C in 1995.

			Numbers of eggs produced	
Station/year	*n*	Shell length (mm)	Real observations	Standard *M. balthica*
1995				
A	7	17.0 ± 1.0	32884 ± 8339	22115 ± 5496
B	12	16.7 ± 1.7	15953 ± 13104	11082 ± 9765
C	14	14.3 ± 2.1	4744 ± 2387	6199 ± 4256
1996				
A	18	16.7 ± 2.4	44950 ± 33612	30587 ± 18886
B	14	15.1 ± 1.3	33696 ± 16485	31909 ± 11322
C	11	16.4 ± 1.4	57780 ± 20887	44782 ± 16952

$$\log(n_{ijk}) = \{? + ?_i + ?_j + (??)_{ij}\} + ?*\log(l_{ijk}) + ?_{ijk} \quad (2).$$

The estimated common slope of $? = 2.906$ was not significantly different from the value of $? = 3$ (Table 4.2). This results in the further simplified model:

$$\log(c_{ijk}) = ? + ?_i + ?_j + (??)_{ij} + ?_{ijk} \quad (3),$$

where $\log(c_{ijk}) = \log(n_{ijk}) - 3*\log(l_{ijk})$

or $c_{ijk} = n_{ijk} * (l_{ijk})^{-3}$, thus representing the number of eggs per unit of shell volume.

Table 4.4. Analysis of variance for the effects of body mass (BMI) and type of data (field or experimentally collected) on egg number, and interaction between BMI and data type (common slope). n.s. means $P > 0.05$.

Source of variation	*SS* (*10^9)	df	*MS* (*10^9)	*F*-ratio	*P*
Type	0.107	1	0.107	0.7	n.s.
BMI	3.740	1	3.740	24.7	< 0.001
Type * BMI	0.137	1	0.137	0.9	n.s.
Error	3.790	25	0.152		

Analysis of variance revealed that site, year and the interaction between site and year all contributed significantly ($P < 0.05$) to the variation of $\log(c_{ijk})$. More eggs were produced in 1996 than in 1995, but the orders of egg number per site differed between 1995 (A > B > C) and 1996 (C > A > B) (Table 4.3). Although the size ranges of the selected animals were similar in the six groups, the mean shell length of one group of females (station C in 1995) was significantly smaller ($P < 0.05$) (Table 4.3). Therefore, it made sense to recalculate the egg numbers for a *M. balthica* with a standardised shell length; the back-transformed estimate of $\log(c_{ijk})$ was multiplied by 15^3 to obtain an estimate of egg numbers produced by a *M. balthica* with a standardised shell length of 15 mm (last column of Table 4.3).

Egg numbers in relation to body mass

The estimated egg numbers of standardised *M. balthica* of 15 mm shell length (Table 4.3) were plotted against BMI values of both the preceding summer (August) and just prior to spawning (March) (Figs 4.2C and D). In contrast to the observed relation of BMI *versus* egg size (Figs 4.2A and B), egg numbers were more closely related to the March BMI values ($R^2 = 0.96$, $P < 0.01$; Fig. 4.2D) than to the preceding August BMI values ($R^2 = 0.58$, $P > 0.05$; Fig. 4.2C). The best linear fit for the field data (n = 6) of the relation between the egg number of a 15 mm *M. balthica* and its BMI just prior to spawning was:

$$\text{egg number} = 5858 * \text{BMI} - 28087 \qquad (4).$$

By manipulation of temperature and tidal level, several groups of *M. balthica* with different BMI values prior to spawning were obtained experimentally (Honkoop and Beukema 1997, Honkoop and Van der Meer

1997). Using egg numbers and BMI values of these *M. balthica* (after recalculation to a standardised shell length of 15 mm) the best linear fit ($R^2 = 0.46$, $P < 0.01$) for the experimental data ($n = 20$) was:

$$\text{egg number} = 8721 * \text{BMI} - 52818 \quad (5).$$

A homogeneity-of-slopes test showed that the two slopes for the relation between egg number and BMI were not significantly different between field and experiment data (Table 4.4). The common slope was significantly different from zero. The intercepts were again not significantly different between the field and laboratory collected data. So the regression lines for the field and the experiments were sufficiently similar ($F_{s\ [2,22]} = 0.5$, $P > 0.05$) to calculate a single fit ($R^2 = 0.54$, $P < 0.001$):

$$\text{egg number} = 7739 * \text{BMI} - 43314 \quad (6).$$

This equation predicts that if the BMI is smaller than 43314 / 7739 = 5.6, no eggs are produced, whereas 7739 eggs are produced per BMI unit above the value of 5.6. All data points, as well as the line defined by equation (6), are shown in Fig.4.3.

Discussion

Stimulation of spawning

Under natural conditions, the trigger initiating spawning is thought to be a rise in seawater temperature (Caddy 1967, De Wilde and Berghuis 1976). We imitated this trigger using a temperature shock to adult animals collected in the field every week in early spring. Our specimens could be stimulated to spawn their eggs only during the one or two weeks before natural spawning took place in the field. Although De Wilde and Berghuis (1976) report a relatively early success in initiating spawning in the lab, we did not (or hardly did so) succeed in making *Macoma balthica* spawn more than two weeks before natural spawning took place. This was also reported by Caddy (1967), who only succeeded in initiating spawning during the period of natural spawning. Therefore, it seems reasonable to suppose that spawning, initiated by means of a temperature shock, is only

successful if animals are ripe and that 'artificially' spawned eggs show their mature size.

Field observations

After the mild winter of 1994 - 1995, body mass values at the three stations varied in the expected way, longer immersion times being linked with higher body mass values. This relationship has been shown earlier by Beukema *et al.* (1977) on the basis of field measurements and by Honkoop and Beukema (1997) on the basis of experimentally collected data. In the latter publication it is also argued that (facultative) deposit feeders such as *M. balthica* can reduce part of their winter loss of body mass by the intake of detritus and / or benthic diatoms, which are present in winter, particularly at higher intertidal levels. Accordingly, both in the August 1994 to March 1995 period and in the August 1995 to March 1996 period, losses of body mass were lowest at the station (station C) that is situated highest in the intertidal area (Table 4.1). Due to the different characters of

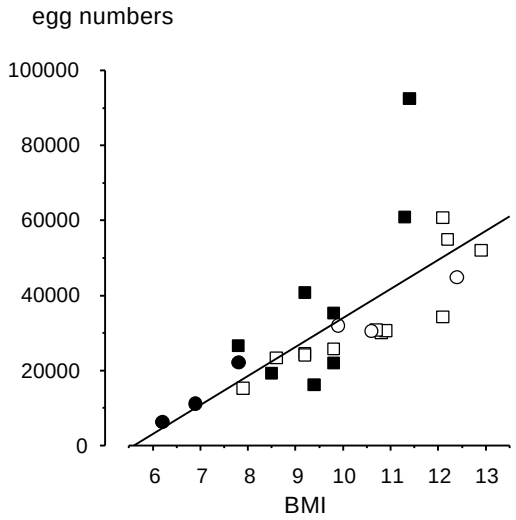

Figure 4.3. Mean egg number produced by a standardised *Macoma balthica* (with a shell length of 15 mm) in relation to body mass (BMI) values measured just prior to spawning. Origin of data: solid and open squares from experimental data obtained in 1994 and 1995, respectively, and solid and open circles from field-collected data in 1995 and 1996. BMI is ash-free dry mass divided by cubic shell length (mg cm^{-3}). The line represents the best linear fit: egg number = 7739 * BMI - 43314.

the two winters (the first was mild, the second was cold), *i.e.* because of the lower metabolic costs at lower temperatures, the loss of body mass was lower at all stations during the cold winter (1995 - 1996) than it was during the mild winter (1994 - 1995). Bayne and Widdows (1978) argued that higher water temperatures cause a more negative scope for growth in the marine mussel *Mytilus edulis*. A lower loss of body mass at (artificially) lowered water temperatures during winter was indeed observed in *M. edulis* and also in two other bivalves, *M. balthica* and *Cerastoderma edule* (L.), by Honkoop and Beukema (1997). It is therefore reasonable to suppose that differences in loss of body mass during the two winters were directly caused by differences in temperature. There is no evidence that feeding conditions are better in cold than in mild winters. Growth rates in *M. balthica* are primarily governed by diatom concentrations (Beukema and Cadée 1991) and in winter these are not related to temperatures (G.C. Cadée pers. comm.). The higher August BMI values in 1995 (as compared to 1994) were probably caused by better feeding conditions during the spring - summer growing season and contributed strongly to the relatively high BMI at all three stations after the winter of 1995 - 1996 (as compared to the values observed after the winter of 1994 - 1995): a large part of the differences observed between years in March BMI (4.0 mg cm^{-3}) already existed in the preceding August (2.8 mg cm^{-3}).

Egg size at the three stations differed significantly (Fig. 4.1). Though the difference between the diameter of the smallest and largest eggs amounted to only about 8 %, it equals a considerable difference in egg volume (about 21 %). In accordance with the better feeding conditions (longer daily foraging times), higher growth rates, and higher body masses at the lower tidal levels (Beukema *et al.* 1977), eggs were largest at the lowest intertidal station. A more direct relationship between egg size and food availability was found in Canadian *M. balthica* populations, with somatic growth rates being higher and eggs being larger in areas where the chlorophyll content of the sediment was higher (Harvey *et al.* 1993).

In contrast to egg sizes (which were better correlated with August BMI values), egg numbers were significantly correlated with March BMI values (Fig. 4.2D). Final egg numbers to be spawned will be determined by both formation and resorption processes. Lack of food prior to the spawning period may be an important factor stimulating resorption. Resorption of gametes during gametogenesis, at relatively high water temperatures and in the absence of food, has been reported in the bivalves

M. edulis (Bayne *et al.* 1978, 1982, Pipe 1987) and *Mya arenaria* (Coe and Turner 1938), but not yet in *M. balthica*, although in this species similar processes were reported in the period immediately after spawning (Caddy 1967, Gilbert 1978). Though no data appear to be available, resorption processes are plausible also in *M. balthica*. At European coasts at temperate latitudes, most *M. balthica* females already have been found to possess mature gonads a few months before spawning (Caddy 1967, Lammens 1967, Chambers and Milne 1975, Madsen and Jensen 1987, Bonsdorff and Wenne 1989), although some other authors have reported this phase to be reached only just before spawning (De Wilde and Berghuis 1976, Gilbert 1978). Therefore, it is possible that eggs are resorbed during winter when food availability is low. As the annual minima of body mass values in *M. balthica* are found in February - March (Beukema and De Bruin 1977), such resorption would continue until March, when food concentrations and *M. balthica* body mass rise again (Cadée 1978, Beukema and Cadée 1996). In this way, a close correlation can arise between egg numbers and March BMI values.

Shell-length and body-mass related reproductive output

After testing the homogeneity of slopes between shell length and number of eggs produced, it was found that a significant relation existed between produced egg number and the third power of shell length. This provides a tool to recalculate egg number to apply to female *M. balthica* individuals with a standardised shell length of 15 mm and to compare fecundities between groups with different mean shell lengths. In this way it was possible to compare experimentally collected data (Honkoop and Van der Meer 1997) with the field-collected data described in the present paper (Fig. 4.3).

To find evidence that immersion time and winter temperature can indeed influence egg numbers, experiments were performed in which immersion time and water temperature during the winter period were manipulated. In groups of *M. balthica*, body mass was measured prior to spawning (Honkoop and Beukema 1997) and egg numbers were estimated (Honkoop and Van der Meer 1997). The combination of these results with the field-collected data described in the present paper showed a causal relationship between both immersion time and water temperature and egg numbers. Thus both environmental factors controlled the fecundity of *M. balthica* populations and their effects were exerted via influences on body

mass. The highly significant relation between BMI prior to spawning and produced egg numbers (Table 4.4, Fig. 4.2D) provides a tool to predict egg number on the basis of the easily measurable BMI. This could make it possible to study relationships between egg production at the population level and subsequent recruitment. No eggs were produced if the BMI value became smaller than 5.6 (Fig. 4.3), indicating that this is a critical value, below which survival apparently becomes more important than the production of offspring.

Life-history aspects

Our results showed that egg size is correlated with body mass in August (Fig. 4.2A) and hardly correlated with body mass in March (Fig. 4.2B). This indicates that egg size is determined at an early stage during gametogenesis. Gonadal development in *M. balthica* takes place during a prolonged period, from early summer until the end of autumn or the beginning of winter (Caddy 1967, Lammens 1967, Chambers and Milne 1975, Pekkarinen 1983, Madsen and Jensen, 1987, Bonsdorff and Wenne 1989, Harvey and Vincent 1989, 1991), although some other authors have reported an even longer lasting gametogenesis, until just prior to spawning (De Wilde and Berghuis 1976, Gilbert 1978). If the availability of food was high (station A and B), eggs larger than those produced in areas where availability of food was more marginal (Station C), were produced. Experiments reported by Honkoop and Van der Meer (1997) showed that egg size could not be influenced by manipulation of water temperature and immersion time during autumn and winter. This explains why the correlation between egg size and March BMI values was low (Fig. 4.2B).

Reproductive output consists of two components, egg size and egg number. According to Smith and Fretwell (1974), Roff (1992) and Stearns (1992), a trade-off between size and number exists and is lineage dependent. Within populations of the same species this trade-off is not always clear (Stearns 1992). This seems to be the case in *M. balthica*, where fewer and also smaller eggs were produced under relatively unfavourable conditions (short immersion times, low food availability, high energy demand). However, there are some examples that this trade-off also holds in some other bivalve species. In two field populations of *M. edulis*, one with a poor and one with a rich food supply during the period of gametogenesis, egg sizes were similar but the group without food produced fewer eggs (Bayne and Worral 1980, Bayne *et al.* 1983). However,

under stressful conditions *M. edulis* can also produce smaller eggs (Bayne *et al.* 1978). In the giant scallop *P. magellanicus* food shortage resulted in low numbers of eggs of normal size (Barber *et al.* 1988). Thus, if food becomes limited, the latter two bivalve species generally decrease their fecundity (*i.e.* egg number), whereas egg size remains unchanged.

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CHAPTER FIVE

Reproductive investment in the intertidal bivalve *Macoma balthica*

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.

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, Holland and Spencer 1973, Bayne 1976, Chu and Webb 1984 Gallager *et al*. 1986, Helm *et al*. 1991, Whyte *et al*. 1992). The size of larval lipid stores 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 5.1, 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.

Table 5.1. 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

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.

Materials and Methods

Experimental design

In 1995, an experiment was performed to study differences in reproductive output of three bivalve species, *Macoma balthica*, *Mytilus edulis*, and *Cerastoderma edule*. Groups of the first two species were collected at Balgzand, a tidal flat area in the south-western part of the Dutch Wadden Sea, whereas *C. edule* were collected at 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.

The set-up was placed outdoors. Fresh sea water from the Marsdiep, the south-western tidal inlet from the Wadden Sea, was pumped into the set-up at a rate of 8 l min^{-1}. To maintain differences in water temperature, the water was continuously cooled (2.5 ?C) for the cold treatment, or remained untreated for the mild treatment. The average water temperature during the months Jan., Febr., and March was 3.1 ?C and 5.7 ?C for the cold and mild treatment, respectively. For more details about the experimental set-up (split-plot design) see Honkoop and Beukema (1997).

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 -35 °C. 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 4 °C 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 12 °C (one individual per beaker), thus experiencing a temperature shock of 8 °C. 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 -35 °C. Prior to the lipid analysis, frozen egg-samples were also freeze-dried for four days.

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 hours at 580 °C, cooled to room temperature and weighed again (W3) and the percentage AFDM of total dry mass ({W2-W3}/{W2-W1}*100 %) 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 4 equal aliquots. The eggs were concentrated, transferred to clean glass-tubes and dried for 4 days at 60 °C in a ventilated stove. After drying, the tubes were weighed (W1) and incinerated at 580 °C for 4 hours, cooled to room temperature ands 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 60 °C 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 hours at 580 °C, 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.

Lipid analysis

The analysis of the lipid content was based on the method of Zöllner and Kirsch (1962) and adapted for a 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 sulfuric 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 waterbath at 100 °C. Then the tubes were cooled in cold water for 5 min. From each tube, 50 mm^3 was placed in polyethylene tubes using a micro pipette 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 using a Hitachi UV-VIS type 181 spectrophotometer, using 50 mm^3 concentrated sulfuric acid (H_2SO_4) 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).

Results

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 *Macoma 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):

$$BMI = m * l^{-3} \quad (1).$$

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:

$$y = 7739(SE = 1463) * \{BMI - 5.6\ (SE = 2.2)\} \quad (2).$$

Equation (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 (4 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 μm^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 μm^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. 5.1A as open points. The best linear fit (R^2 = 0.58, P < 0.001) for these data points was:

$$\text{egg mass} = 1.869\ (\text{SE} = 0.329) * \{\text{BMI} - 5.6\ (\text{SE} = 2.0)\} \qquad (3),$$

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 Equation 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 Equation (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. 5.1B 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 equation 3). This means that the average mass of an individual egg in the experimental and field groups was (1.869 / 7739) * 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).

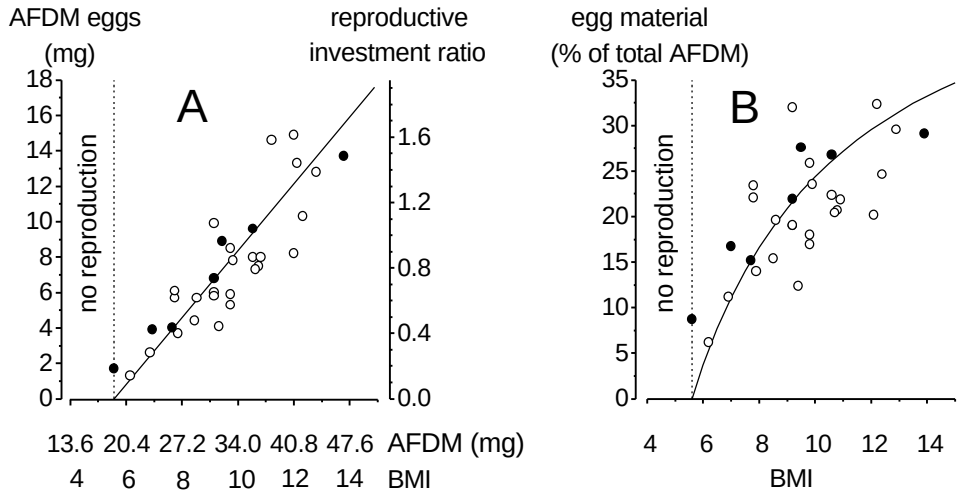


Figure 5.1. (A) Investment in egg material by female *Macoma balthica* with a standard shell length of 15 mm at a range of BMI values, expressed both in weight of egg mass (mg AFDM) (left Y-axis), and in relative reproductive investment, *i.e.* energy in gonads, eggs and extra somatic tissue divided 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 (open dots). Solid dots represent investment in egg-mass calculated from measurements made by De Wilde and Berghuis (1978).

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. 5.1. The best linear fit (Fig. 5.1A, $R^2 = 0.97$, $P < 0.0001$) for these data was:

$$\text{egg mass} = 1.495\ (\text{SE} = 0.117) * \{\text{BMI} - 4.5\ (\text{SE} = 0.8)\} \qquad (4).$$

Statistical analysis revealed that the slopes of the lines of the two data sets available (described by equations 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, $F_{[1,29]} = 0.08$, $P > 0.1$).

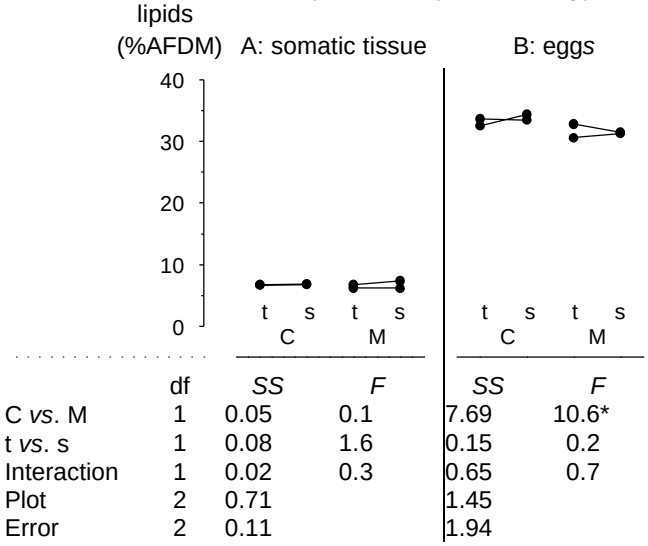
Energy value of reproductive mass

Mean lipid contents of non-gonadal (*i.e.* somatic) tissues of mature females of *M. balthica* are shown in Fig. 5.2A. The mean values of the 8 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. 5.2B).

Because no significant differences in lipid content in somatic tissue was observed between experimental groups subjected to different treatments (Fig. 5.2A), mean lipid percentages could be calculated for this part of the body. The differences between treatments in lipid content of the eggs (Fig. 5.2B) 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 equation (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 * 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



	df	A: somatic tissue SS	A: somatic tissue 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	

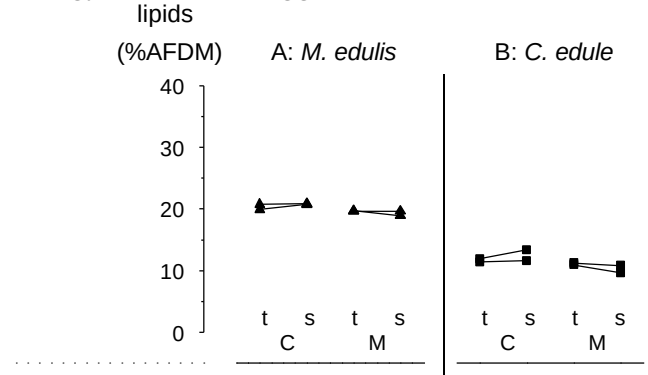
Figure 5.2. Lipid contents (as percentage of the total Ash-Free Dry Mass) just prior to spawning of (A) somatic tissue of 8 pooled individuals per sub-plot per treatment, and (B) freshly spawned eggs of experimental groups of female *Macoma balthica*. Each line connects the lipid percentage of the tidal (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 as ** if $P < 0.05$ and * if $P < 0.10$.

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 (BMI - 5.6) * 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 in Fig.5.1A right-hand axis.

Comparison with C. edule *and* M. edulis

The lipid contents of freshly spawned eggs in *M. edulis* (20.0 % of AFDM, Fig. 5.3A) and *C. edule* (11.4 % of AFDM, Fig. 5.3B) were lower than the lipid content of egg spawned by *M. balthica* (32.5 % of AFDM, Fig. 5.2B). Per unit of mass, *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 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 5.2) indicate that



	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	

Figure 5.3. The lipid contents (as percentage of the total Ash-Free Dry Mass) of freshly spawned eggs of (A) *Mytilus edulis* and (B) *Cerastoderma edule*, in experiments performed in 1995. Each data point is the mean of pooled eggs spawned by 2 to 7 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 as ** if $P < 0.05$ and * if $P < 0.10$.

Table 5.2. The calculated energy content per egg of freshly spawned eggs of *Cerastoderma edule*, *Macoma balthica*, and *Mytilus edulis* as observed in 1995. 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. 5.2.

Species	Size (µm)	Volume (x10^{-7} cm^3)	Mass (µg)	Lipid (%)	Lipid (µg)	Lean mass (µg)	Energy (x10^{-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

the eggs of *M. balthica* contained about three times more energy ($5.9 * 10^{-3}$ J egg^{-1}) than the eggs of *C. edule* or *M. edulis* ($1.97 * 10^{-3}$ and $1.78 * 10^{-3}$ J egg^{-1}, respectively).

Discussion

Reproductive investment of M. balthica

The body of a *Macoma 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 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 (Equation 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 (BMI = 14), 636 J is invested in gonadal material, eggs and extra somatic tissue. The eggs released at BMI = 14 (about 15 mg eggs, equation 5) 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 = 0.4 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).

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 *Cerastoderma edule* and *Mytilus 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 early in spring, makes larval growth slower than at 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 12.5 °C in *M. balthica*, and 2 and 1.5 day at 15 °C 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 1986, Cadée and Hegeman 1979). 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, Holland and Spencer 1973, Bayne 1976, Chu and Webb 1984, Gallager *et al*. 1986, Helm *et al*. 1991, 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. 5.2)). If the differences in egg size between the species are also taken into account (Table 5.2), the between-species differences are even larger. With an amount of $5.9 * 10^{-3}$ J egg^{-1}, *M. balthica* produces relatively energy-rich, and thus expensive, eggs compared to the other species ($1.97 * 10^{-3}$ J egg^{-1} and $1.78 * 10^{-3}$ J egg^{-1} in *C. edule* and *M. edulis*, respectively). The energy per egg of *M. edulis* ($1.78 * 10^{-3}$ 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 5.2) 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 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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CHAPTER SIX

Does temperature-influenced egg production predict the recruitment in the bivalve *Macoma balthica*?

Abstract

As in most populations of bivalves, year-to-year variation in recruitment is large in the tellinacean *Macoma balthica* (L.). During the period of observation (1973 - 1996), high densities of recruits (numbers of spat, 0-year-class) per m² in late summer were observed after 3 severe winters (1979, 1985, and 1987) and also in 1991, which was a normal winter, whereas recruitment failed after all of the 5 mildest winters of the period of observation (1974, 1988, 1989, 1990, and 1995). As fecundity (the number of spawned eggs per female) also varies strongly in response to winter temperature in *M. balthica*, we studied to what extent the large year-to-year variation in recruitment can be explained in terms of temperature-influenced variation in fecundity. To this end, we related both mean annual egg numbers per female (fecundity) and per m² (egg density) to subsequent recruitment data during the 1973 - 1996 period in a population at Balgzand, a 50 km² tidal flat area in the southwestern part of the Dutch Wadden Sea. Water temperatures in winter influenced individual egg production (winter temperature was negatively correlated with fecundity) and, consequently, total egg production of the population (stock size was not related to winter temperature). Although a substantial part (37 %) of the year-to-year variation in recruit densities could be explained by interannual variation in winter temperatures, only a minor part of recruitment variation (7 %) was explained by variation in egg density. Thus, the numbers of adults and the total number of eggs spawned in a certain year are poor predictors of subsequent recruit abundance. The significant effect of winter temperature on recruitment cannot be explained by the winter-temperature-governed fecundity and total egg production.

Introduction

An important topic in marine ecology is the still poorly understood interannual variation in recruitment of many marine species, including

economically important fish and shellfish species. So far, different definitions for recruitment are in use. In fish ecology, the term recruitment is commonly used for the year-class strength at the fishable stage (Miller 1994). As a consequence, the period between spawning and recruitment can be very long, sometimes even years (Hancock 1973). In our study on bivalve ecology, we adopt the following definition: recruitment is the number of juveniles (spat, 0-year-class individuals, which can be distinguished from 1-year old juveniles by their size and the lack of a year-ring) retained on a sieve with a mesh-size of 1 mm, present per m^2 in the first summer after spring spawning. The period between spawning and recruitment is relatively short (3 - 5 months) and the factors affecting recruitment success can be studied within a short period.

Variation in bivalve recruitment can be very large. Examples of long-term data series of recruitment success include the cockle *Cerastoderma edule* (L.) (Kristensen 1957, Beukema 1982, Möller and Rosenberg 1983, Ducrotoy *et al.* 1991, Beukema *et al.* 1993), the mussel *Mytilus edulis* (L.) (Beukema 1982, McGrorty *et al.* 1990, Beukema *et al.* 1993), the clam *Mya arenaria* (L.) (Beukema 1982, Möller and Rosenberg 1983), and the tellinaceans *Macoma balthica* (L.) (Beukema 1982, Beukema *et al.* 1998), *Scrobicularia plana* (da Costa) (Essink *et al.* 1991), and *Abra tenuis* (Wood) (Dekker and Beukema 1993). All these studies show that year-to-year differences of 2 to 3 orders of magnitude are not uncommon.

In fish, only a minor part of the interannual variability in recruitment can usually be explained by variability in adult stock size or egg numbers (Parrish 1973, Corten and Van de Kamp 1979). Data about the stock-recruitment relationship in shellfish and other marine invertebrates appear to be scarce (Hancock 1973) and show a lack of correlation between size of spawning stock and subsequent recruitment. An exception is the common cockle *C. edule* (L.), for which a (weak) negative relationship between the stock, defined as the total number (Hancock 1973) or biomass of adult year classes (Van der Meer 1997), and recruitment was observed.

One of the factors which seems to influence variation in recruitment is winter temperature. For a number of bivalve species, relatively successful recruitment has been reported after cold winters, whereas recruitment after mild winters usually fails. Among these species are *M. edulis* (Beukema 1982, 1992a, Jensen and Jensen 1985, McGrorty *et al.* 1990, Young *et al.* 1996), *C. edule* (Kristensen 1957, Hancock 1973, Beukema 1982, 1992a, Möller and Rosenberg 1983, Jensen and Jensen 1985, Yankson 1986,

Ducrotoy *et al.* 1991, Young *et al.* 1996), *M. arenaria* (Beukema 1982, 1992a, Möller and Rosenberg 1983, Jensen and Jensen 1985), and *M. balthica* (Beukema 1982, 1992a, Jensen and Jensen 1985).

In this study, we present detailed data on the egg number-recruitment relationship in the Balgzand (The Netherlands) population of the bivalve *M. balthica*. Data on numbers and biomass of aged specimens of this population have been collected since 1970 at 15 tidal-flat Wadden Sea stations (Beukema 1974, 1988). It has already been shown that there is no correlation between the stock size of this population, defined as the total *M. balthica* ash-free dry mass per m², and the subsequent recruitment (Van der Meer 1997). In an earlier study, the dependence of fecundity (number of spawned eggs per female) in *M. balthica* on its body mass and shell size was established (Honkoop and Van der Meer 1997). Taking these variables into account, we modify the relevant index of adult stock size to total number of eggs produced per m² (*i.e.* egg density). Using this index we examine the stock-recruitment relationship to determine how far the interannual variation in *M. balthica* recruitment can be explained by year-to-year variation in egg production.

In earlier studies it was shown that variation in fecundity in *M. balthica* and *C. edule* can be very large, and that winter temperature is one of the main factors influencing variation in individual egg production (Honkoop and Van der Meer 1997, 1998); low winter temperatures appear to have a positive effect on fecundity. Thus, winter temperature seems to affect both individual egg production and subsequent recruitment in a similar way. Therefore, the following question arises: are egg production and recruitment closely connected and can winter temperatures thus explain a significant part of the variation in recruitment via temperature-induced variation in fecundity? To answer this question, we estimated which part of the year-to-year variation in egg density and recruitment can be explained by variation in winter temperature.

Materials and methods

Study area and monitoring

Each year, since 1970, benthic invertebrates have been quantitatively sampled at the Balgzand, a 50 km² tidal-flat area in the southwestern part of the Dutch Wadden Sea. Mostly twice a year, in March and August, 15 fixed sites scattered over the whole area were sampled: 12 transects 1 km in

length (each with 50 sub-sampling points) and 3 squares of 900 m² (each with 18 sub-sampling points). At each sub-sampling point a core with a known area was taken from the sediment down to a depth of 20 cm. The cores were washed through a sieve with a mesh-size of 1 mm, and all animals were sorted, identified, counted, dried and incinerated. Numbers and biomass are expressed per m². For further details about the sampling method see Beukema (1974, 1988). At each site the following variables were measured or calculated for *Macoma balthica*: (1) shell length (mm), each individual was aged, by counting year-rings, and measured to the nearest mm, using a calliper; (2) density (m^{-2}), for each year-class (6 year-classes), which is defined as a group of individuals born in the same year (individuals older than 5 years were pooled into the same year-class); (3) body mass index (BMI) within each year-class for each mm-class, *i.e.* ash-free dry mass per cubic shell length (mg cm^{-3}); and (4) recruitment, which is defined as the number of spat (0-year-class individuals, no year-ring) retained on a 1 mm mesh-size sieve in August. Annual recruitment data are available from 1973 up to and including 1996 (n = 24 years). The year 1991 was exceptional in the Dutch Wadden Sea with respect to the density of filter-feeding bivalves, the adult body masses, and the recruitment of some bivalve species (Beukema and Cadée 1996). Because 1991 was such an outlying year we did not use data from this one year in our statistical analyses.

Water temperatures were obtained from measurements of surface water in the Marsdiep, the neighbouring major tidal channel; each day at 08.00 h the water temperature was measured to the nearest 0.1 °C. The winter water temperature is defined as the mean of the daily measurements in January, February, and March in each year.

Calculation of egg production

To estimate the number of eggs spawned by an individual female with a standard shell length of 1.5 cm, the following relationships were used:
(1) total body mass m (mg) is proportional to cubic length:

$$m = c * l^3 \quad (1),$$

where l = shell length (cm);
c = body mass index (mg cm^{-3}).

(2) based on the relationship described by Honkoop and Van der Meer (1997), the number of spawned eggs per adult female, y (fecundity), is related to the difference between total body mass (cl^3) and minimal (structural) body mass for any egg production ($? l^3$) (*i.e.* the minimum body mass, excluding stores, necessary for a functional normal life; Van der Meer and Piersma 1994) of the female individual.
Hence:

$$y ? ? \frac{l^3}{s^3}(c ? ?) \qquad (2),$$

where $?$ = structural body mass in BMI units (mg cm^{-3}),
✍ = number of spawned eggs per BMI unit for a standard individual of 1.5 cm shell length (cm^3 mg^{-1}), and
s = shell length of a standard female (cm).

Values for ✍ (7739), $?$ (43314 / 7739 = 5.6), and s (1.5 cm) were given by Honkoop and Van der Meer (1997).
Additional assumptions were:
(3) if $c < 5.6$, egg production was set to zero (thus excluding negative values for fecundity);
(4) only females > 10 mm reproduce (reproduction rarely occurs at smaller shell lengths; Caddy 1967, Gilbert 1978, pers. obs.);
(5) on average, half of the adult population (all individuals except the 0-year class) are females (Caddy 1967, De Wilde and Berghuis 1976, Gilbert 1978, Brousseau 1987).
Then, total egg production x_j in year j is,

$$x_j ? \sum_{i?1}^{15} \sum_{k?1}^{6} \frac{n_{ijk}}{2} y_{ijk} ? \sum_{i?1}^{15} \sum_{k?1}^{6} \frac{n_{ijk}}{2} ? \frac{l_{ijk}^3}{(1.5)^3}(c_{ijk} ? ?) \qquad (3),$$

where i = sampling site;
k = year class;
n_{ijk} = number of adults larger than 10 mm for site i, year j, and year-class k;
c_{ijk} = average body mass index for site i, year j, and year-class k;

l_{ijk} = average length for site i, year j, and year-class k,
y_{ijk} = number of spawned eggs by a female for site i, year j, and year-class k.

Results

Winter water temperature and egg production

Using average March BMI values of each year-class at each station, individual egg production y for a standard female with a shell length of 1.5 cm was calculated for each year (using equation 2). A negative relationship between winter water temperature and individual egg production y was observed, in accordance with previous experiments (Honkoop and Van der Meer 1997, 1998). Variation in mean winter (January - March) temperature explained 68 % of the total among-years variance in (log-transformed) egg numbers produced by a single standard *Macoma balthica* ($P < 0.0001$, $R^2 = 0.68$; Fig. 6.1A).

Total egg production (estimated with equation 3) also depended on the numbers of reproducing females (as well as their age and size distribution). Linear regression of (log-transformed) total egg production

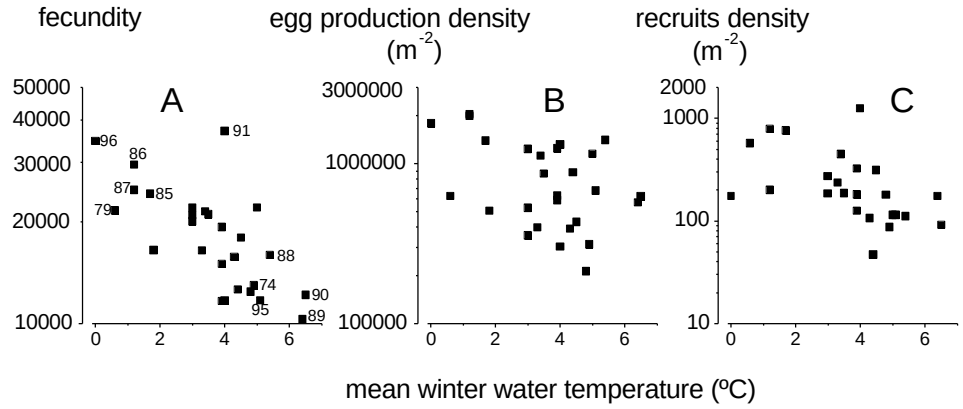

Figure 6.1. Relationships between winter water temperature (mean water temperature of the January - March period) and log-scaled (A) individual egg production at Balgzand of a standard female *Macoma balthica* with a shell length of 15 mm, (B) total egg production of *M. balthica* at Balgzand, and (C) recruit numbers of *M. balthica* at Balgzand. The numbered points in (A) refer to 5 cold winters (1979, 1985, 1986, 1987, and 1996), 5 mild winters (1974, 1988, 1989, 1990, and 1995), and the exceptional year 1991.

against winter water temperature (Fig. 6.1B) revealed a negative relationship, but the explained variance was much lower ($R^2 = 0.23$, $P < 0.05$). Yet, relating subsequent (log-transformed) recruitment to winter water temperature (Fig. 6.1C) revealed a somewhat better fit ($R^2 = 0.37$, $P < 0.01$).

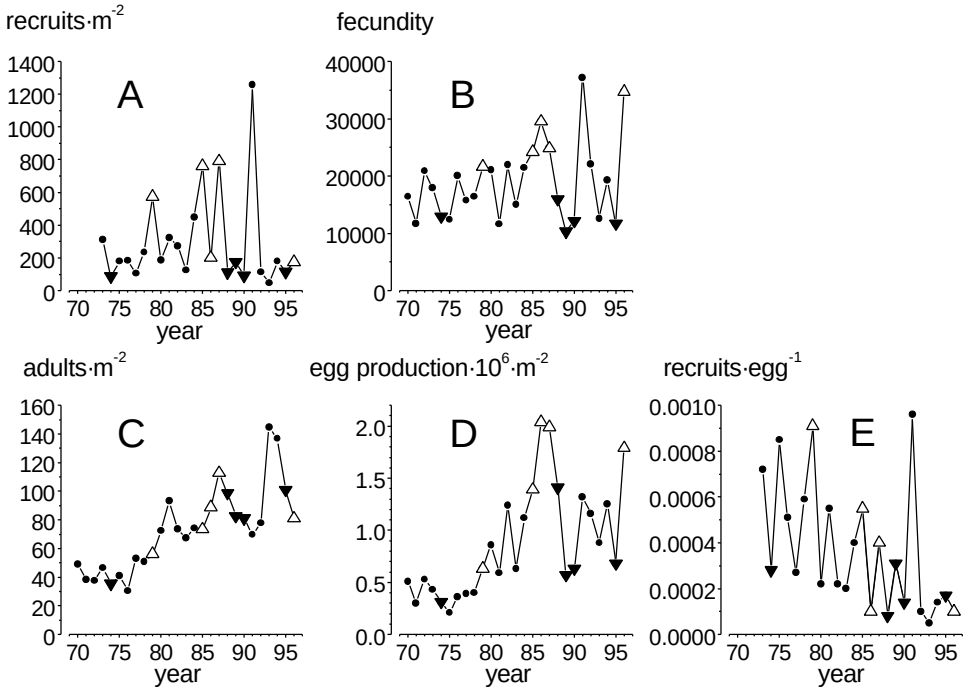

Figure 6.2. Annual variability of some population characteristics of *Macoma balthica* (A) Average number of recruits per m² on the tidal flats of Balgzand in August of each of the years 1973 - 1996, (B) number of eggs spawned by a standard female with a shell length of 15 mm for each year in the period 1970 - 1996, (C) density of reproducing individuals (thus males + females) per m² for the same period, (D) calculated egg production per m² for the same period, and (E) survival from egg to recruit for the 1973 - 1996 period. Open triangles refer to the 5 years with cold winters (mean January - March water temperature < 1.8 °C), and solid triangles refer to the 5 years with mild winters (mean January-March water temperature > 4.8 °C) during the 1970 - 1996 period.

Egg production and subsequent recruitment.

M. balthica recruitment at Balgzand was low during most years of the study period, except for a few years with relatively large recruitment, *viz.* 1979, 1985, 1987, and 1991 (Fig. 6.2A).

In most years fecundity amounted to values between 10 000 and 20 000 spawned eggs per standard 1.5 cm female, but in some years it was roughly twice this amount (1985, 1986, 1987, 1991, and 1996) (Fig. 6.2B). Egg density per year, which also depends on adult density (Fig. 6.2C), showed a somewhat larger variation among years (Fig. 6.2D), with approximately an order of magnitude difference among years. Survival of eggs to recruits (recruit / egg ratio) varied between 0.0001 and 0.001 and showed an almost continuous decrease throughout the study period (Fig. 6.2E). Only 7 % of the interannual variation in (log-transformed) numbers of recruits could be explained by variation in (log-transformed) egg density (Fig. 6.3). The estimate of the regression slope was much lower than 1 ($b = 0.31$, $SE = 0.24$), which implies that high egg production results in low survival of eggs to recruits.

Thus, although variation in winter water temperature accounts for a relatively large part (37 %) of variation in recruitment, this cannot be explained by temperature effects on egg production, as only 7 % of the variation in recruitment could be attributed to (temperature-related) variation in egg density. The effect of winter water temperature operates on survival from egg to recruit.

Discussion

Stock-recruitment relationship

A study of a stock-recruitment relationship such as the present one for the *Macoma balthica* population at Balgzand has its inherent limitations, primarily because it is an open system that has been studied. Therefore, it is not certain that all Balgzand recruits originated from eggs of this population. It might be that (1) part of the eggs were produced by nearby populations and (2) eggs of the Balgzand population contributed to recruitment somewhere else (*e.g.* the North Sea). A real distortion of the relationships shown is not expected, as (1) the other populations will have experienced similar winter temperatures, and (2) their densities will have fluctuated similarly to those of the Balgzand population (Desprez *et al.* 1991, Beukema *et al.* 1996).

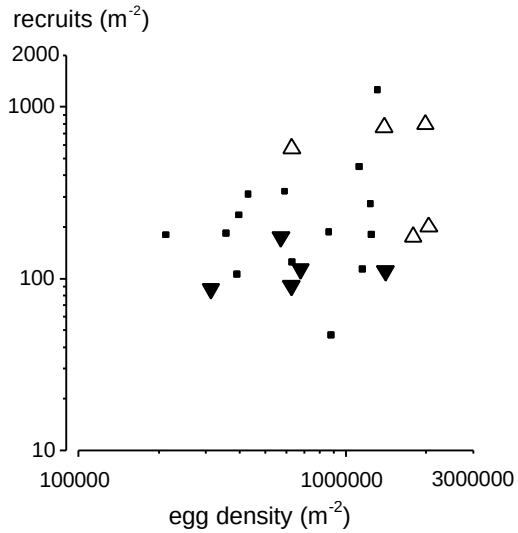

Figure 6.3. Relationship (log-scales) between the total number of eggs produced per m² in spring and the number of recruits per m² observed in the following August. Each data point represents one year during the 1973 - 1996 period. Open triangles refer to the 5 years with the coldest winters (mean January-March water temperature < 1.8 °C), and solid triangles refer to the 5 years with the mildest winters (mean January-March water temperature > 4.8 °C) during the 1970 - 1996 period.

Fecundity of *M. balthica* is highly variable and depends on body mass and shell length (Honkoop and Van der Meer 1997, 1998). Therefore, these two factors have to be taken into account when calculating total numbers of eggs produced. To this end we used a previously described relationship (Honkoop and Van der Meer 1997). The total number of eggs spawned is not exactly equivalent to the product of the fecundity of a standard individual times the overall female stock size, as variation in age and size distribution are ignored. However, 92 % of the variance in total egg number (as calculated using equation (3), which takes into account differences in age and size distribution) was explained by this simple approximation, leaving only 8 % to be explained by annual differences in age and size distribution.

The lack of significant correlation between total egg number and recruitment (Fig. 6.3) and the observation of an increasing adult density throughout the studied period (Fig. 6.2C) imply that there must be a negative relationship between adult densities and survival of eggs to

recruits. The opposing trends shown in Figs 6.2C and 6.2E illustrate this point. It is not clear whether this relationship is based on an effect of the adults on survival of eggs, larvae, or post-larvae. In several species such a relationship has been shown (*M. balthica*: Bachelet 1986, Bonsdorff *et al.* 1986; *Cerastoderma edule*: Kristensen 1957, André and Rosenberg 1991, André *et al.* 1993; *Mya arenaria*: André and Rosenberg 1991) but a possible direct mechanism has been suggested only in *C. edule*, in which the adults inhaled their own offspring (André *et al.* 1993).

Winter temperature and recruitment

Recruitment after mild winters during the period of observation (Figs 6.1C and 6.2A, solid triangles) was consistently low. In these years, fecundity was invariably low (Fig. 6.2B), but adult densities happened to be high in most years (Fig. 6.2C), resulting in egg numbers which were generally not particularly low (Fig. 6.2D). Survival of eggs to recruits in these years was always relatively low (Fig. 6.3). This may have been due to predation by juvenile shrimps *Crangon crangon*, important predators on post-larval (*i.e.* early spat) *M. balthica* (Beukema *et al.* 1998) that generally appear on tidal flats earlier in springs after mild than after cold winters (Beukema 1992b).

Recruitment after cold winters (Figs 6.1C and 6.2A, open triangles) was less consistent, being high after 3 out of the 5 years with a cold winter (1979, 1985, and 1987), and low after the others (1986 and 1996). In 1986, low recruitment can possibly be explained by shrimp summer density which was the highest observed during the studied period, ~210 ind. m^{-2}, which is on average twice as high as in other years. Why recruitment success was low after the severe winter of 1996 is not known. An exceptionally high recruitment was observed in the summer of 1991: it was the highest observed in the studied period and followed high fecundity values in spring (Fig. 6.2B), but a close-to-average total egg production (Fig. 6.2D). The year 1991 was exceptional in several ways. Due to intensive bottom-fishery activities and 3 successive years of recruitment failure in almost all large filter-feeding bivalves (particularly the important species *C. edule* and *Mytilus edulis*), bivalve stocks were exceptionally low in late 1990 and in early 1991. This resulted in increased food supply, phytoplankton densities are significantly reduced at high filter-feeding densities (Prins *et al.* 1995), and therefore high body mass values for reproducing *M. balthica* (Beukema and Cadée 1996). Moreover, shrimp

densities in spring 1991 were exceptionally low, ~25 ind. m^{-2} (Beukema *et al.* 1998).

Fecundity was significantly negatively correlated with winter temperature (Fig. 6.1A), resulting in a large percentage of the variation in egg production being explained by variation in water temperature (Fig. 6.1B). However, the relationship between winter temperature and recruitment (Fig. 6.1C) cannot be directly explained from temperature-determined differences in egg production. Egg density was weakly correlated with recruit numbers (Fig. 6.3) and only a small percentage (7 %) of the variation in recruitment was explained by temperature-influenced egg production. This suggests that the temperature-influenced variation in recruit numbers (Fig. 6.1C) must have been caused by temperature-affected variation in factors other than egg numbers. One possibility is the predation by juvenile shrimps *C. crangon* and juvenile shore crabs *Carcinus maenas*. It has been shown that both shore crabs and shrimps arrive earlier on the tidal flats and, moreover, crabs arrive in higher numbers after a mild than after a cold winter (Beukema 1991, 1992b). Also, the density of juvenile shrimps in spring was significantly positively correlated with the temperatures of the preceding winter (Beukema *et al.* 1998). Although almost nothing is known about the intensity and timing of predation, this process may have decisively affected survival of all early life stages (eggs, larvae, and post-larvae) and may have overruled the effects of initial egg numbers.

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