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Growth and reproduction in bivalves

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Cardoso, J. F. M. F. (2007). *Growth and reproduction in bivalves: An energy budget approach*. s.n.

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Growth and Reproduction in Bivalves

An energy budget approach

The research reported in this thesis was carried out at the Department of Marine Ecology and Evolution (MEE) of the Royal Netherlands Institute for Sea Research (NIOZ) and financially supported by the project 'Praxis XXI', grant BD/21799/99, from 'Fundação para a Ciência e a Tecnologia (FCT)', Portugal.

Cover design and thesis layout: Joana Cardoso
Figures: Henk Hobbelink, Bert Aggenbach and Joana Cardoso
Printed by: PrintPartners Ipskamp

RIJKSUNIVERSITEIT GRONINGEN

Growth and Reproduction in Bivalves
An energy budget approach

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 21 september 2007
om 14.45 uur

door

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geboren op 6 november 1975
te Porto, Portugal

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ISBN: 978-90-367-3140-9

Fundo do mar

No fundo do mar há brancos pavores,
Onde as plantas são animais
E os animais são flores.

Mundo silencioso que não atinge
A agitação das ondas.
Abrem-se rindo conchas redondas,
Baloiça o cavalo-marinho.
Um polvo avança
No desalinho
Dos seus mil braços,
Uma flor dança,
Sem ruído vibram os espaços.

Sobre a areia o tempo poisa
Leve como um lenço.

Mas por mais bela que seja cada coisa
Tem um monstro em si suspenso.

Sophia de Mello Breyner Andresen

To my family

Contents

CHAPTER 1

General introduction

Objective

Bivalves are a main component of the benthic fauna of many marine and estuarine areas. Due to their high abundance (Dame 1996, Gosling 2003, and references therein), they are important as food source for other species, such as shorebirds, fishes, crustaceans (Braber and De Groot 1973, Hulscher 1982, Phil and Rosenberg 1984, Fréchette and Bourget 1985, Offringa 1993, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Leopold 1996, Zwarts et al. 1996, Van der Veer et al. 1998, Mascaró and Seed 2000, Hiddink et al. 2002, Fox 2003, Beukema and Dekker 2005) as well as humans. Bivalve species can be found all around the globe in a variety of environments, from the poles to the tropics (Tebble 1966, Hayward and Ryland 1995, Dance and Ward 2002). Over such a range, differences in environmental conditions such as in water temperature, salinity, food availability and water current occur. These differences influence growth, survival and reproduction and, ultimately, they limit and determine the distribution of species.

At a latitudinal scale, physiological processes in bivalves are mainly affected by temperature. Latitudinal differences in growth rate of bivalves have been frequently related to latitudinal gradients in temperature (Gilbert 1973, Bachelet 1980, Appeldoorn 1983, 1995; Beukema and Meehan 1985, Hech et al. 2002, Fiori and Morsán 2004). Also gametogenesis and spawning are affected by temperature. In many bivalve species, spawning occurs once a specific threshold temperature is reached (Loosanoff and Davis 1963, Lammens 1967, De Wilde and Berghuis 1978, Giese and Kanatani 1987, Drent 2004). Overall, temperature is seen as a key factor directly or indirectly affecting physiological processes. After severe winters, the amount of settled bivalve larvae (spat) on the seafloor appears to be higher than after mild winters (Reise 1987, Beukema et al. 2001). In this case, temperature acts indirectly by reducing the amount of predators that feed on early life stages (Beukema 1992, Strasser and Günther 2001, Strasser 2002, Philippart et al. 2003).

At a local scale, physiological processes in bivalves are affected by other environmental factors as well. Food quality and quantity, tidal level and sediment type are known to influence growth and reproduction, and these seem to have locally a more important role (Newell and Hidu 1982, De Montaudouin 1996, Beukema and Cadée 1997, Honkoop and Beukema 1997, Beukema et al. 2002, Carmichael et al. 2004). Food availability is considered the most important factor influencing growth in bivalves at a local scale. For example, higher growth, body condition and reproductive output are usually observed at lower intertidal (exposed during low tide) areas than at higher ones (Jones et al. 1978, Guevara and Niell 1989, Roseberry et al. 1991, Jensen 1992, Wanink and Zwarts 1993, De Montaudouin 1996, Honkoop and Beukema 1997), due to the longer submersion time and thus longer possibility of food intake at lower intertidal areas. Furthermore, numerous intra- and interspecific interactions between bivalve species and other benthic fauna also occur within an area and these include competition for food (Hummel 1985, Kamermans et al. 1992, Kamermans 1993, Herman et al. 1999, Cognie et al. 2001), predation (Van der Veer et al. 1998) or disturbance by other benthic organisms such as worms (Flach 1992). As a consequence, bivalve species experience differences in local conditions and, to be able to survive in a variable environment, they have strategies to maximize growth, reproduction and survival. These life history strategies include the timing of reproduction, their feeding and also their use of the different tidal levels.

In the shallow Wadden Sea, the largest estuarine area in northern Europe, in size around 10,000 km² (Wolff 1983), a few bivalve species constitute more than 65% of the total biomass of macrobenthos, both at intertidal and subtidal (always submersed) areas (Dekker 1989, Beukema 1991, Dekker et al. 2002, 2003; Dekker and Waasdorp 2004, 2005, 2006). These species (Fig. 1.1) include the Baltic tellin *Macoma balthica*, the edible cockle *Cerastoderma edule*, the blue mussel *Mytilus edulis*, the soft-shell clam *Mya arenaria*, the razor shell *Ensis americanus* and a recent invading species the Pacific oyster *Crassostrea gigas* (Beukema 1976, Wolff 1983, Dankers et al. 2006, Dekker and Waasdorp 2006).

Cerastoderma edule *Mya arenaria* *Mytilus edulis*

Macoma balthica *Ensis americanus* *Crassostrea gigas*

Fig. 1.1. Most common bivalve species of the western Wadden Sea.

Despite the similarities in their life cycles, there are differences in habitat use among the various species. In some of these species, such as *M. balthica* and *C. gigas*, spat is almost exclusively found in high densities in the intertidal zone, whereas in *C. edule*, *M. arenaria*, *E. americanus* and *M. edulis* settlement of spat is observed over a wider range in the intertidal and subtidal. Adults of all species are generally found over a wider tidal range (intertidal and subtidal) than the juveniles (Beukema et al. 1978, Beukema 1993). Differences in habitat use between the various species imply that differences in their life history characteristics must be involved. Therefore, spatial differences in food and growing conditions between tidal areas are expected to result in differences in, for example, age at maturity, maximum age and maximum size.

On the one hand, the fact that these species are widespread and common illustrates the strong persistence of the populations (c.f. Sinclair 1988) and hence their survival from year to year. On the other hand, environmental conditions in the Dutch Wadden Sea hardly seem favourable for growth and survival of bivalves, since food conditions appear to be suboptimal during most of the year. For example, low temperatures and low food availability during winter lead to weight loss in bivalve species such as *M. balthica*, *M. arenaria* and *C. edule* (Zwarts 1991). Additionally to these environmental stresses, there is also a high predation pressure and competition for food between bivalve species (De Vlas 1979, Kamermans et al. 1992). Therefore, despite their survival in the Dutch Wadden Sea, their yearly energy balance can be negative in some years, as seen in *M. balthica* (Hummel 1985).

The main aim of this thesis is to get insight on how different bivalve species are able to persist in an apparently unfavourable environment. First, variation in life history strategies among bivalve species in relation to environmental conditions is studied. The objective is to identify whether interspecific differences occur with respect to feeding, growth, reproductive investment and timing of spawning. Second, intraspecific differences in the various bivalves are analysed, whereby the outcome of the life history strategy of a species in different habitats is studied. Since food quantity, food quality and temperature are the main factors influencing physiological rates, such as feeding rates and respiration rates (Widdows 1973a, b; Newell and Bayne 1980, Møhlenberg and Kiørboe 1981, Smaal et al. 1997), this has been translated into a study of the physiology of bivalve species. Environmental conditions affect the energy available for growth and reproduction and therefore, differences in life history strategies among species are expected to result in differences in success of species.

Approach

This thesis focuses on growth and reproduction of bivalve species in different habitats of the western Dutch Wadden Sea and North Sea coastal zone in relation to environmental conditions. This approach allows a further analysis of intra- and interspecific interactions (such as competition for food) between those bivalve species. Such a comparative bio-energetic study should, ideally, be based on a general framework that can be applied to all species. The Dynamic Energy Budget (DEB) theory developed by Kooijman (1988, 2000) offers this framework. By applying the DEB model, it is possible to quantitatively describe the energy flow through an individual and to analyse the allocation of energy over growth and reproduction in relation to environmental conditions and food intake. The same model can be applied for different species whereby differences among species are expressed as differences in parameter values. The DEB model has already been successfully applied to analyse the effects of toxic components in mussels (Van Haren and Kooijman 1993), to compare life history strategies of marine flatfish species (Van der Veer et al. 2001) and to simulate growth and reproduction in oysters (Bacher and Gangnery 2006, Pouvreau et al. 2006). The DEB model is applied in this thesis in two ways: [1] for the prediction of growth under various food and temperature conditions and [2] for the reconstruction of food intake under current temperature conditions and with observed bivalve growth in the field.

The work presented in this thesis is based on extensive field studies on growth and reproduction of various bivalve species in combination with DEB model simulations with the final aim to analyse differences in life history strategies among and within species in relation to habitat characteristics. This thesis consists of thee parts:

[1] Field studies,

[2] Estimation of DEB model parameters for the various bivalve species,

[3] Application of the DEB model for the reconstruction of food conditions in the field to analyse intra- and interspecies competition.

Field studies

Studied species and their characteristics

Five bivalve species were studied in this thesis: the Baltic tellin *Macoma balthica*, the edible cockle *Cerastoderma edule*, the blue mussel *Mytilus edulis*, the soft-shell clam *Mya arenaria* and the Pacific oyster *Crassostrea gigas* (see Fig. 1.1). All species have a wide range of distribution, from cold to temperate or even subtropical waters (Tebble 1966, Hayward and Ryland 1995). Although in the Dutch Wadden Sea and adjacent coastal waters these species occupy different habitats (Wolff 1983), they share fundamental similarities in their life cycles. After fertilization in the water column and a short egg stage of maximum a few days, a free swimming planktonic larval period occurs. Usually, after three to four weeks in the water column, larvae are ready to settle on the seafloor. Once these bivalve spat have settled for a few weeks, it is common for them to redistribute over the intertidal or subtidal range.

M. balthica occurs from the upper regions of the intertidal and subtidal areas in the Dutch Wadden Sea to the outer parts of the tidal inlets in the coastal zone. *C. edule* and *M. edulis* occur from the intertidal to the shallow subtidal areas in the estuary. *M. arenaria* is present mainly from the upper intertidal to the shallow subtidal but it may be found, in offshore areas, at considerable depths. *C. gigas*, a native species from Japan, is now quite abundant and expanding in intertidal areas of the estuary, although it can also be found in the subtidal. Considerable differences in ultimate size are found between these species: *M. balthica* reaches a maximum shell length of 2.5 cm, *M. arenaria* of 10-15 cm, *C. edule* of 5 cm, *M. edulis* up to 15 cm and *C. gigas* up to 20 cm (Tebble 1966, Hayward and Ryland 1995, Reise 1998). All species live half or totally burrowed in muddy to sandy sediments although mussels and oysters always have to attach to hard substrate such as rocks or dead shells, being often found on stones of harbour piers. *M. balthica* and *M. arenaria* are able to bury deep in the sediment and protrude their long siphons over the surface for feeding and respiration. All species except for *M. balthica* are suspension-feeders, filtering food particles from the water column. Although *M. balthica* is capable of suspension-feeding, it is mainly a deposit-feeder, feeding on benthic algae from the sediment surface.

Studied locations and methods

Bivalve species were sampled at selected intertidal (above Low Low Water Spring (LLWS)) and subtidal (between LLWS and LLWS – 5 m) areas of the western Dutch Wadden Sea and in the North Sea coastal zone (called in this thesis “offshore”, between LLWS - 5 m and LLWS - 10 m), in such a way that representative samples could be collected (Fig. 1.2). Sediment composition varied from sand, in the vicinity of gullies and deeper channels, to muddy areas near the shore.

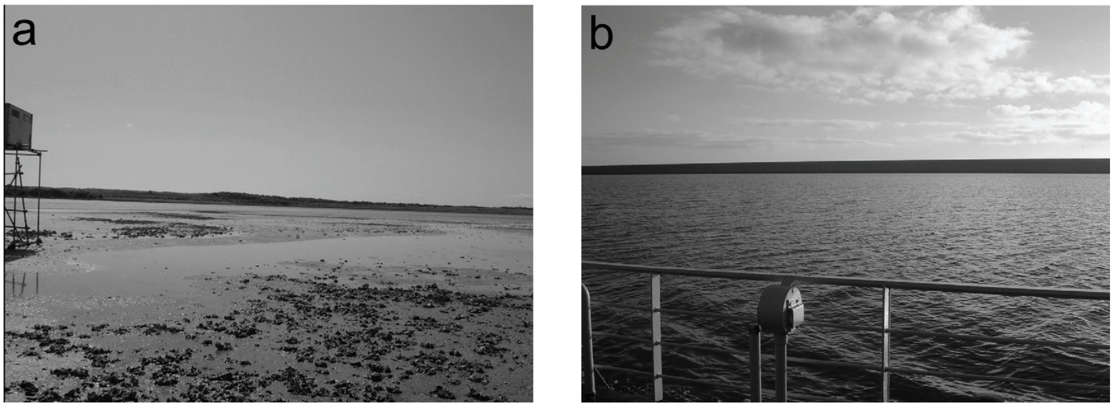

Fig. 1.2. Two of the sampled stations in this study: a) intertidal, b) subtidal.

Intertidal sampling areas were emerged for about 4 to 5 hours per tidal cycle. In these areas, sampling was done during low tide, by walking directly from the shore to the sampling area or by travelling first by boat to the tidal flat. On the tidal flat, *M. edulis*, *C. edule*, *M. arenaria* and *C. gigas* were collected by hand, by digging out with a fork, while *M. balthica* was sampled with a hand core (Fig. 1.3a). At subtidal areas, sampling was done by boat. *M. balthica* and *M. arenaria* were sampled with a ‘Reineck’ box corer (Fig. 1.3b) and *C. edule* was sampled with a 1.9 m beam trawl (Fig. 1.3c). At offshore areas, *M. balthica* was sampled by boat with a ‘Van Veen’ grab (Fig. 1.3d). Samples were then sieved over 1 mm mesh size. In addition to the sampling on the Wadden Sea, *C. gigas* was also sampled in an estuary in the south of the Netherlands (Oosterschelde estuary) and on the coast near La Rochelle in France. At each sampling station and sampling date, water temperature and salinity were measured.

After samples were collected, they were transported to the laboratory and stored in a refrigerator at 5 ºC. During the following 48 hours, all individuals were processed. From each individual, shell length, shell height and shell width were measured with an electronic calliper. Live bivalves were opened and flesh was removed. Flesh mass was immediately weighed in a balance while the shell was left to air dry and weighed the day after.

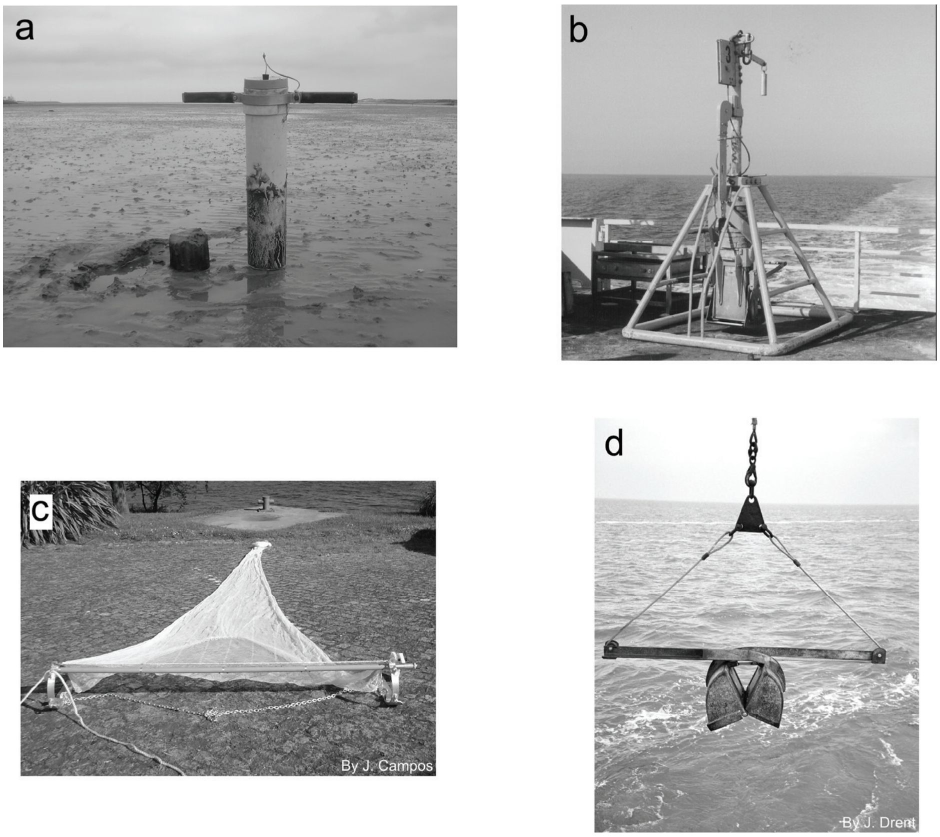

Fig. 1.3. Gear used to sample the different species: a) Hand core, b) Box corer, c) Beam-trawl and d) ‘Van Veen’ grab.

Reproductive tissue (gonad) was separated from the rest of the body tissue (soma) under a microscope and each part was put individually in ceramic pots. Tissues were then dried for 4 days in an oven at 60 ºC and incinerated for 4 h in a furnace at 560 ºC. After drying and incinerating, each pot was weighed to the nearest 0.01 mg. The ash-free dry mass (AFDM) of somatic and gonadal tissues was then determined by subtracting the ash weight from the dry weight. This AFDM was used to analyse differences in body condition and reproductive investment along the year among species and, within one species in different sampling locations.

The field data collected was used not only to test the predictions of the DEB model simulations with field data, but also to gather information on the life history strategies of the different species in relation to their current status in the Wadden Sea.

The DEB model

DEB model structure

The DEB theory is built on dynamic systems and quantifies the energy flow through an individual during its life-time. Key processes are feeding, digestion, storage, maintenance, growth, development, reproduction and ageing. The quantitative aspects of energy budgets were taken to follow the rules as specified by the Dynamic Energy Budget (DEB) theory (Kooijman 2000). The DEB model describes the energy flow through an animal (Fig. 1.4) and the changes in this flow in environments in which food densities and temperatures vary. The DEB model distinguishes three life stages: embryos, which neither feed nor reproduce; juveniles, which feed but do not reproduce; and adults which both feed and reproduce. In addition, also three main body fractions are considered: structural biovolume, or somatic tissue; stored reserves; and gonads, or stored energy reserves allocated to reproduction. The contribution of these body fractions to total biovolume changes with time. Storage materials (such as glycogen in bivalves) are continuously used and replenished while structural materials (such as proteins) are continuously degraded and reconstructed. The chemical composition of each of these fractions is taken to remain constant (homeostasis).

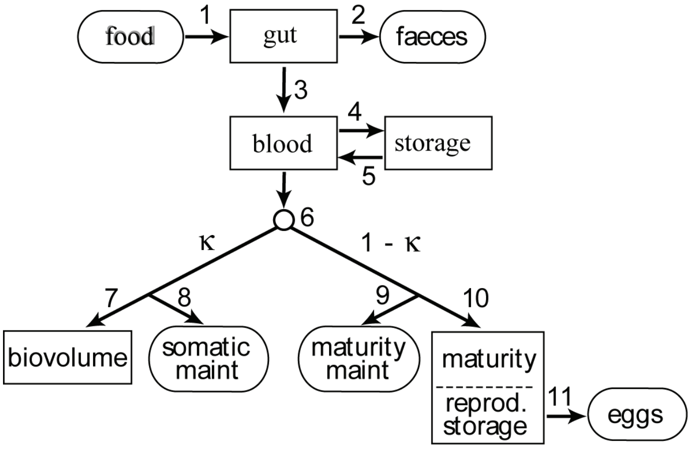

Fig. 1.4. Energy flow through an organism in the DEB model, after Van Haren (1995). Rates: 1 ingestion (uptake), 2 defecation, 3 assimilation, 4 demobilisation of energy into reserves, 5 mobilisation of energy from reserves, 6 utilisation, 7 growth, 8 somatic maintenance, 9 maturation maintenance, 10 maturation, 11 reproduction. The rounded boxes indicate sources or sinks; the rectangles indicate state variables.

The state of a system at a given moment is described by state variables. The DEB theory characterises an individual using two state variables: structure, quantified as body volume, and energy reserves, quantified as reserve density. State variables change with time and can be described by a set of differential equations which have parameters (Table 1.1). The notation and symbols used here are the same as in Kooijman (2000):

1. variables are indicated by symbols and lower case symbols frequently relate to upper case ones via scaling;
2. quantities are expressed per unit of volume with square brackets [ ]; per unit of biosurface area with braces { }; and per unit of mass with angles < >;
3. rates have dots, indicating the dimension per time.

Table 1.1. Parameter set of primary and compound parameters of the DEB model. Notation after Kooijman (2000).

Symbol	Dimension	Interpretation
Primary parameters		
T_A	K	Arrhenius temperature
$\{\dot{J}_{Xm}\}$	J cm^{-2} d^{-1}	Maximum surface area-specific ingestion rate
ρ	—	Losses due to digestion
$\{\dot{p}_{Am}\}$	J cm^{-2} d^{-1}	Maximum surface area-specific assimilation rate
$[\dot{p}_M]$	J cm^{-3} d^{-1}	Volume-specific maintenance costs
$[E_m]$	J cm^{-3}	Maximum storage density
$[E_G]$	J cm^{-3}	Volume-specific costs of growth
$[E_V]$	J cm^{-3}	Volume-specific structural energy content
κ	—	Fraction of utilized energy spent on maintenance plus growth
δ_m	—	Shape coefficient
Compound parameters		
$\dot{v} = \{\dot{p}_{Am}\}/[E_m]$	cm d^{-1}	Energy conductance
$\dot{k}_M = [\dot{p}_M]/[E_G]$	d^{-1}	Maintenance rate constant
$g = [E_G]/\kappa[E_m]$	—	Investment ratio
$\dot{r}_B = \left(3/\dot{k}_M + 3fV_m^{1/3}/\dot{v}\right)^{-1}$	y^{-1}	Von Bertalanffy growth rate

Parameters are constants that are assumed to have a fixed value but may change with time. For example, parameters related to physiological rates (growth, filtration, respiration) depend on temperature and therefore, remain constant as long as temperature does not change. If temperature changes, then parameters change as well. With this respect, it is assumed that temperature affects all physiological rates in the same way, and a species-specific parameter (the Arrhenius temperature) is used to correct physiological rates measured at different temperatures (Box 1). Intraspecific variability is caused by differences in state variables not in parameter values and in contrast, interspecific variability is the result of differences in parameter values. The elegance of the DEB model is the limited number of parameters which can fully describe the state of an organism.

Box 1 - The Arrhenius temperature

Temperature has an effect on all physiological rates. Each species can only tolerate temperature within a specific temperature range. Within part of the temperature tolerance range (i.e. the optimal temperature range), rates increase exponentially with increasing temperature. Outside the optimal temperature range, both at low and high temperatures, rates are reduced. In the DEB theory, the description proposed by Arrhenius describes the effect of temperature on physiological rates with acceptable accuracy within the temperature tolerance range of a species. The Arrhenius relationship is described by the equation

$$\dot{k}(T) = \dot{k}(T_1)e^{\left\{ {T_A}/{T_1} - {T_A}/{T} \right\}}$$

in which $\dot{k}$ is a physiological rate, T is the ambient temperature (in Kelvin), T1 is a chosen reference temperature (in this thesis all model simulations were done at 20 °C, that is 293 K) and T_A is the Arrhenius temperature. This relationship is used to correct different physiological processes for differences in temperature. By plotting $\ln \dot{k}$ against T^{-1}, the result is a straight line with slope T_A. The Arrhenius temperature is species-specific, which means that within one species, the relationship between different physiological rates and temperature is the same.

The DEB model is based on a set of general assumptions regarding food uptake, storage and utilisation (Table 1.2). Food uptake is assumed to be proportional to the organisms' surface area and to follow a type II functional response curve (Holling 1959), in which the intake rate depends hyperbolically on the food density in the environment (Box 2). Food is assumed to enter first a reserve pool and later mobilized reserve is allocated to growth, to somatic and maturation maintenance and to maturation or reproduction. That is, a fixed fraction (κ) of utilised reserve is allocated to growth plus somatic maintenance while the remainder fraction (1-κ) is allocated to maturity maintenance plus maturation or reproduction. This rule is called ‘κ-rule’. The different life stages utilize energy in a different way. Juveniles have to mature and become more complex (i.e. develop new organs and regulation systems) while adults do not increase in complexity but reproduce. Therefore, the energy spent on development in juveniles is spent on reproduction in adults. If conditions are poor, reproduction is blocked and all energy is allocated to growth and somatic maintenance. However, maintenance has priority over growth, and hence growth ceases when all reserve that is allocated to somatic maintenance plus growth is required for somatic maintenance.

Box 2 - The functional response

Functional responses describe the relationship between consumption rate of an individual and food density. In the DEB model, the link between food conditions in the field and ingestion rate $\left(\dot{J}_X\right)$ as a function of food density is described by a hyperbolic functional response (Kooijman 2000):

$$\dot{J}_X = \left\{\dot{J}_{Xm}\right\} f V^{2/3}$$

where $\left\{\dot{J}_{Xm}\right\}$ is the maximum ingestion rate per unit of surface area; $V^{2/3}$ is the body surface area and f is the scaled functional response. This response curve corresponds with the type II response curve as proposed by Holling (1959). The type II functional response assumes that a predator spends its time either searching for a prey or handling a prey. Handling includes chasing, killing, eating and digesting. In this type of functional response, ingestion (consumption) rate increases with food density at a decelerating rate, until a maximum is reached above which ingestion rate remains constant (Fig. 1.5). The initial slope of the function is given by the searching rate while the asymptote is given by the reciprocal of the handling time. In bivalves, filtration rate decreases with increasing food concentration, leading to a constant ingestion rate because, at a certain moment in time, ingestion rate cannot increase further due to the time necessary for food digestion (Fig. 1.5). Values of the scaled functional response vary between 0 (starvation) and 1 (optimal, food *ad libitum*).

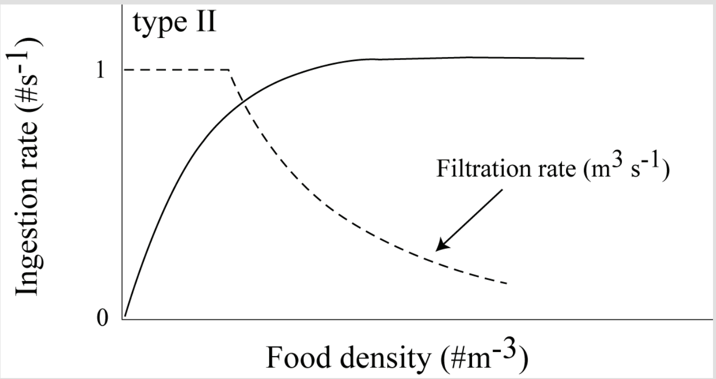

Fig. 1.5. Schematic representation of the type II functional response proposed by Holling (1959) about the relationship between food density and food ingestion (solid line) or filtration in bivalves (striped line).

Maintenance is defined as the energy requirement of an organism, excluding the energy necessary for growth of structural mass, reproduction and development. Maintenance costs are species-specific and depend on the size of the organism and on body temperature (Box 3).

A more detailed description of the DEB model can be found in Kooijman (2000).

Table 1.2. The basic assumptions of the DEB model (from Van der Meer 2006, following Kooijman 2000).

a. An organism is characterised by a structural body and a reserve density (i.e. amount of reserves per amount of structural body). The chemical composition of both structural body and reserves is constant, which is called the assumption of strong homeostasis.

b. Each organism starts its life as an embryo (which does not feed and does not reproduce). When the embryo has reached a certain degree of maturation, it changes into a juvenile (which feeds, but does not reproduce). Similarly, a juvenile changes into an adult (which feeds and reproduces) when it exceeds a given threshold value.

c. Ingestion is proportional to the surface area of the organism and depends upon food density by a Holling type II functional response. Recall that embryos do not feed.

d. A fixed fraction of the ingested food is assimilated and enters a storage pool, which is characterised by the reserve density.

e. The regulation of the reserve density follows a first-order process.

f. A fixed fraction κ of the utilisation rate goes to somatic maintenance, heating (for endotherms) and growth of the structural body (with a priority for maintenance), and the rest goes to maturity maintenance and (for embryos and juveniles) maturity or (for adults) reproduction.

g. Maintenance rate is proportional to structural volume and heating rate is proportional to the surface area of the organism.

Estimation of parameters and DEB model simulations

Applying the DEB model requires the estimation of a set of parameters for the various species according to a standard procedure. For the estimation of parameters, published data on the various species was collected and, when data sets were lacking, a newly established standard procedure was used to determine missing parameters.

For each species, the DEB model can be used for: 1) the simulation of growth in the field if food and temperature conditions are known, 2) the reconstruction of the food level in the field if temperature conditions and growth are known, or 3) the reconstruction of temperature conditions in the field if food conditions and growth are known. In this thesis, DEB model simulations were done first to simulate annual growth by using the prevailing (current) temperature conditions at different scenarios of food level and compared to field results. Second, the DEB model was used to reconstruct the seasonal pattern in food level for the various species in the different habitats. Food conditions in the field were reconstructed by back-calculating food from observed growth in the field at the current temperature conditions.

Finally, the existence of food competition between species was analysed by comparing the estimates of the back-calculated food conditions for the various species.

Box 3 - Maintenance

In the DEB theory, maintenance of both somatic and gonadal tissues is proportional to body volume. Maintenance costs are species-specific and depend on the size of the organism. This is because a cell is a volume that uses a fixed amount of energy per unit of time for maintenance, i.e. to maintain its structure (or volume). In this way, a larger bivalve (with more cells) needs more energy to maintain its cells than a smaller one and the required energy is proportional to the amount of cells (volume) it needs to maintain. Maintenance costs $(\dot{p}_M)$ are described by:

$$\dot{p}_M = [\dot{p}_M] * V$$

where $[\dot{p}_M]$ is the volume-specific maintenance costs and V is the body volume.

This implies that the volume-specific maintenance costs are independent of volume. This is because the size or volume of a cell is related to its complexity, therefore, the amount of energy necessary to maintain a cell is the same regardless of the size of the individual. Since the complexity of cells is similar between related species such as bivalves, the volume-specific maintenance costs are also considered to be similar.

Outline of the thesis

This thesis is divided in a number of components: field data collection (Chapters 2 to 5), DEB model predictions for bivalves (Chapter 6), estimation of DEB parameters (Chapter 7), simulation exercises and combination of DEB model simulations and field data (Chapter 8) and a synthesis with a final discussion (Chapter 9).

Chapter 2

The blue mussel *Mytilus edulis* is one of the most common bivalves in the Wadden Sea. From mid 1980's to late 1990's, strong declines in mussel populations have been observed. Part of the decrease in mussel densities has been due to fishing activities for seed mussels. Although measures were taken to protect mussel beds, densities are still much lower than in the 1970's. The slow recovery of mussel beds in the intertidal of the western Dutch Wadden Sea suggests that factors involved in the survival of adults or recruitment success could be acting too. Water temperature is an important factor influencing various physiological processes in bivalves. In the Wadden Sea, lower body condition has been observed in several species after mild winters in comparison to severe winters. Therefore, the observed increase in water temperatures in the Dutch Wadden Sea suggests that an effect of temperature on the population dynamics of mussels might be expected. It is known that high winter temperatures enhance predation pressure on larvae and post-larvae. However, it is also possible that the increasing trend in water temperature during the last decades could have a negative effect on the reproductive output of mussels. In order to test whether reproductive failure could be the cause for the low recruitment of *M. edulis* in the western Dutch Wadden Sea, the reproductive investment of the mussel was studied during one year and a long-term data series on body condition was analysed to assess whether the decrease in recruitment success could be due to a negative impact of increasing temperatures on body condition and, hence on reproductive output.

Chapter 3

In the Dutch Wadden Sea estuary, the common cockle *Cerastoderma edule* and the soft-shell clam *Mya arenaria* are found in intertidal and subtidal areas. These species present large differences in maximum size and age: 15 cm and 28 years in *M. arenaria* vs. 5 cm and 10 years in *C. edule*. In the western Dutch Wadden Sea, recruitment success of *C. edule* is usually higher than in *M. arenaria*. These differences in recruitment success can result from a number of factors, starting with differences in reproductive output or differences in larval and post-larval processes (such as growth, pelagic stage duration and mortality). The fact that egg size is similar in these species (57 µm in *M. arenaria* vs. 65 µm in *C. edule*) suggests that larval size and pelagic stage duration will also be similar. Therefore, differences in larval processes between species are not likely to be responsible for the observed differences in recruitment. As a starting point, this chapter focuses on the reproductive investment of *C. edule* and *M. arenaria* in two habitats of the western Wadden Sea by analysing the timing of spawning and the reproductive output of each species. The aim was to find out if differences in reproductive investment between these two species could be the cause for the observed differences in recruitment success in the western Wadden Sea.

Chapter 4

The bivalve *Macoma balthica* is an abundant species in intertidal and subtidal areas of the Dutch Wadden Sea and adjacent coastal waters. However, from an energetic point of view, at least the environmental conditions for the intertidal population hardly seem favourable due to exposure to high temperatures during the summer and the fact that significant food intake can only occur during submersion. Therefore, it is possible that recruitment in the Wadden Sea originates mainly from the subtidal and/or offshore stocks. In order to evaluate the importance of the different habitats to the persistence of the *M. balthica* population in the western Dutch Wadden Sea, growth and reproductive output in various habitats (intertidal, subtidal, offshore) was analysed.

Chapter 5

The Pacific oyster *Crassostrea gigas* is an invading species of the Dutch Wadden Sea which has become abundant during the last years. Since its recent expansion seems to be related to an increase in mean water temperature, it is possible that the latitudinal decrease of temperature towards the poles might potentially determine the ultimate northern limit of the geographical distribution of *C. gigas*. As a first approach to analyse the physiological performance of this species along a latitudinal gradient, spatial and temporal variation in growth and reproductive output were studied in three *C. gigas* populations from France to the Netherlands. Focus was on the energy allocation to growth and reproduction of the various populations in relation to environmental conditions, with the aim of finding out how this species has been able to successfully invade and expand in the Dutch Wadden Sea.

Chapter 6

The DEB model predicts a set of body-size scaling relationships for the comparison of species. Body-size scaling relationships describe relationships between physiological variables (egg size, growth rate, size at settlement and maturation) and body size. The DEB model was used to analyse body-size scaling relationships that can be applied to predict species characteristics in case basic information is lacking. In addition, the discrepancies between DEB predictions based on energetic constraints and field observations were analysed to identify potentially important factors in the life history strategy of bivalves.

Chapter 7

The various DEB parameters were estimated for a number of bivalve species that are abundant in northern European waters, including the Dutch Wadden Sea. First, a brief overview was presented on how the various parameters can be estimated and what types of data sets are required. Next, a protocol was introduced on how to deal with missing estimates in case data sets are lacking and how to achieve consistency between various estimates. Finally, a complete and consistent set of DEB parameters was presented for various bivalve species.

Chapter 8

In chapter 8, the energy flow in the various bivalve species in different habitats of Dutch coastal waters was analysed by comparing growth observations in the field with model simulations by means of the DEB theory. The final objective was to reconstruct food conditions for the various species and habitats by applying the DEB model. For that, field data collected in the previous chapters were used, for the analysis of seasonal variation in somatic mass per age group for each species and location. In addition, the parameters estimated in chapter 7 were used to run the model, in combination with temperature and food conditions as input variables. At the end, DEB simulations were run to reconstruct food conditions for the various species at the various locations under the current water temperature conditions, in order to have an idea of the interactions between bivalve species in terms of food competition.

Chapter 9

In this chapter, the main conclusions from the previous chapters were summarized. Limitations for using the DEB model in relation to the quality of datasets available were discussed. Focus was put into the problem of age determination in bivalves and on the variability of species-specific parameters. In addition, suggestions for further research were presented.

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CHAPTER 2

Is reproductive failure responsible for reduced recruitment of intertidal *Mytilus edulis* L. in the western Dutch Wadden Sea?

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Abstract

The mussel *Mytilus edulis* is an abundant bivalve in the Dutch Wadden Sea, both in intertidal areas as well as in subtidal culture plots. From mid 1980's to late 1990's, strong declines in mussel intertidal populations have been observed, both in terms of occupied area and in biomass. Despite the efforts since 1999 to preserve intertidal mussel beds, abundance and biomass of mussels in the Dutch Wadden Sea are at present still much lower than in the 1970's. In the present paper, we tested whether reproductive failure could have been the cause for the low recruitment of *M. edulis* in the western Dutch Wadden Sea. Water temperature is an important factor influencing growth and reproduction in bivalves. The observed increase in mean temperature in the Dutch Wadden Sea during the last decades suggests that an effect of temperature on the population dynamics of mussels might be expected. Nevertheless, no significant relationship was found between body condition and mean winter temperature. Therefore, a decrease in body condition and, consequently, in reproductive output, due to increasing temperature, will not have been a cause for the observed low recruitment in recent years. The seasonal patterns of individual growth supported this view: most adults developed gonads during the spawning season suggesting that reproductive (gametogenic) failure was not a cause for the low recruitment. Nevertheless, reproductive investment by the mussel population studied was not optimal since complete spawning hardly occurred. In addition, the high gonadosomatic ratio at the end of the summer did not result in heavy spawning, suggesting resorption of gonadal tissue due to unfavourable environmental conditions (too high temperature and/or food limitation during summer). Reduced recruitment could be due to poor spawning but other factors that exert their influence after spawning (predation on larvae or postlarvae, lack of settlement substrate) or on the adult population (decrease in number of spawners) are also likely to be involved in the observed low recruitment during the last years.

Introduction

The blue mussel *Mytilus edulis* is one of the most common inhabitants of the Dutch Wadden Sea, where it occurs in natural (wild) beds in intertidal and subtidal areas. Mussel beds have an important role in the ecology of the Wadden Sea, since they serve as a habitat and food source for various species (De Jonge et al. 1993, Dankers et al. 1999, De Vlas et al. 2005). The large filter-feeding activity of mussels results in the deposition of large amounts of suspended matter in the form of faeces and pseudofaeces (Dankers et al. 1999). The breakdown of organic matter produces ammonia and silicates which may stimulate primary production (Asmus and Asmus 1991) while pseudofaeces provide a food source for various species, such as deposit-feeding worms (Dankers and Koelemaij 1989, Dankers et al. 1999). In addition, mussels provide hard substrate for species such as barnacles and macroalgae, and are an important food source for a variety of shorebirds and ducks (Zwarts 1991, Ens et al. 1993, Nehls et al. 1997). From the 1950's onwards, commercial mussel plots were introduced in subtidal parts of the western Dutch Wadden Sea. Initially, this was done to avoid the impact of the mussel parasite *Mytilicola intestinalis* on mussel stocks, but in the late 1950's, commercial plots were laid as a compensation for the loss of cultivating area in the Delta waters in the south-western part of The Netherlands (Dankers et al. 1989, Van der Veer 1989). In the early years the total area available for culture steadily increased from about 10 km^2 in 1951 to about 70 km^2 in 1960 and remained stable since then (Van der Veer 1989, Dankers and Zuidema 1995). The so-called seed mussels (spat of about 1 year old) are dredged from wild beds and spread on the subtidal culture plots, where they can reach a commercial size in about one year (Dankers and Zuidema 1995).

In the Wadden Sea, mussel beds have been studied for a long time and until now (Kuenen 1942, Maas Geesteranus 1942, Verweij 1952, Havinga 1960, Beukema 1976, Asmus 1987, Dekker 1989, Asmus and Asmus 1991, Dankers et al. 1999, Buschbaum and Saier 2001, Munch-Petersen and Kristensen 2001, Herlyn and Millat 2002). From mid 1980's to late 1990's, strong declines in mussel intertidal populations have been observed, both in terms of occupied area and in biomass (Dankers et al. 1989, Dijkema et al. 1989, Herlyn and Michaelis 1996, Kristensen 1996, Herlyn and Millat 1997, Dankers et al. 1999). Winter storms, predation and intensive fisheries for spat may have had a negative impact on mussel beds (for an overview see Dankers et al. 2004). In addition, poor spatfall and unsuccessful recruitment could also have contributed to population decline. Since mussel larvae tend to settle where mature beds used to occur or even on top of existing mussel beds (De Blok and Geelen 1958, Dankers et al. 1999), measures were taken in 1999 to preserve intertidal mussel beds in the Dutch Wadden Sea. As a result, the surface area and biomass of intertidal mussel beds increased since 2001, although values are still much lower than in the 1970's (Dankers et al. 2003, Steenbergen et al. 2003, Dankers et al. 2004, Steenbergen et al. 2004). This suggests that at least part of the decrease in mussel densities has been due to fishing activities for spat.

However, the slow and restricted recovery of mussel beds in the intertidal points to the idea that other factors involved in the survival of adults or recruitment success might be acting too.

Already in the 1940's, food limitation for mussels in the intertidal of the Dutch Wadden Sea was suggested by Kuenen (1942) and, subsequently, more detailed studies have supported this suggestion (Asmus et al. 1990, Asmus and Asmus 1991, Kamermans 1993, Beukema and Cadée 1997, Beukema et al. 2002, Van de Koppel et al. 2005). Under food limitation, unfavourable environmental conditions will have a strong impact on the energy available for the various physiological processes, such as growth and reproduction. The observed increase in water temperatures in the area (Van Aken 2003; http://www.nioz.nl, go to Research, Scientific Departments, Physical Oceanography, Ferry and Jetty Observations) suggests that an effect of temperature on the population dynamics of mussels might be expected. In this respect, Beukema & Dekker (2005) and Philippart et al. (2003) concluded that the recruitment trends of bivalve species in the Wadden Sea are governed primarily by natural processes, especially by temperature-mediated predation processes. For several bivalve species, the decrease in recruitment during the last decades seems to be due to an increase in predation pressure, which is enhanced by high winter temperatures. In *M. edulis*, such mechanisms have also been reported (McGrorty et al. 1990, Beukema 1982, 1992, Young et al. 1996).

Nevertheless, this does not exclude an impact by temperature-mediated physiological processes. An effect of temperature on body mass and egg production has been observed in bivalve species of the Dutch Wadden Sea. Not only loss of body mass is generally higher after mild than after severe winters (Honkoop and Beukema 1997) but also a positive relationship between body mass indices and egg numbers has been observed (Honkoop and Van der Meer 1998). In *M. edulis*, a lower body condition has been observed after mild winters (Honkoop and Beukema 1997), suggesting that the increasing temperature trend during the last decades might also have a negative effect on its reproductive output.

In the present paper, we aim to test whether reproductive failure could be a cause for the low recruitment of *M. edulis* in the western Dutch Wadden Sea. For that, we followed the approach of Honkoop et al. (1998), in which egg production, used as a measure of reproductive investment, was calculated from body condition in spring. In this study, we used a long-term data series from the intertidal western Wadden Sea (Balgzand area) in late winter (see Beukema and Dekker 2005). In case reproductive failure is occurring, we expect a decrease in mean body condition of *M. edulis* as a result of the increase in mean winter temperature. In addition, the seasonal pattern in gonadal mass was followed to check [1] whether body condition in late winter was a good indicator of the reproductive investment in *M. edulis*; and [2] to analyse the variability in reproductive investment and assess whether reproductive failure occurred in individual mussels. Reproductive failure is analysed here in terms of failure in gametogenesis.

Materials and Methods

Growth and reproductive investment

From September 2001 to October 2002, *M. edulis* were collected, if possible every month, on a wild mussel bed in the intertidal Wadden Sea (52° 55’ N and 4° 48’ E; Fig. 2.1). This site is emerged for an average of 4 h per tidal cycle and the sediment has a silt content of about 17.5%. At each sampling, around 100 individuals were collected randomly by hand at low water, transported to the laboratory within a few hours, stored in seawater at 5 ºC and processed within the next 48 hours.

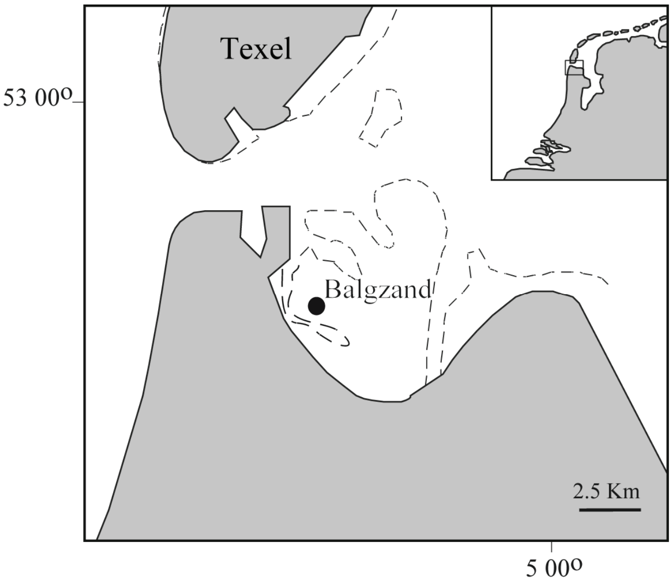

Fig. 2.1. Sampling location of *M. edulis* on the intertidal flats of Balgzand in the western Dutch Wadden Sea. Striped lines indicate the limits of tidal flats.

Each individual shell was weighed to the nearest 0.01 g and its length was measured to the nearest 0.01 mm with electronic callipers. Age was estimated by counting the shell’s external growth rings (for more details see discussion). All bivalves were opened and flesh was removed. Reproductive tissue (gonadal mass) was separated from the rest of the body mass under a microscope. Ash-free dry mass (AFDM) of each part was determined to the nearest 0.01 mg. Body condition was determined by calculating the Body Mass Index (BMI), expressed as the total body AFDM (soma + gonads) divided by shell length^3. The investment in somatic and gonadal mass was determined by estimating the Somatic Mass Index (SMI) and the Gonadal Mass Index (GMI). SMI (mg cm^{-3}) was estimated as the AFDM of the soma divided by shell length^3 and GMI (mg cm^{-3}) was expressed as the gonadal AFDM divided by shell length^3. The relative investment in reproduction (reproductive effort) was determined by calculating the Gonadosomatic Ratio (GSR), described as the gonadal AFDM divided by the total body AFDM.

By dividing mass by cubic shell length, animals of different size could be compared in terms of condition. The extent to which variability in condition could be accounted for by seasonal variability and by differences among age classes was examined by using analysis of variance ANOVA. A sinus function was chosen to represent the seasonal variability in mass along the year. For that, a linear trend over time was used in combination with a sinusoidal seasonal effect. The overall time effect looked, therefore, like

$$\beta_1\, Time + \beta_2 \sin(\, 2\pi\, ((Month - \beta_3)/\, 12)),$$

in which β_1, β_2, and β_3 are parameters, *Time* is a continuous variable that runs from the first day of observation till the last day, and *Month* is a continuous variable that runs from the first month of observation till the last month. Note that this model is only a linear model when β_3 is known beforehand. For that, we ran this linear model for all 12 possible values of β_3 (i.e. the values 1 to 12) and selected the model with the lowest residual mean squares. Subsequently, the selected model was used to correct somatic and gonadal mass indices for seasonal and age differences. In order to obtain Normality, GMI data were transformed using the squared root transformation and therefore, only individuals that contained gonadal mass were included in the estimation of this index.

For the analysis of growth rates, shell length (mm), total body mass (mg AFDM), somatic (mg AFDM) and gonadal (mg AFDM) masses were plotted against age (in years). Von Bertalanffy growth (VBG) curves were fitted to shell length-at-age curves according to the expression:

$$L_t = L_\infty * (1 - e^{-k*t})$$

where L_∞ is the estimated maximum length (mm), k is the growth rate constant (d^{-1}), t is the age (days) and L_t is the observed length at age t. VBG parameters L_∞ and k were iteratively estimated.

All statistical analyses were made using the software package SYSTAT (Wilkinson 1996).

Long-term trends in body condition

Long-term data on average total body masses per length class in winter (February/March) were available for the intertidal western Dutch Wadden Sea (Balgzand area) from the early 1970's till now. For information on sampling methods see Beukema et al. (2001). From these data series, mean body mass index (see above) was determined per year. Long-term trends in body condition were then analysed and related to water temperature data. Temperature data were obtained from the long-term series from the Marsdiep (via http://www.nioz.nl, go to

Research, Scientific Departments, Physical Oceanography, Ferry and Jetty Observations; Van Aken pers. com. 2006)

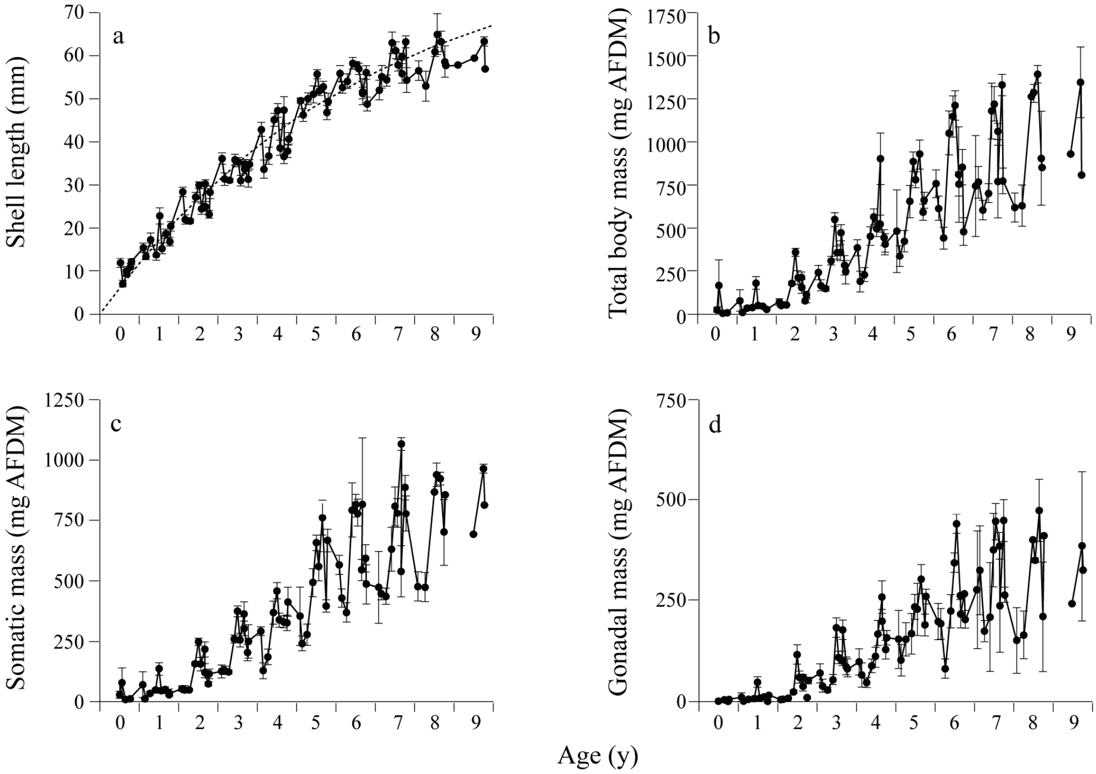

Fig. 2.2. a) Shell length (mm, mean±SE), b) total body mass (mg AFDM, mean±SE) and c) somatic (mg AFDM, mean±SE) and d) gonadal mass (mg AFDM, mean±SE) of *M. edulis* at Balgzand plotted against age (years). Von Bertalanffy growth (VBG) curve was fitted for shell length-at-age data (a). The transition between two age groups is considered to be on the 1st of January.

Results

Growth and seasonal patterns

Fig. 2.2 shows shell length, total body mass, somatic mass, and gonadal mass for each age class along the studied year. In the studied area, maximum age and shell length observed were respectively 9 years and around 70 mm, while maximum observed total body, somatic and gonadal masses (considering all individuals sampled) were about 2160 mg AFDM, 1130 mg AFDM and 760 mg AFDM, respectively (Fig. 2.2). For all age classes, growth in shell length occurs generally in spring (Fig. 2.2a). The sometimes observed decrease in shell length of a certain age class, between successive sampling occasions, will have been due to sampling errors. A general pattern of periods of growth in mass was alternated with periods of decrease in mean values (Fig. 2.2b,c,d). Growth in somatic and gonadal mass occurs in spring and beginning of summer, after which there is a decrease (Fig. 2.2c,d). Large variability was seen in individual total body mass, somatic and gonadal mass. The estimated maximum size was about 86.4 mm shell length ($k = 0.409*10^{-3}$, n = 735, $r^2 = 0.90$).

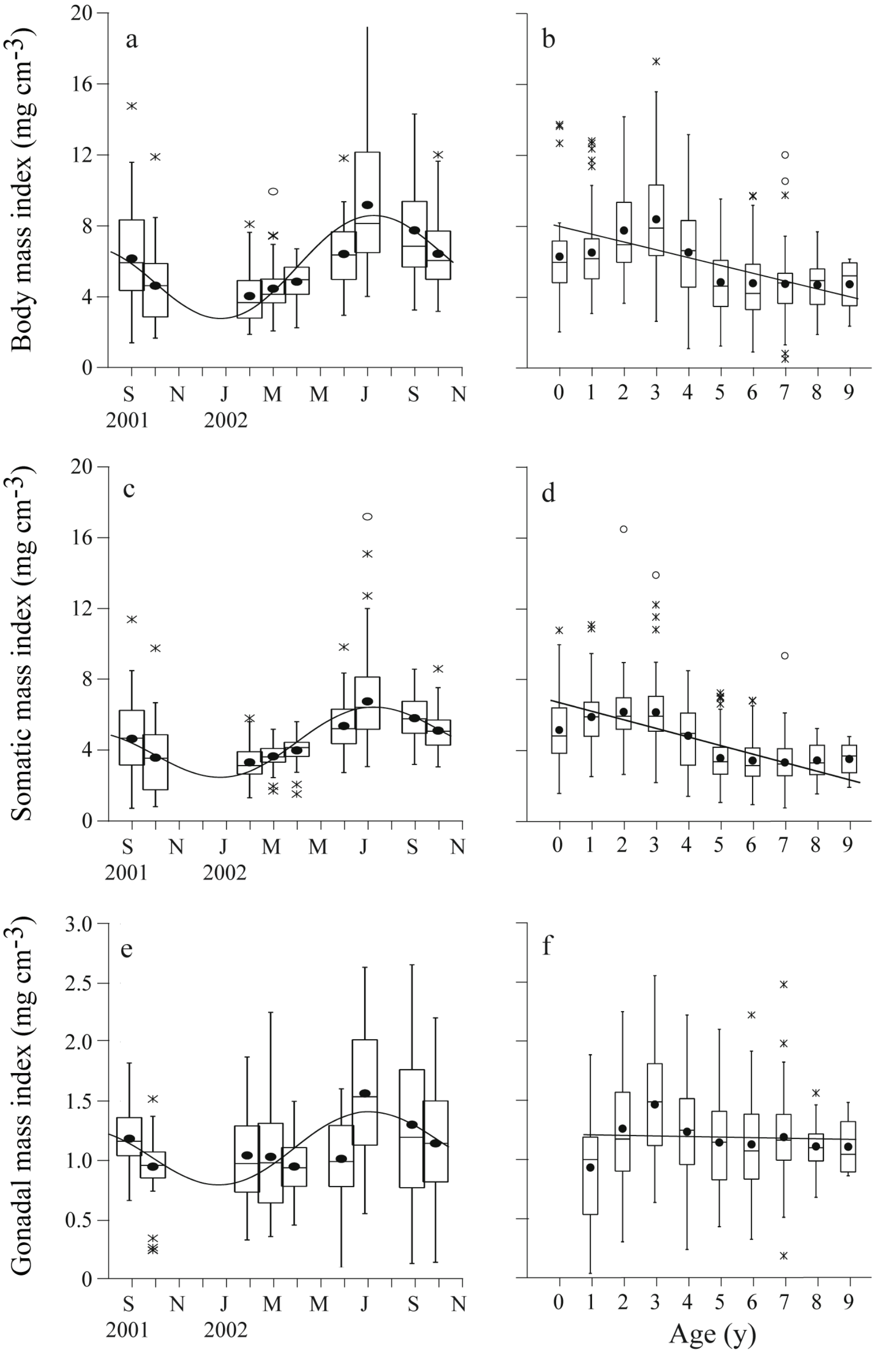

Fig. 2.3. a, b) Body mass index (mg cm^{-3}); c, d) somatic mass index (mg cm^{-3}); and e, f) gonadal mass index (mg cm^{-3}) of *M. edulis* at Balgzand along the year (left panel) and between different age groups (right panel). Values of gonadal mass index were square root transformed and only animals which developed gonads were included. Curves and lines are model predictions.

In all analyses sexes are treated together, as no differences in body mass index (BMI), somatic mass index (SMI) and gonadal mass index (GMI) were found between them (ANOVA, BMI: $F_{(1,292)} = 2.171$, $p = 0.142$, $r^2 = 0.55$; SMI: $F_{(3,294)} = 2.115$, $p = 0.147$, $r^2 = 0.62$; GMI: $F_{(3,295)} = 1.381$, $p = 0.241$, $r^2 = 0.25$). A clear seasonal pattern was seen in body, somatic and gonadal mass indices (Fig. 2.3). Season and age affect significantly BMI, SMI and GMI (ANOVA, BMI: $F_{(3,641)} = 116.494$, $p = 0.000$, $r^2 = 0.35$; SMI: $F_{(3,644)} = 165.117$, $p = 0.000$, $r^2 = 0.44$; GMI: $F_{(3,572)} = 27.294$, $p = 0.000$, $r^2 = 0.13$). For BMI and SMI, this is due to significant effect of season (ANOVA, t-statistic, BMI: $t = -13.384$, $n = 645$, $p = 0.000$; SMI: $t = -13.289$, $n = 648$, $p = 0.000$; 2-tailed) and age (ANOVA, t-statistic, BMI: $t = -8.948$, $n = 645$, $p = 0.000$; SMI: $t = -14.795$, $n = 648$, $p = 0.000$; 2-tailed). However, significant differences in GMI were found along the year (ANOVA, t-statistic, $t = 7.271$, $n = 576$, $p = 0.000$; 2-tailed) but not between age groups (ANOVA, t-statistic, $t = -0.526$, $n = 576$, $p = 0.599$; 2-tailed). A peak in body and somatic mass index occurred in July while the period with lowest mass was between December and February (Fig. 2.3a,c). Gonadal mass index also showed maximum values around July and low values in the beginning of the year (Fig. 2.3e). According to the reading of external shell marks, the oldest age found at the studied location was 9 years old. Individuals of all age groups, from 0-group to maximum age were present. BMI and SMI showed an increase until an age of 3 years old, decreasing from 3 to 5 years old and being more or less constant from then on (Fig. 2.3b,d).

The smallest individual that developed gonads was 14.6 mm shell length and 1 year old. From the decrease in GMI in 2002, spawning seemed to occur between July and September. However, a small decrease in GMI was also observed in April (Fig. 2.3e). The gonadosomatic ratio (GSR), used as a measure of reproductive investment, also reached a maximum mean value in July, where total body mass consisted of about 27% of gonads (Fig. 2.4). Another smaller peak of about 21% GSR occurred in April, suggesting the occurrence of two spawning periods.

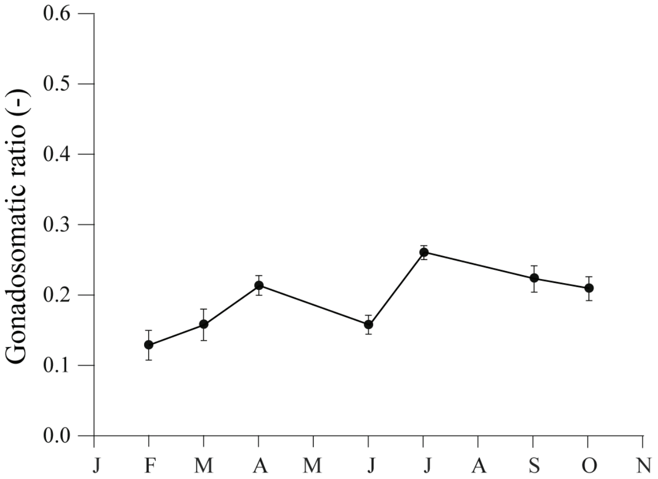

Fig. 2.4. Gonadosomatic ratio (-) of *M. edulis* at Balgzand in 2002. Bars are 95% confidence intervals.

Trends in body condition

The body mass index in winter (February and March) showed a significantly positive relationship with the gonadosomatic ratio (ANOVA, February: $F_{(1,27)} = 26.988$, $p = 0.000$, $r^2 = 0.52$, March: $F_{(1,37)} = 7.578$, $p = 0.009$, $r^2 = 0.18$) (Fig. 2.5). This means that, for these months, GMI can be estimated from data on body condition with reasonable accuracy. Long-term trends of mean body condition in late winter (February/March), before the spawning season, and of mean winter (January-March) water temperature, from the late 1960's until 2006, are shown in Fig. 2.6. Body condition did not show a significant trend along the years (Spearman rank correlation test: $r_s = 0.137$, n = 37, $p = 0.412$, 1-tailed; Fig. 2.6a) while winter temperature (January- March) showed a significant increase (ANOVA, $F_{(1,36)} = 4.585$, $p = 0.039$, $r^2 = 0.11$; Fig. 2.6b).

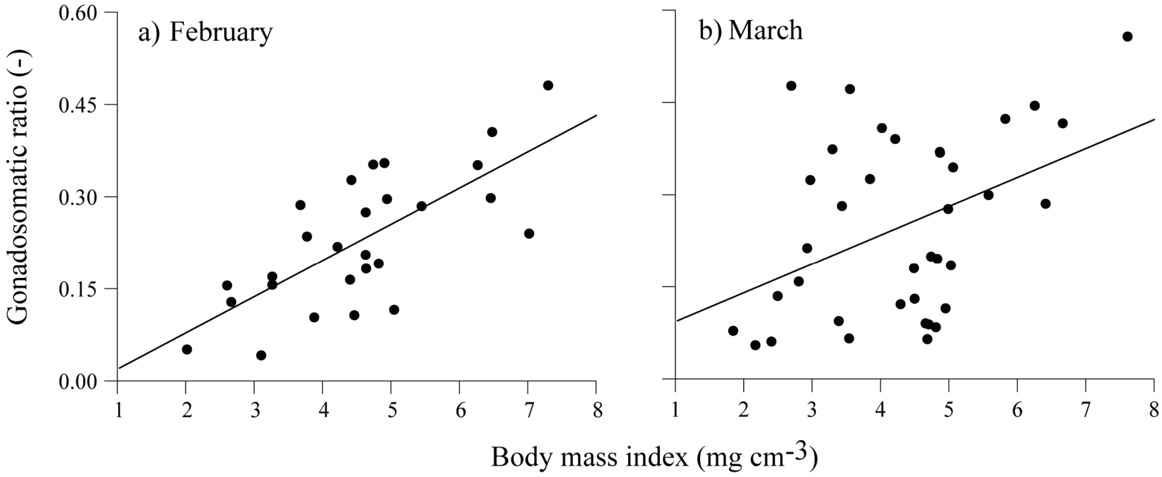

Fig. 2.5: Relationship between gonadosomatic ratio (-) and body mass index (mg cm^{-3}) of individual *M. edulis* at Balgzand in a) February and b) March.

Although a slight negative correlation was found between BMI and temperature (Fig. 2.6c), it was not significant (Spearman rank correlation test: $r_s = -0.047$, n = 37, $p = 0.785$, 1-tailed). However, two periods of different trends in temperature and body mass could be seen. Until the late 1980's mean winter temperatures were low (average 1969-1989 was 3.3 ºC) and body mass index significantly decreased ($r_s = -0.514$, n = 21, $p = 0.021$, 1-tailed), while after that period, mean winter temperatures were higher (average 1989-2006 was 4.5 ºC) and body mass index significantly increased ($r_s = 0.650$, n = 16, $p = 0.012$, 1-tailed).

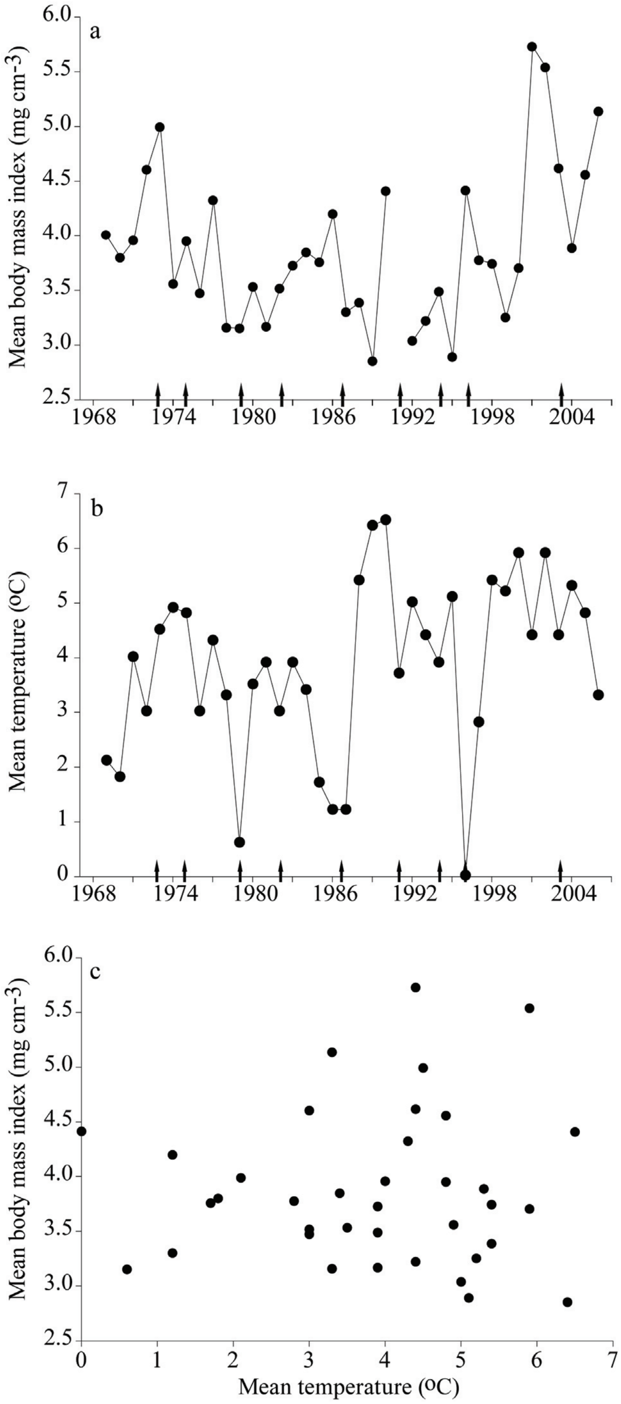

Fig. 2.6. a) Long-term series of mean body mass index (mg cm^{-3}) of *M. edulis* at Balgzand in late winter (February-March); b) Long-term series of mean winter water temperature (January-March, °C) the western Dutch Wadden Sea; c) Mean winter temperature (°C) plotted against mean body mass index (mg cm^{-3}) of *M. edulis*. Arrows show years with good mussel spatfall at Balgzand (densities of six months old individuals higher than 20 ind. m^{-2}). Temperature data were obtained via http://www.nioz.nl (go to Research, Scientific Departments, Physical Oceanography, Ferry and Jetty Observations) and Van Aken (pers. comm. 2006).

Discussion

Growth and seasonal patterns

Age determination was done by counting the external winter growth marks, a method validated by Lutz (1976) using shell sections, and used recently by Cusson and Bourget (2005) and by Ozernyuk and Zotin (2006) to study growth in *Mytilus edulis*. The use of external growth rings for age estimations has been under debate. Growth rings are usually seen on the shell surface of mussels but whether such rings are annual is dependent mostly on the seasonal variation in water temperature. Mussels from the English North Sea coast have clearly shown annual growth marks that are related to the absence of growth during winter (Seed 1969a). In the western Wadden Sea, growth of bivalves also stops during winter (Lammens 1967, Pieters et al. 1979, Beukema et al. 1985). Therefore, visible annual growth rings are expected to be seen in mussels from this area. A maximum age of 9 years old was found in the present study, which is in line with the information that the studied mussel bed originated from the spatfall of 1992 (“Bank 101” described in Dankers et al. 2004). However, it should be kept in mind that external counts might be biased because disturbance (stress) marks are not always visually distinguishable from annual growth marks. This will mainly occur in old individuals, since growth marks are very close to each other and difficult to visualize. As a result, an overestimation of age and underestimation of the growth rate is likely to occur. Shell erosion, which occurs mainly in old individuals, may also lead to errors in age determination. On the other hand, unclear marks may have been overlooked.

The seasonal trend in somatic mass index followed similar patterns as described before for other bivalves from the Wadden Sea area (Cardoso et al. 2007, Cardoso et al. in press, Zwarts 1991, Chapters 3, 4 and 5). In general, body condition tends to increase from early spring to mid summer. In the mussel *M. edulis*, growth in terms of body mass started in February, earlier than observed by Honkoop and Beukema (1997) some years before at the same location. Body and somatic mass indices increased from January until July, which coincided with the increase in water temperature from about 5 °C in January to 20 °C in July (not shown). Gonadal mass index also reached a peak in July suggesting that the main spawning peak occurred shortly after this time. However, the peak settlement of spat occurs usually at around June (De Vooys 1999), leading to the appearance of young mussels by the end of June and beginning of July (R. Dekker pers. obs.). Spat originating from late summer is usually not abundant in the Dutch Wadden Sea (Dankers pers. comm. 2006). Although the largest decrease in GMI and gonadosomatic ratio occurs at this time, the contribution to recruitment is low and, therefore, part of the decrease in gonadal mass between July and September could be due to a general decrease in body condition at the end of the summer. This suggests that the main spawning event of mussels in the Wadden Sea occurred between April and June. These results support the observations of De Vooijs (1999), in which a major larval peak was observed in May, followed by smaller peaks along the summer.

Factors affecting mussel recruitment

Several factors can be involved in the decreasing trend in mussel recruitment (as shown in Fig. 1c of Beukema et al. 2001). The decrease in eutrophication due to the cleaning-up of the river Rhine, suggested by the decrease in phytoplankton primary production and chlorophyll-a (Cadée and Hegeman 1993, Cadée and Hegeman 2002) could lead to a decrease in macrozoobenthic biomass and eventually to a decrease of spawners. In addition, periods with low salinity due to freshwater input in the Wadden Sea originating from the IJssel Lake (Anon. 2005), can lead to problems in fertilization and larval development (Bayne 1965) as well as low growth (Remane and Schlieper 1971) in mussels.

Food availability is the most important factor controlling growth in bivalves. By the analysis of growth curves in somatic and gonadal mass, it seems that conditions for individual growth in the western Wadden Sea are rather good. Maximum observed length in this study was around 70 mm, at an age of 9 years old. Smaller maximum sizes, between 50-60 mm shell length, were found in intertidal mussels from the northern German Wadden, the Danish Wadden Sea and the English North Sea coast (Seed 1969b, Buschbaum and Saier 2001, Munch-Petersen and Kristensen 2001). In addition, recruitment has also been occurring every year, since all age classes from the last 9 years are present in the samples. Therefore, an effect of food quantity/quality on individual growth and reproductive investment does not seem a probable explanation for reduced recruitment.

The expansion of the invading oyster species *Crassostrea gigas* (Diederich et al. 2005, Diederich 2006), which, unlike the mussel, benefits from warm summers (Diederich et al. 2005), could also be responsible for decrease in recruitment. This species settles on former mussel beds and forms massive reefs which seem to be more resistant to environmental stress than mussel beds (Diederich 2006). The large filtering capacity of oysters (Gerdes 1983, Dupuy et al. 2000, Bougrier et al. 1995) also suggests that they may filter larvae from other bivalve species. In large oyster reefs, this may have a negative impact on recruitment of nearby bivalve populations. Therefore, the fast growth and reproductive output of the oysters (Cardoso et al. 2007a, Diederich 2006) may be a competitive disadvantage to mussels, especially if food and/or space are limiting.

Up to now, evidence of an effect of winter temperature on recruitment has been observed for several species (Beukema 1982, 1992; Strasser et al. 2001, Strasser 2002, Philippart et al. 2003, Nehls 2006). Mild winters seem to enhance the presence of predators, such as shrimps and crabs, which feed on newly settled larvae, leading to low recruitment success (Beukema 1991, Strasser and Gunther 2001, Strasser 2002, Beukema and Dekker 2005). Besides the high numbers of predators during mild winters, also the temporal mismatch between bivalve settlement and predator settlement is shorter because bivalve larvae tend to appear later on the tidal flats (Beukema 1991, Strasser and Gunther 2001, Philippart et al. 2003). In the Dutch Wadden Sea, highest shrimp densities occur in May/June (Beukema and Dekker 2005). Since mussel spat settlement also occurs in May/June, it is possible that spat will suffer heavy predation pressure.

Moreover, mild winters also lead to a lower body condition in spring (Honkoop and Beukema 1997). Body condition is not only positively related to gonadal mass index, as seen in this study, but also animals with low body condition produce fewer eggs (Honkoop and Van der Meer 1998). Therefore, a negative effect of increasing winter temperatures on body condition and, consequently, on reproductive output can be expected. Despite the increase in mean winter temperature during the last decades, no clear trend in body condition was seen (neither through time nor with temperature). An effect of higher winter temperatures on body condition and, consequently, on reproductive output does not seem to be a cause for the observed low recruitment in recent years. However, body mass indices from 1969 to 2006 seem to follow a trend from low body mass index in some years, to higher index after a few years, which decreases again to low values. Often, these periods of low body mass index occur after years with good recruitment (years with densities of six months old individuals higher than 20 ind. m^{-2}). It is possible that after years with good recruitment, the body mass index of the population is low, because young individuals have a lower body size in relation to shell size (as seen in Fig. 4b). During the following years, the body mass index increases. From the results in this paper, the body mass index is highest in 2-3 year old individuals, and decreases in older ones. A general decrease in condition and mortality in old individuals could lead to a decrease of the body mass index of the population. At the moment, body condition of the population is relatively high, suggesting that during the next years, with the mussel population getting older, a decrease in condition is expected.

Is reproductive failure responsible for reduced recruitment?

No clear correlation was found between body mass index and temperature. Considering that the ratio gonadal mass/total body mass has not changed over time, this suggests that the low recruitment success over the last decades was not caused by a decrease in body condition and, hence, on the energy available for reproduction (gametogenesis), due to an increase in mean water temperatures. Unfortunately no information on long-term data sets of gonadal mass in relation to body mass was found for mussels and, therefore, we could not evaluate possible variability in gonads/body ratio through time. In *Macoma balthica*, no correlation was found between the amount of eggs produced and subsequent recruitment (Honkoop et al. 1998), suggesting that egg production (reproductive investment) does not govern recruitment. Nevertheless, the number of adult mussels in beds has been declining since the mid 1980's (Dankers et al. 1989, Dijkema et al. 1989, Dankers et al. 1999). At very low densities the spawning adult stock might become a limiting factor and this could ultimately cause a decrease in the amount of eggs produced by the population, leading to a decline in recruitment. Moreover, the decrease in adult mussels in recent years might also have lead to a shortage of suitable settlement substrate.

In terms of individual growth, reproductive (gametogenic) failure does not seem to have been a cause for the low recruitment because most adults developed gonads during the spawning season. However, reproductive output is not optimal, since most individuals did not spawn completely during the spawning season. Unfortunately, no data on reproductive output of mussels was found in literature. However, Honkoop and Van der Meer (1998) have also observed incomplete spawning in mussels of the same area. More than one decade earlier, Sprung (1983) observed complete spawning in mussels offshore the German coast, where two spawning peaks were observed (a small one in April and a strong one at the end of June). At high summer temperatures, local food limitation could have lead to the observed decline in body condition. Since food availability is the most important factor controlling gonad growth in mussels (Bayne and Worrall 1980, Kautsky 1982), resorption of gonadal tissue might have caused a low reproductive output at the end of the summer. Resorption of gonads has been previously observed in *M. edulis* (Bayne et al. 1978, 1982; Pipe 1987, Toro et al. 2002). Whether poor spawning (due to unfavourable conditions) could be potentially a cause for low recruitment can not be accessed in the present paper.

Both the long-term series and the individual growth study suggest that reproductive failure in terms of failure in gametogenesis was not the cause for the observed low recruitment during the last years. Reduced recruitment could be due to resorption of gonads but other factors that execute their influence after spawning or on the adult population could also be involved.

Acknowledgments: Thanks are due to Ewout Adriaans, skipper of RV 'Stern' for assistance during sampling, and to Wim Wolff, Norbert Dankers, Jan Beukema and two anonymous reviewers for providing critical comments on earlier versions of the paper.

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CHAPTER 3

Reproductive investment of the bivalves *Cerastoderma edule* (L.) and *Mya arenaria* L. in the Dutch Wadden Sea

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Abstract

Cerastoderma edule and *Mya arenaria* are two common bivalve species in European waters. In the Dutch Wadden Sea, these species are abundant in intertidal and subtidal areas, and recruitment success is usually higher in *C. edule* than in *M. arenaria*. Differences in reproductive output or in larval and post-larval processes could be responsible for the observed differences in recruitment success between species. In the present paper, the timing of spawning and the reproductive investment of the two bivalve species was studied at intertidal and subtidal areas of the western Dutch Wadden Sea. *M arenaria* showed higher growth than *C. edule*, in both locations. This resulted in a maximum gonadal mass of 60 mg AFDM in *C. edule* and 2000 mg AFDM in *M. arenaria* at the intertidal, and 20 mg AFDM in *C. edule* and 1300 mg AFDM in *M. arenaria* at the subtidal. Individual reproductive investment was higher in *M. arenaria* than in *C. edule*. *M. arenaria* invested more energy in gonadal mass than *C. edule* both in the intertidal (20% versus 15%) and in the subtidal (13% versus 10%). Timing of spawning was similar in both species, around May, except for *M. arenaria* in the intertidal which seemed to spawn in August. Most *C. edule* showed empty gonads after the spawning season while *M. arenaria* did not spawn completely. However, due to the differences in mass composition between species, absolute reproductive output of the *M. arenaria* population in the western Wadden Sea must be much higher than that of the *C. edule* population. The observed differences in recruitment success between the two species cannot be due to differences in reproductive output since *M. arenaria* has a higher reproductive output. Since egg and larval stage duration are similar between species, differences in post-larval processes are more likely to be the cause for the observed recruitment patterns.

Introduction

The Dutch Wadden Sea, in north-western Europe, is an important nursery area for many pelagic and epibenthic organisms, and a feeding area for migrating shorebirds (Wolff 1983). Bivalves play an important role in the ecosystem, both in terms of biomass as well as in terms of productivity, whereby a few species account for most of the numbers and biomass (Beukema 1991, Dekker et al. 2002, 2003; Dekker and Waasdorp 2004). The life cycle of the various bivalve species is in general similar: after the release of gametes in the water column, a planktonic larval stage occurs, followed by settlement on the seafloor and a demersal juvenile and adult stage. Nevertheless, the various species show large differences in distribution and abundance patterns over the various habitats in the Dutch Wadden Sea. For instance, settlement of the Baltic tellin *Macoma balthica* occurs mostly at intertidal areas, but before or during their first winter, spat migrates in the opposite direction and redistributes into deeper intertidal, subtidal and coastal waters (Beukema 1993). On the other hand, the Pacific oyster *Crassostrea gigas* is more restricted to the intertidal area (Dekker and Waasdorp 2005, 2006) while the blue mussel *Mytilus edulis* can be found in wild beds in the intertidal and subtidal (Dankers and Koelemaij 1989, Dankers and Zuidema 1995, Dankers et al. 1999).

In two other species, the common cockle *Cerastoderma edule* and the soft-shell clam *Mya arenaria* such an intermediate distribution pattern is also observed. *C. edule* and *M. arenaria* are common suspension-feeding bivalves along the European coast. *M. arenaria* occurs from the White Sea to the Gironde estuary and *C. edule* can be found from the western Barents Sea to Mauritania in West Africa (Tebble 1966, Wolff unpubl. observ.). They present large differences in maximum size and age: *M. arenaria* reaches a maximum shell length of about 15 cm while *C. edule* usually grows until a maximum size of 5 cm (Tebble 1966, Hayward and Ryland 1995); and the oldest age reported for *M. arenaria* is 28 years (MacDonald and Thomas 1980) in contrast to 10 years in *C. edule* (Seed and Brown 1978).

In the western Wadden Sea, spat settlement of these species occurs in intertidal and subtidal areas and, as a result, juveniles and adults of both species are found in these areas (Dekker 1989, Dekker et al. 2002, 2003; Dekker and Waasdorp 2004, 2005). Biomass of *C. edule* shows strong fluctuations from year to year, from more than 6 g m^{-2} ash-free dry mass (AFDM) in some years to almost 0 in others. Biomass of *M. arenaria* has been increasing during the last decade, from almost 0 g m^{-2} AFDM in 1991 to more than 30 g m^{-2} AFDM in recent years, in some areas of the western Wadden Sea (Dekker and Waasdorp 2006). In terms of recruitment success, both species show fluctuations from year to year (Beukema et al. 2001). However, during the last 40 years, recruit numbers in intertidal and subtidal areas at the end of the summer are usually higher in *C. edule* than in *M. arenaria* (Beukema et al. 2001, Dekker et al. 2002, 2003; Dekker and Waasdorp 2004, 2005, 2006). Differences in recruitment can result from a number of factors, starting with differences in reproductive output and/or differences in larval and post-larval processes (such as pelagic stage duration, mortality, growth). Egg size is rather similar between these species (*C. edule*: 65 µm; *M. arenaria*: 57 µm), considering that egg sizes in bivalves can vary between 40 µm and 270 µm (Loosanoff and Davis 1963, Brousseau 1979, 1987; Nott 1981, Strathman 1977, Honkoop and Van der Meer 1998, Kenchington et al. 1998, Cardoso et al. 2007a,b). The fact that egg size of *C. edule* and *M. arenaria* is similar suggests that larval size and pelagic stage duration should also be similar (Kooijman 2000).

Therefore, differences in larval processes between species are not likely to be responsible for the observed differences in recruitment. Lower reproductive success in *M. arenaria* could be related to lower recruitment, although post-larval processes such as higher predation on settled larvae can not be excluded. In the present paper, we focussed, as a starting point, on the reproductive output of *C. edule* and *M. arenaria* in two habitats of the western Wadden Sea. The aim was to find out if differences in reproductive investment between these two species could be the cause for the observed differences in recruitment success in the western Wadden Sea. Regular sampling of both species was carried out in intertidal and subtidal areas to reveal:

[1] the timing of spawning by analysing the seasonal patterns in gonadal mass; and

[2] the reproductive investment by determining the energy investment into somatic and gonadal mass in relation to size and age.

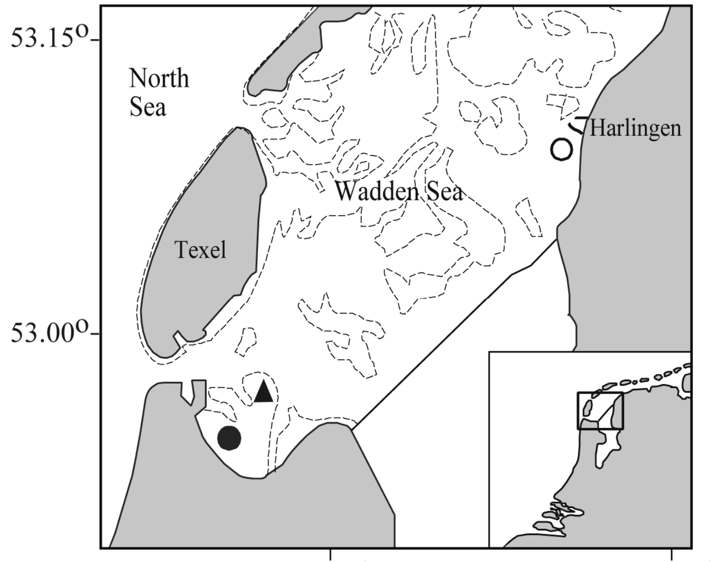

Fig. 3.1. Sampling locations of intertidal *C. edule* (●) and *M. arenaria* (▲). Subtidal sampling was done in the same area for both species (○). Striped lines mark the limits of the intertidal areas.

Materials and Methods

Field sampling

From September 2001 to April 2003, *Mya arenaria* and *Cerastoderma edule* were collected, if possible, every month in intertidal and subtidal areas in the western Wadden Sea (Fig. 3.1; for characteristics see Table 3.1). At each sampling date, around 100 individuals were sampled in an area of a few km^2. Individuals of all size classes were sampled. Intertidal *M. arenaria* and *C. edule* were collected by hand at the Balgzand area during low tide. The intertidal sites are emerged for an average of 4 h per tidal cycle. Subtidal samples were collected near Harlingen during high tide. Since *M. arenaria* lives buried in the sediment, sampling was done with a 'Reineck' box corer (0.06 m^2). For *C. edule*, which lives on the sediment surface, sampling was done with a 1.9 m beam trawl (for description see Van der Veer and Witte 1999). In the laboratory, all animals were stored in seawater at 5 ºC and processed within the next 48 h.

Table 3.1. Characteristics of the locations where *C. edule* and *M. arenaria* were sampled. Depth values are expressed as the difference between MTL and the mean intertidal level of each sampling site.

Species	Location	Latitude (°N)	Longitude (°E)	Depth (m)	Density (ind m^{-2})*^{1}
C. edule	intertidal	52° 56'	4° 48'	-0.4	19
C. edule	subtidal	53° 09'	5° 19'	-2.5	103
M. arenaria	intertidal	52° 57'	4° 55'	-0.5	14
M. arenaria	subtidal	53° 09'	5° 19'	-2.5	102

*1 data from Dekker and Waasdorp (2003)

Data analysis

Of each individual bivalve, shell length was measured to the nearest 0.01 mm with electronic callipers, and subsequently, bivalves were opened and all flesh was removed. Gonads were separated from somatic mass under a microscope. Ash-free dry mass (AFDM) of each part was determined to the nearest 0.01 mg. Age was estimated by counting the external year marks on the shell (following Seed and Brown 1978, Brousseau 1979, Iglesias and Navarro 1990, Van Moorsel 2003). Shell length (mm), somatic AFDM (mg) and gonadal AFDM were plotted against age. Von Bertalanffy Growth (VBG) parameters were iteratively estimated for length-at-age growth curves.

The energy investment in somatic and gonadal mass was determined by estimating, respectively, the Somatic Mass Index (SMI), expressed as the AFDM of the soma divided by cubic shell length (cm^3), and the Gonadal Mass Index (GMI) expressed as the gonadal AFDM divided by cubic shell length (cm^3). The relative investment in reproduction was determined by calculating the Gonadosomatic Ratio (GSR), described as the gonadal AFDM divided by the total body AFDM (soma + gonads).

By dividing mass by cubic shell length, animals of different size could be compared in terms of condition. The extent to which variability in condition was accounted for by seasonal variability and by differences among age classes and among locations was examined by analysis of variance ANOVA. Due to an unbalance in the sampling scheme over the year, the effect of time could not be described in terms of differences among all sampling months (that is, by using sampling month as a categorical variable). Instead we used a linear trend over time in combination with a sinusoidal seasonal effect. The overall time effect was modelled as:

$$\beta_1\,Time + \beta_2 \sin(\,2\pi\,((Month - \beta_3)/\,12)),$$

in which β_1, β_2, and β_3 are parameters, *Time* is a continuous variable that runs from the first day of observation till the last day, and *Month* is a continuous variable running from the first month of observation till the last month. Note that this model is only a linear model when β_3 is known beforehand. For that, we ran this linear model, which further included the factor site and the covariate age, for all 12 possible values of β_3 (i.e. the values 1 to 12) and selected the model with the lowest residual mean squares. Subsequently, the selected model was used to correct somatic and gonad mass indices for seasonal and age differences, and adjusted to the average month and age. In order to obtain Normality, GMI data were transformed using the squared root transformation.

All statistical analyses were made using the software package SYSTAT (Wilkinson 1996).

Results

Growth

In both species, shell length, somatic and gonadal mass showed an increase over time (Fig. 3.2). A decrease in mean shell length between successive sampling occasions was sometimes observed but this was due to sampling errors (Fig. 3.2a and 3.2d). In *C. edule*, growth in somatic mass occurred from mid spring to the end of the summer (Fig. 3.2b). Weight loss occurred in winter, as indicated by the decrease in mean somatic mass values. Gonadal mass increased in spring, after which it rapidly decreased due to spawning (Fig. 3.2c). For most year classes, a slight increase in gonadal mass was also observed at the end of the summer. In autumn, most individuals had empty gonads. Patterns of growth in shell length were similar between intertidal and subtidal individuals but somatic growth in the subtidal did not show very clear patterns due to the variability in data. Gonadal mass in the subtidal followed the same pattern as in the intertidal but only one peak in mass was observed in mid spring, except for 1 year old individuals which showed a peak in gonads in late spring.

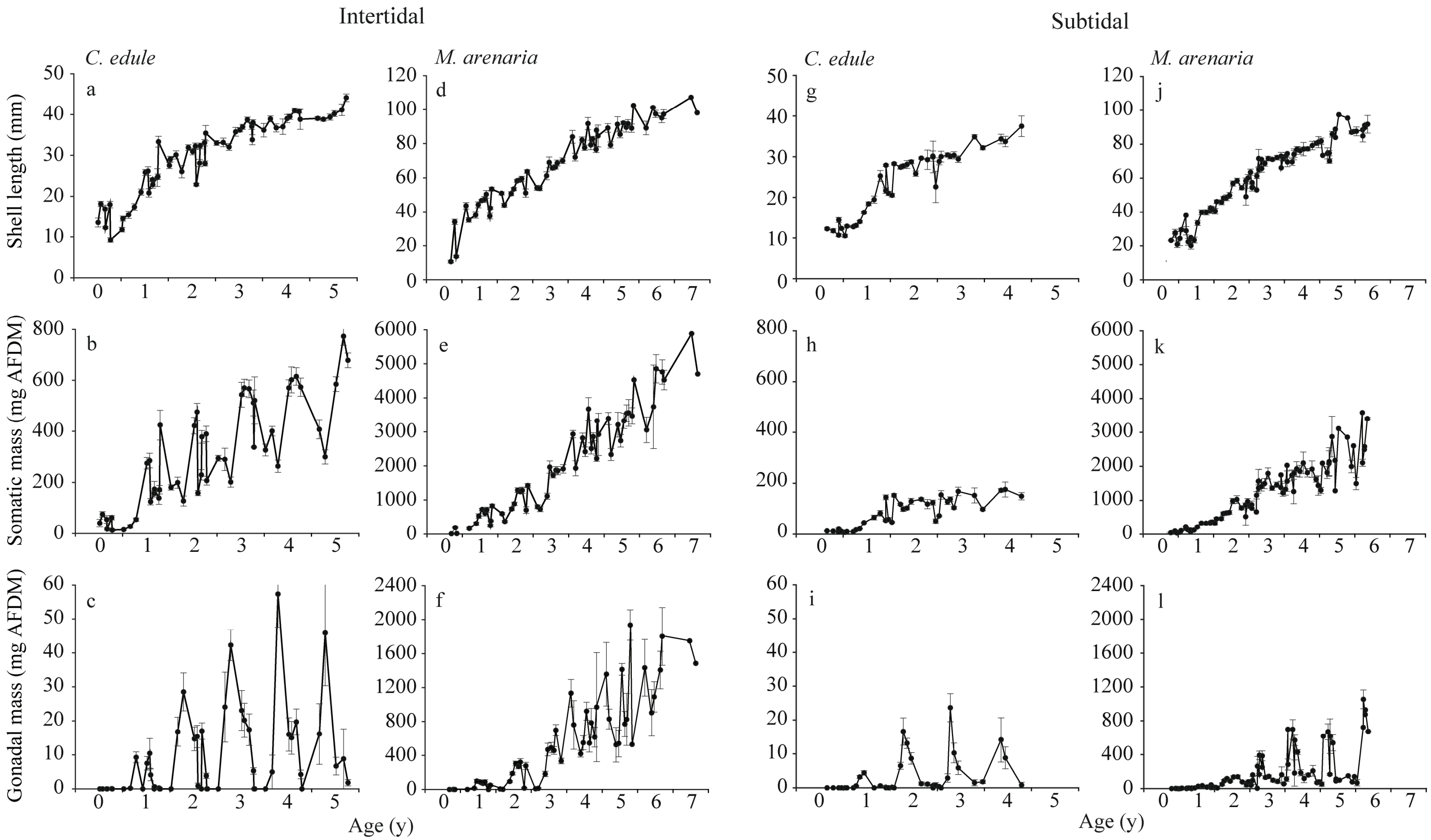

Fig. 3.2. Shell length (mm, mean ± SE), somatic mass (mg AFDM, mean ± SE) and gonadal mass (mg AFDM, mean ± SE) of *C. edule* and *M. arenaria* plotted against age (years) in intertidal (a-f) and subtidal (g-l) locations. The transition between two age classes is considered to be on the 1st of January.

The observed maximum mean shell length, somatic mass and gonadal mass for *C. edule* were, respectively, about 43 mm, 700 mg AFDM and 60 mg AFDM in the intertidal, and 41 mm, 100 mg AFDM and 20 mg AFDM in the subtidal. The estimated maximum length for the intertidal and subtidal stations was, respectively, 43.4 mm and 43.3 mm (Table 3.2).

Table 3.2. Parameters of the Von Bertalanffy growth curve for maximum length (mm) of *C. edule* and *M. arenaria* at the subtidal and intertidal locations. L_∞ is the estimated maximum shell length (mm), k is the Von Bertalanffy growth coefficient and $\omega = L_\infty * k$.

Species	n	L_∞ (±SE, mm)	$10^3 * k$ (±SE, d^{-1}) r^2	ω (mm d^{-1})
Intertidal				
C. edule	56	43.40±1.42	0.33±0.10	0.90±0.06
M. arenaria	55	119.34±5.88	0.68±0.06	0.93±0.08
Subtidal				
C. edule	40	43.26±3.19	1.04±0.13	0.90±0.05
M. arenaria	77	115.74±5.24	0.65±0.05	0.95±0.08

In *M. arenaria*, growth in somatic mass occurred earlier, from spring to mid summer at the two stations, although the large variability in older age classes partly disturbed the patterns, especially in the intertidal (Fig. 3.2e). Weight loss occurred usually between the end of the summer and the beginning of the following year. Large variability in gonadal mass was also observed in the intertidal location. In the intertidal, highest gonadal masses were found in mid summer (Fig. 3.2f). In the subtidal, gonadal mass reached maximum values in spring. Complete spawning was only observed during the first years of life at both locations. Maximum shell length, somatic mass and gonadal mass were in the intertidal 108 mm, 6000 mg ADFM and 2000 mg AFDM, respectively, while in the subtidal they were 100 mm, 4800 mg AFDM and 1300 mg AFDM, respectively. Length-at-age curves resulted in an estimated maximum length of 119.3 mm in the intertidal and 115.7 mm in the subtidal (Table 3.2).

C. edule reached a maximum age of 5 years in the intertidal and 4 in the subtidal while in *M. arenaria* the maximum age was 7 years in the intertidal and 6 in the subtidal. For both species and locations, individuals of all size ranges from 0-group to maximum age were found. To compare growth rates between the two species, the parameter ω was estimated by multiplying L_∞ with the Von Bertalanffy growth rate k (Appeldoorn 1983, Beukema and Meehan 1985). *M. arenaria* had higher growth rates than *C. edule*, both in intertidal and subtidal locations (Table 3.2).

Body mass cycles

For both species, sexes were treated together because no differences in somatic mass index (SMI) and gonadal mass index (GMI) were found between males and females (ANOVA, $p >$

0.05). Somatic mass index showed a clear seasonal pattern in both species (Fig. 3.3) although *C. edule* showed a stronger pattern than *M. arenaria*. The peak in SMI was in July at intertidal locations for both species (Fig. 3.3a and 3.3c), and in June/July in subtidal *C. edule* (Fig. 3.3b) and between April and June in subtidal *M. arenaria* (Fig. 3.3d).

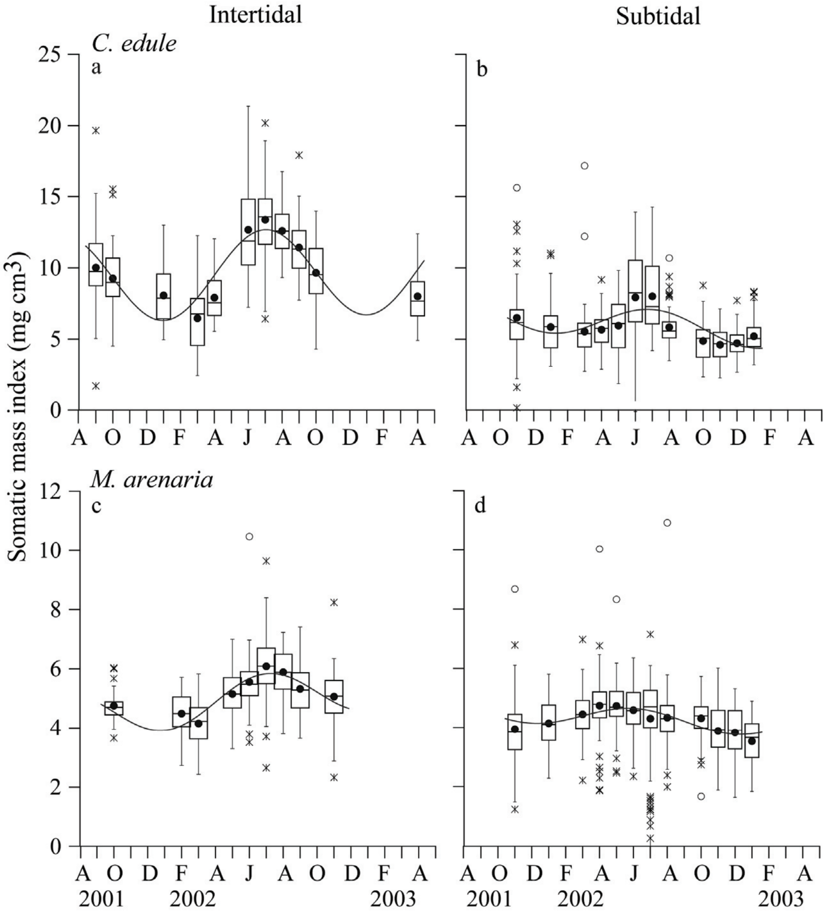

Fig. 3.3. Somatic mass index (mg cm^{-3}) of *C. edule* (a and b) and *M. arenaria* (c and d) along the year in intertidal and subtidal locations.

The period with lowest somatic mass index was between December and February for *M. arenaria* and around February/March for *C. edule*. Both in *M. arenaria* and *C. edule*, significant differences in SMI between intertidal and subtidal stations were found between months (ANOVA, $p<0.001$) but not between age groups (ANOVA, $p>0.05$). In *C. edule*, SMI increased during the first year of life and decreased from then onwards (not shown) while in *M. arenaria,* SMI increased during the first 2 to 3 years of life (not shown).

In relation to the gonadal mass index, clear seasonal cycles were also seen in the two species (Fig. 3.4). *C. edule* presented, for both stations, a peak in April and minimum values in October/November (Fig. 3.4a and 3.4b). *M. arenaria* showed maximum values around July in the intertidal and April in the subtidal (Fig. 3.4c and 3.4d). GMI decreased with age in *C.*

edule (not shown) while it increased with age in *M. arenaria* (not shown). In *C. edule*, differences in GMI between stations were significant with age (ANOVA, $p<0.001$) but not between months (ANOVA, $p>0.05$). In contrast, *M. arenaria* showed significant differences both between months and age groups (ANOVA, $p<0.001$).

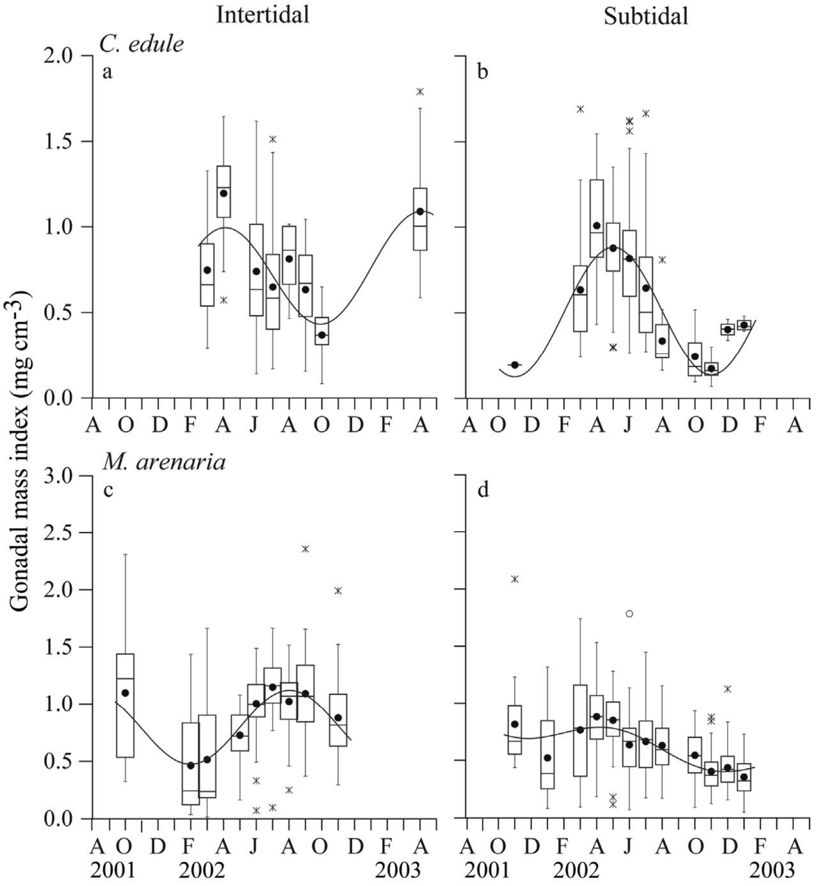

Fig. 3.4. Gonadal mass index (mg cm^{-3}) of *C. edule* (a and b) and *M. arenaria* (c and d) along the year in intertidal and subtidal locations. GMI values are square-root transformed. Note that only individuals that had gonadal mass are represented.

Timing of spawning and gonadosomatic ratio

From the decrease in GMI after the peak in spring/summer, spawning was estimated to occur around May for subtidal *M. arenaria* and for intertidal and subtidal *C. edule*, and around August for intertidal *M. arenaria* (Fig. 3.4). In *C. edule*, there was a suggestion of a second spawning period in September, as seen by an increase in GMI between July and August and a decrease in September (Fig. 3.4a).

The seasonal pattern in gonadosomatic ratio (GSR) was different between species. Overall, *M. arenaria* had a higher GSR than *C. edule*, both in the intertidal and the subtidal (Fig. 3.5). In the intertidal, the amount of gonads in *M. arenaria* was about 20% of the total body mass while in *C. edule*, about 15% of the body was composed of gonadal mass. In the subtidal, the investment in gonads amounted to roughly 13% of the total body mass in *M. arenaria* and only about 10% in *C. edule*. Overall, *M. arenaria* invested more energy in gonadal mass than *C. edule*. However, most *C. edule* seemed to have spawned completely during the spawning season, as suggested by the very low or even null values in gonadal mass after the spawning season (Fig. 3.5a). In contrast, most *M. arenaria* did not seem to spawn completely, due to the relatively high values in GSR after the spawning season (Fig. 3.5b).

An overview of the main data collected in the present study is presented in Table 3.3.

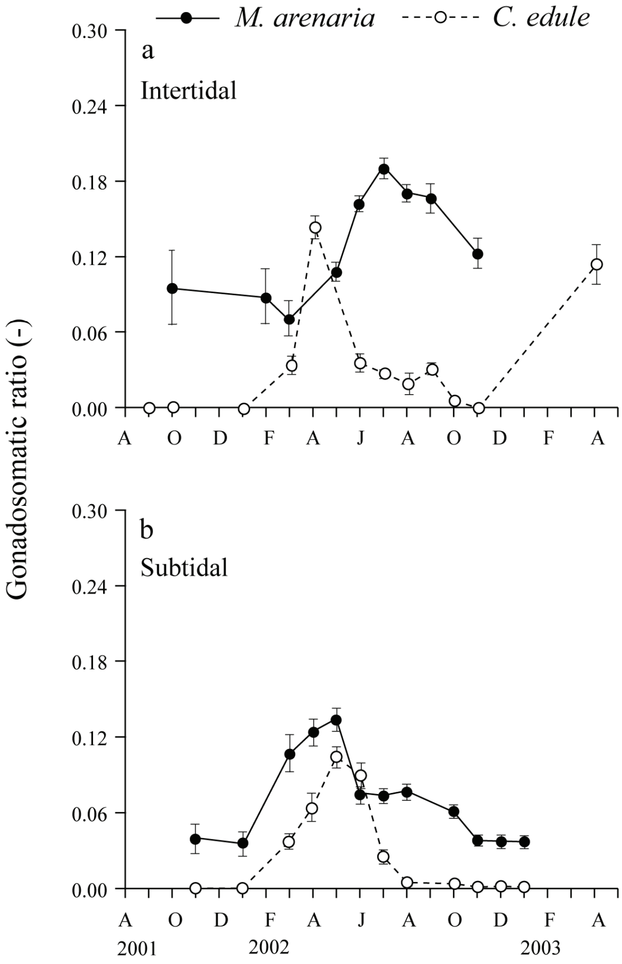

Fig. 3.5. Gonadosomatic ratio (-) of *C. edule* (a) and *M. arenaria* (b) along the year, in intertidal and subtidal locations.

Table 3.3. Overview of data from this study.

	Intertidal	Subtidal
Maximum observed length		
C. edule	43 mm	41 mm
M. arenaria	108 mm	100 mm
Max. observed somatic mass		
C. edule	700 mg AFDM	100 mg AFDM
M. arenaria	6000 mg AFDM	4800 mg AFDM
Max. observed gonadal mass		
C. edule	60 mg AFDM	20 mg AFDM
M. arenaria	2000 mg AFDM	1300 mg AFDM
Individual reproductive investment		
C. edule	15%	10%
M. arenaria	20%	13%
Timing of spawning		
C. edule	May	May
M. arenaria	August	May

Discussion

Age determination

Age determination in *C. edule* and *M. arenaria* was done by counting the external shell marks. Growth marks on shells are usually related to cessation of growth during winter months, due to low food availability and low temperatures. In Dutch waters, growth of bivalves stops during winter and they usually loose weight in autumn/winter (Lammens 1967, Pieters et al. 1979, Beukema et al. 1985, Zwarts 1991, Honkoop and Beukema 1997). In the present study, this seasonal pattern in growth was confirmed by the decrease in somatic mass during winter and the increase in shell length and somatic mass during the spring/summer. Therefore, a clear winter mark on the shell surface can be expected. However, sudden changes in temperature or food conditions, spawning, and other stressing factors may also lead to a temporary cessation of growth and the formation of disturbance marks. Such marks are not always visually distinguishable from real growth marks and can be mistaken for year rings, leading to errors in age determination. This will mainly occur in older individuals, in which the last growth marks are very close to each other and difficult to visualize. An overestimation of age will result in an underestimation of the growth rate. Nevertheless, examples from the literature show that in these two species, the use of external shell marks to determine age does not cause a large error and is mostly correct (Newcombe 1936, Seed and Brown 1978, Brousseau 1979, MacDonald and Thomas 1980, Brêthes and Desrosiers 1981, Brousseau and Baglivo 1987, Iglesias and Navarro 1990, Roseberry et al. 1991, Maximovich and Guerassimova 2003). However, Van Moorsel (2003) found that reading internal shell rings in *M. arenaria* yielded better results. But he analyzed only 6 clams.

A comparison of the age data of *M. arenaria* from this study with data from the 1991 cohort (Dekker unpubl. data), which was a year with strong recruitment, and data of Philipp et al. (2005) for the same subtidal location, illustrates the large variability in the estimated size-at-age in *M. arenaria* (Fig. 3.6).

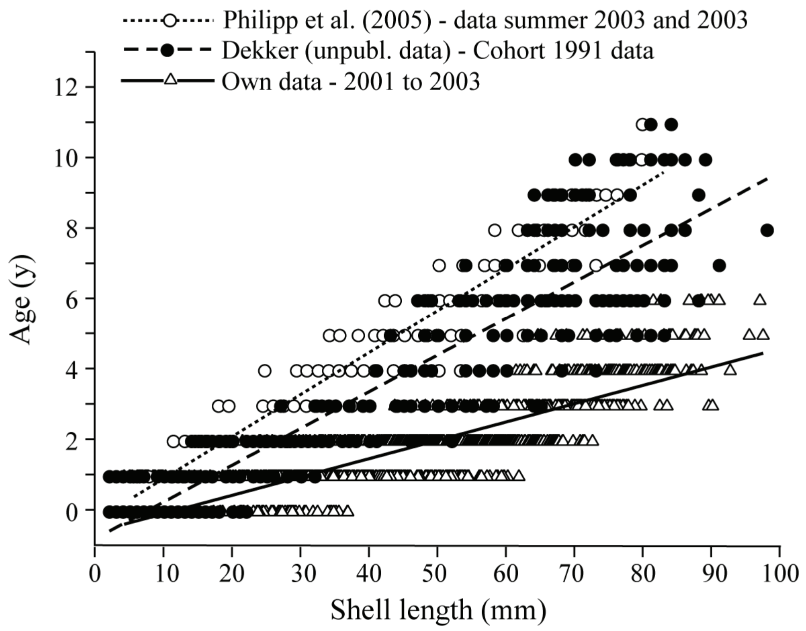

Fig. 3.6. Shell length (mm) – age (y) relationship for subtidal *M. arenaria* resulting from different methods of age determination. •: data of Dekker (unpubl. data; 1991 cohort); Δ: own data (2001-2003); ○: data from Philipp et al. (2005; summer 2002 and 2003).

In relation to the 1991 cohort data, age estimated in the present study seems to be underestimated, especially in older individuals, while age determined by Philipp et al. (2005) seems to be overestimated. Although Philipp et al. (2005) validated the growth rings in the umbo with isotope analysis, this was done in only 4 individuals. It should also be kept in mind that variability between datasets can be due to the time difference. However, for the first years of life the differences are relatively small. The large difference in age determination between different methods points out to the necessity of validating age determination in *M. arenaria* for different areas.

Growth

In both species, 0-group individuals were found in intertidal and subtidal areas. This means that recruitment of *M. arenaria* and *C. edule* occurs in both areas as observed by Dekker et al. (2003) and Dekker and Waasdorp (2004, 2005, 2006). The reported maximum age of *C. edule* varies along the European coast: 7 years old in northern Spain (Iglesias and Navarro 1990), 6 in the Dutch Wadden Sea (Dekker et al. 2003), 9 in Wales (Jones 1979) and 10 in Ireland (Seed and Brown 1978). In *M. arenaria*, maximum observed age in the southern Baltic Sea and East American coast was 13 years (Brousseau 1979, Kube 1996), in Canada 28 years (MacDonald and Thomas 1980) and in the White Sea 17 years (Maximovich and Guerassimova 2003). In the present study, the oldest *M. arenaria* was 7 years old and the oldest *C. edule* was 5. However, only two locations were sampled during a little bit more than a year. For an overview of the population dynamics of these species in the western Wadden Sea, more locations should be sampled over a longer time period. In addition, age determination in *M. arenaria* is preliminary until age has been studied in more detail and validated (see above).

Within each habitat, differences in growth between species could be due to competition for food or differences in predation pressure. Competition for food is likely to occur between bivalve species living in the same area (Kamermans 1994). *C. edule* and *M. arenaria* are both suspension-feeding species and therefore they compete for the same food source. Density-dependent regulation of growth rates, by local depletion of phytoplankton, has been observed in populations of suspension-feeding bivalves (Peterson 1982, Olafsson 1986, Vincent et al. 1989, Jensen 1992, Peterson and Black 1993, De Montaudouin and Bachelet 1996). However, body condition and growth of *C. edule* was seen to be independent of density (Kamermans et al. 1992, De Montaudouin 1996), except when high densities occur over areas of several km^2 (Jensen 1992). Intraspecific competition in 10 mm *C. edule* seems to occur only at densities above 2000 individual m^{-2} (Jensen 1992, 1993), which are much higher densities than observed in the present study. In *M. arenaria*, significant negative effects of increasing clam density on growth were observed in individuals between 9 and 12 mm, especially at densities above 1000 individual m^{-2} (Beal and Kraus 2002). Since densities of both species were similar during the study period, an effect of density on growth of *C. edule* and *M. arenaria* does not seem likely to have occurred.

The fact that *M. arenaria* lives deeply buried in the sediment while *C. edule* lives just under the sediment surface, suggest that differences in predation pressure may occur between species. Two different types of predation are possible: predation on complete individuals, resulting in direct mortality, and predation on regenerating body parts such as siphon and foot tips, which affects food intake and condition. Predation on complete individuals occurs by birds (Fréchette and Bourget 1985, Wanink and Zwarts 1993, Zwarts et al. 1996), shrimps *Crangon crangon*, crabs *Carcinus maenas* and fish species (Seed and Brown 1978, De Vlas 1979, Phil and Rosenberg 1982, Möller and Røsenberg 1983, Kube 1996, Van der Veer et al.

1998, Mackenzie and McLaughlin 2000, Mascaró and Seed 2000, Beukema and Dekker 2005). Various fish species, shrimps and crabs are also responsible for predation on regenerating body parts (De Vlas 1979). It is possible that by burying shallower, *C. edule* is more susceptible to predation.

Timing of spawning and reproductive investment

Seasonal patterns in somatic and gonadal mass indices, as well as in gonadosomatic ratio, were used to determine the timing of spawning and the reproductive investment of each species. As observed in other intertidal areas of the Wadden Sea (Zwarts 1991, Honkoop and Beukema 1997), body mass indices increased from early spring to mid summer and decreased in winter. Somatic growth started in early spring in both species, corresponding with the annual April/ May peak in chlorophyll-*a* in the water (Cadée and Hegeman 2002). At this time of the year, the amount of chlorophyll-*a* (from benthic and pelagic algae) in the stomachs of bivalves was also high (Kamermans, 1994). The increase in gonadal mass started in the beginning of the year suggesting that gametogenesis takes place during the beginning of the growing season, as observed in other areas (Newell and Bayne 1980, Rosenblum and Niesen 1985, Iglesias and Navarro 1991). Gonadal mass index showed a stronger pattern in *C. edule* than in *M. arenaria*. In the intertidal, timing of spawning was earlier in *C. edule* (around May) than in *M. arenaria* (around August). In the subtidal, both species spawned in the same period (around May). In a nearby intertidal area, *C. edule* was also seen to spawn in May/June (Honkoop and Van der Meer 1998). Spawning in late spring (around May) has the advantage that temperatures and algal concentration in the water are relatively high and, therefore, larval growth can be fast. The fact that gonadal mass index in *M. arenaria* increases with age also indicates that large animals, suffering lower predation, are able to invest more energy in reproduction.

GSR was higher in *M. arenaria* than in *C. edule*, both in the intertidal and subtidal. Despite the similarities in egg size between the two species, within the same habitat conditions *M. arenaria* invests more energy in reproduction than *C. edule*. During the peak in gonadosomatic ratio, *M. arenaria* showed a maximum of about 20% of gonadal mass in relation to the total body mass in contrast to *C. edule* with a maximum of about 15%. Although intertidal *M. arenaria* spawns in late summer, this does not seem to affect its reproductive output. However, in contrast to *M. arenaria,* most *C. edule* spawned completely during the spawning season. In terms of individual reproductive output, *C. edule* seems to be more successful than *M. arenaria*, since all the energy put in gonadal mass is released in the form of gametes. Nevertheless, the absolute amount of gonadal mass produced by individual is more than 30 times higher in *M. arenaria* than in *C. edule*. If densities of both species are similar, then the amount of gametes released by the *M. arenaria* population, both in the intertidal and subtidal, must be higher than the amount released by the *C. edule* population.

Long-term trends in recruit density in the western Dutch Wadden Sea show that densities of *C. edule* are, most of the years, larger than those of *M. arenaria* (Beukema et al. 2001, Dekker et al. 2003, Dekker and Waasdorp 2004, Beukema and Dekker 2005). Since absolute reproductive investment is higher in *M. arenaria* than in *C. edule*, differences in recruitment success between species cannot be caused by differences in reproductive output. Due to the similarity in egg size between species, characteristics of the larvae (such as size and pelagic stage duration) are also thought to be similar. Therefore, differences in post-larval processes (such as differential predation pressure in early life stages) are more likely to be the reason for the observed differences in recruitment between *C. edule* and *M. arenaria*.

Acknowledgments: We would like to thank Oscar Bos and Mardik Leopold for their helpful comments on earlier versions of this chapter.

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CHAPTER 4

Habitat related growth and reproductive investment in estuarine waters, illustrated for the tellinid bivalve *Macoma balthica* (L.) in the western Dutch Wadden Sea

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Abstract

In estuarine areas, bivalve species can be found in a variety of environments, where they experience large differences in environmental conditions. In the present paper, the importance of different habitats (intertidal, subtidal, adjacent coastal waters) for the persistence of the population was evaluated for the bivalve *Macoma balthica* (L.) in the western Dutch Wadden Sea estuary. Intra-specific variation in growth and reproductive output were followed during the year and related to local abiotic conditions. Significant differences in growth and reproductive investment were found between locations. Young individuals were mostly found in the intertidal area, where growth in terms of somatic mass was good. These areas were not favourable for adult individuals, since growth in shell length was low and many individuals did not reproduce. In the subtidal, where the highest densities were found, somatic and gonadal mass indices were low. Coastal areas had the lowest densities and showed high growth in terms of shell length and body mass. The habitat with the highest reproductive effort per individual was not the most important habitat in terms of reproductive output due to differences in density and in size of the habitat type. For *M. balthica*, the subtidal habitat contributed most to the reproductive output of the western Dutch Wadden Sea population although the highest reproductive output per individual was in the coastal area.

Introduction

Bivalve species can be found in a variety of environments, such as estuaries and coastal waters. In these areas, species experience differences in environmental conditions which influence their physiological processes. At a latitudinal scale, differences in growth rate of bivalves have been frequently related to latitudinal gradients in temperature (Gilbert 1973, Bachelet 1980, Appeldoorn 1983, 1995; Beukema and Meehan 1985, Hech et al. 2002, Fiori and Morsán 2004). However, at a local scale, other factors such as food quality and quantity, tidal level and sediment type seem to have a more important role (Newell and Hidu 1982, De Montaudouin 1996, Beukema and Cadée 1997, Honkoop and Beukema 1997, Beukema et al. 2002, Carmichael et al. 2004). An example is the observed higher growth, body condition and reproductive output in bivalves living at lower intertidal areas than at higher ones (Jones et al. 1978, Guevara and Niell 1989, Roseberry et al. 1991, Jensen 1992, Wanink and Zwarts 1993, De Montaudouin 1996, Honkoop and Beukema 1997), due to the longer submersion time and thus longer possibility of food intake at lower intertidal areas.

In the present paper, we aim to analyse intra-specific variation at a local scale for processes such as growth and reproduction. The tellinid bivalve *Macoma balthica* (L.) was selected for this study. *M. balthica* is a dominant species in many estuaries and coastal areas along the Atlantic coast (Dankers and Beukema 1983, Thompson and Nichols 1988, Harvey and Vincent 1989). *M. balthica* is a broadcast-spawning bivalve with separate sexes. After the release of gametes in the water column where fertilization occurs and a short planktonic larval period of about 3 to 4 weeks, larvae are ready to settle (Drent 2002). Initial settlement occurs mainly in the lower intertidal zone but during the summer animals migrate to the upper intertidal zone, where predation pressure of shrimps and crabs is relatively low (Armonies and Hellwig-Armonies 1992, Beukema 1993, Hiddink et al. 2002a). Before or during their first winter, spat migrates in the opposite direction and redistributes into deeper intertidal, subtidal and coastal waters (Beukema 1993). As a result, in Dutch waters, *M. balthica* is present in the intertidal and shallow subtidal areas in estuaries as well as in adjacent coastal waters (Beukema and De Bruin 1977, Dekker 1989).

The fact that *M. balthica* is widespread and common, illustrates a strong persistence of the population in these specific geographic spaces (c.f. Sinclair 1988), which would suggest favourable environmental conditions for the species. However, from an energetic point of view, at least the environmental conditions for the intertidal population hardly seem favourable, since for most of the year the energy balance is negative (Hummel 1985). During the summer, the high energy demand due to relatively high temperatures (Hummel 1985) and the suboptimal feeding conditions in the intertidal (only substantial food intake during submersion, Kamermans 1994), lead to weight and energy losses (Beukema and De Bruin 1977). Only during a relatively short period in spring, *M. balthica* is able to gain sufficient energy to compensate for these losses and even to grow and reproduce (Honkoop and Van der

Meer 1997). In subtidal and coastal areas, food conditions might be more suitable due to the fact that food intake is never hampered because of emersion. The fact that, during the summer, the decline in body condition is stronger at high intertidal levels than at low intertidal levels (Honkoop and Beukema 1997), suggests that at subtidal and coastal areas this difference may be even more marked. Whether spawning at the intertidal contributes substantially to recruitment is at present unclear and it cannot be excluded that the main contribution and hence persistence of the population originates from recruitment from the subtidal and/or coastal stocks.

Therefore, the final aim is to analyse the importance of the different habitats (intertidal, subtidal, coastal) for the persistence of the *M. balthica* population in the western Dutch Wadden Sea estuary. Growth and reproductive investment of *M. balthica* were analysed in the various habitats in order to test whether subtidal and coastal stocks are more important to the persistence of the population than intertidal ones. By analyzing age composition and following changes in somatic and gonadal mass in each habitat in relation to abiotic and biotic conditions, the energy investment into somatic and gonadal mass and, thus, the reproductive investment could be determined. In addition, the occurrence of gonad resorption during periods of unfavourable environmental conditions, as experimentally observed by Drent (2004) for this species, could also be studied by the analysis of seasonal patterns in gonadal mass.

Table 4.1. Characteristics of the locations sampled. Depth values are expressed as the difference between MTL and the mean intertidal level of each sampling site. Density ranges are shown between brackets.

Station	Latitude (ºN)	Longitude (ºE)	Depth (m)	Density (ind. m^{-2})
Intertidal	52° 55’	4° 48’	+0.1 – +0.3	74 (0 – 222)
Subtidal	53° 10’	5° 22’	-2.1 – -2.6	142 (17 – 167)
Offshore	52° 52’	4° 38’	-8 – -12	56 (5 – 75)

Materials and Methods

Field sampling

The sampling area was divided into three different habitat types based on water depth (Fig. 4.1, Table 4.1): the intertidal of the Wadden Sea, defined as the area above Low Low Water Spring (LLWS); the subtidal of the Wadden Sea (between LLWS and LLWS – 5 m); and the coastal zone of the North Sea (called here “offshore” and defined as the area between LLWS - 5 m and LLWS - 10 m).

Selection of sampling stations in the different habitats was based on information from long-term monitoring programmes. The sampling station in the intertidal was selected at the Balgzand, a large intertidal area which has been monitored since the 1970’s by Beukema and co-workers (Beukema et al. 1978). The area between squares A and B of Beukema (1988, 1993) was selected because this area is considered to be representative for the intertidal in the western Wadden Sea (Beukema 1988, 1993; Beukema pers. comm.). At the subtidal, surveys in the western Wadden Sea have been carried out in 1982 and 1986. Densities of *M. balthica* were on average 42 ind. per m^2 in 1982 (Dekker 1989) and around 162 ind. per m^2 in 1986 (Van der Veer and Witte 1993, Van der Veer unpubl. data). An exploratory inventory in the largest subtidal area, Kimstergat, resulted in densities of *M. balthica* in line with previous numbers found in the subtidal in the western Wadden Sea (between 100 – 150 ind. m^{-2}) and therefore this large subtidal area was selected. In the offshore area, the distribution of *M. balthica* has been described by Daan and Mulder (2002, 2003, 2004) and Drent (2004). An exploratory inventory showed a similar distribution in recent years and the sampling area was selected within the areas sampled by these authors (Grote Keeten).

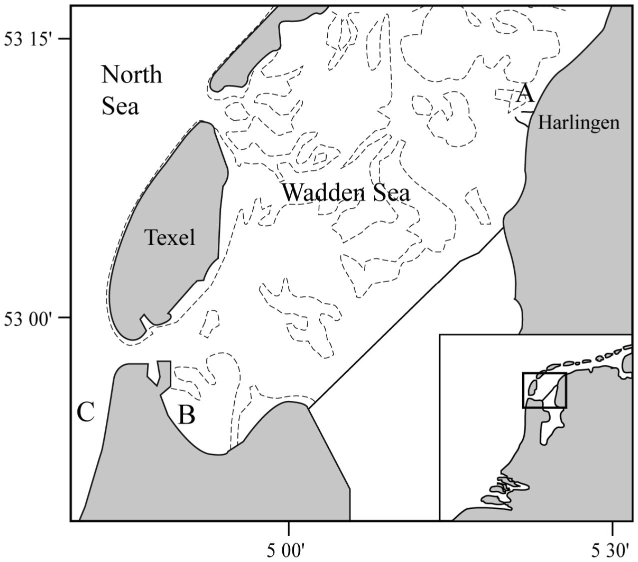

Fig. 4.1. Sampling locations of *M. balthica*: A) subtidal area; B) intertidal area and C) offshore area.

From September 2001 to April 2003, around 100 individuals were collected randomly in an area of a few km^2, if possible once a month. Intertidal samples were collected with hand cores (0.018 m^2) of approximately 30 cm deep during low tide. The intertidal site is submerged for an average of 5 h per tidal cycle. Subtidal samples were collected with a 'Reineck' box corer (0.06 m^2) during high tide. At the offshore station, due to low densities of a few animals per box corer, it was decided to sample with a 'Van Veen' grab (0.2 m^2). Previous research had shown that the distribution of *M. balthica* offshore is restricted to the first few cm in the sediment and that numbers caught by the grab were similar to those from the box corer, after correction for the differences in surface area (Beukema 1974). In the laboratory, all *M. balthica* were stored in seawater at 5 ºC and processed within the next 48 hours.

Data analysis

Of each individual *M. balthica*, shell length was measured to the nearest 0.01 mm with electronic callipers, and subsequently bivalves were opened and all flesh was removed. Animals that were parasitized with the trematode *Parvatrema affinis* (Swennen and Ching 1974) were discarded. Age was estimated by counting the shell's year marks (following Lammens 1967). Gonads were separated from body mass under a microscope and the ash-free dry mass (AFDM) of each part was determined to the nearest 0.01 mg, by drying for 4 days at 60 ºC and incinerating for 4 hours at 560 ºC. The difference between dry and ash mass represented the AFDM. For each individual, the condition index was determined by the Body Mass Index (BMI) as the total body AFDM mass (somatic mass + gonadal mass) divided by shell length^3. The investment in gonadal and somatic mass was determined by calculating the Somatic Mass Index (SMI) and the Gonadal Mass Index (GMI). SMI was defined as the AFDM of the soma divided by shell length^3 and GMI as the gonadal AFDM divided by shell length^3. The relative investment in reproduction was analysed by calculating the Gonadosomatic Ratio (GSR), expressed as the gonadal AFDM divided by the total body AFDM.

By dividing mass by cubic shell length, animals of different size could be compared in terms of mass. The extent to which variability in mass could be accounted for by seasonal variability and by differences among age classes and among locations was examined by using analysis of variance (ANOVA). Due to an unbalance in the sampling scheme over the year, the effect of time could not be described in terms of differences among all sampling months (that is, by using sampling month as a categorical variable). Instead we used a linear trend over time in combination with a sinusoidal seasonal effect. The overall time effect was, therefore, represented by:

$$\beta_1\, Time + \beta_2 \sin(\, 2\pi\, ((Month - \beta_3)/\, 12)),$$

in which β_1, β_2, and β_3 are parameters, *Time* is a continuous variable that runs from the first day of observation till the last day, and *Month* is a continuous variable that runs from the first month of observation till the last month. Note that this model is only a linear model when β_3 is known beforehand. For that, we ran this linear model, which further included the factor site, for all 12 possible values of β_3 (i.e. the values 1 to 12) and selected the model with the lowest residual mean squares. Subsequently, the selected model was used to correct body, somatic and gonadal mass indices for seasonal and age differences, and adjusted to the average month and age. In order to obtain normality, only the GMI data needed to be transformed using the squared-root transformation.

In order to compare growth rates between populations, shell length (mm), somatic AFDM (mg) and gonadal AFDM (mg) were plotted against age. In order to avoid an underestimation of maximum mass due to seasonal periods of decrease in mass, growth curves of somatic mass were estimated for the period of the year in which SMI was maximal. The Von Bertalanffy growth parameters were iteratively estimated and comparison between stations was made using the F-test. For that, non-linear regressions were run for all stations together, each station separately and for combinations of two stations. Multiple comparisons were done between the different regression models with the F-test.

Each sampling allowed an estimation of the instantaneous rate of annual decrease (M, y^{-1}), whereby constant mortality with age was assumed (i.e. exponential decrease in numbers). M was estimated by linear regression after ln transformation of the data, in which M corresponded with the slope of the regression. For that, the decrease in abundance from the age with the peak abundance to the oldest age was considered. This corresponded to individuals older than 0 years for the intertidal location, older than 2 years for the subtidal location and older than 4 years for the offshore location. Significant differences among locations were analysed using ANOVA and Fisher's LSD post-hoc test.

All statistical analyses were made using the software package SYSTAT (Wilkinson 1996).

Results

Age composition

The age composition of *M. balthica* differed between locations (Fig. 4.2). At the intertidal station individuals between 1 and 5 years old were present, with a high incidence of 1 year-old individuals. In the subtidal, most of the individuals were between 2 and 6 years old, with a maximum age of 11 years. In the offshore location, the largest age group consisted of 5 year old individuals and the maximum age was also 11 years, but relatively more individuals of 5 years were found compared to the subtidal location. After the summer, from July onwards, 0-group individuals were seen in the intertidal but not in the subtidal and offshore (Fig. 4.2).

The instantaneous rate of annual decrease (M) was 1.27±0.17 y^{-1} at the intertidal location, 0.39±0.16 y^{-1} at the subtidal location and 0.45±0.21 y^{-1} at the offshore location. This corresponded with an annual decrease of about 72% in the intertidal, 32% in subtidal and 36% offshore. Differences among locations were significant, due to significant differences between the intertidal and the subtidal/offshore locations (ANOVA, $F_{(2,31)} = 8.642$, $p = 0.001$). Between subtidal and offshore, M was not significantly different (Fisher's LSD post-hoc test, $p = 0.805$).

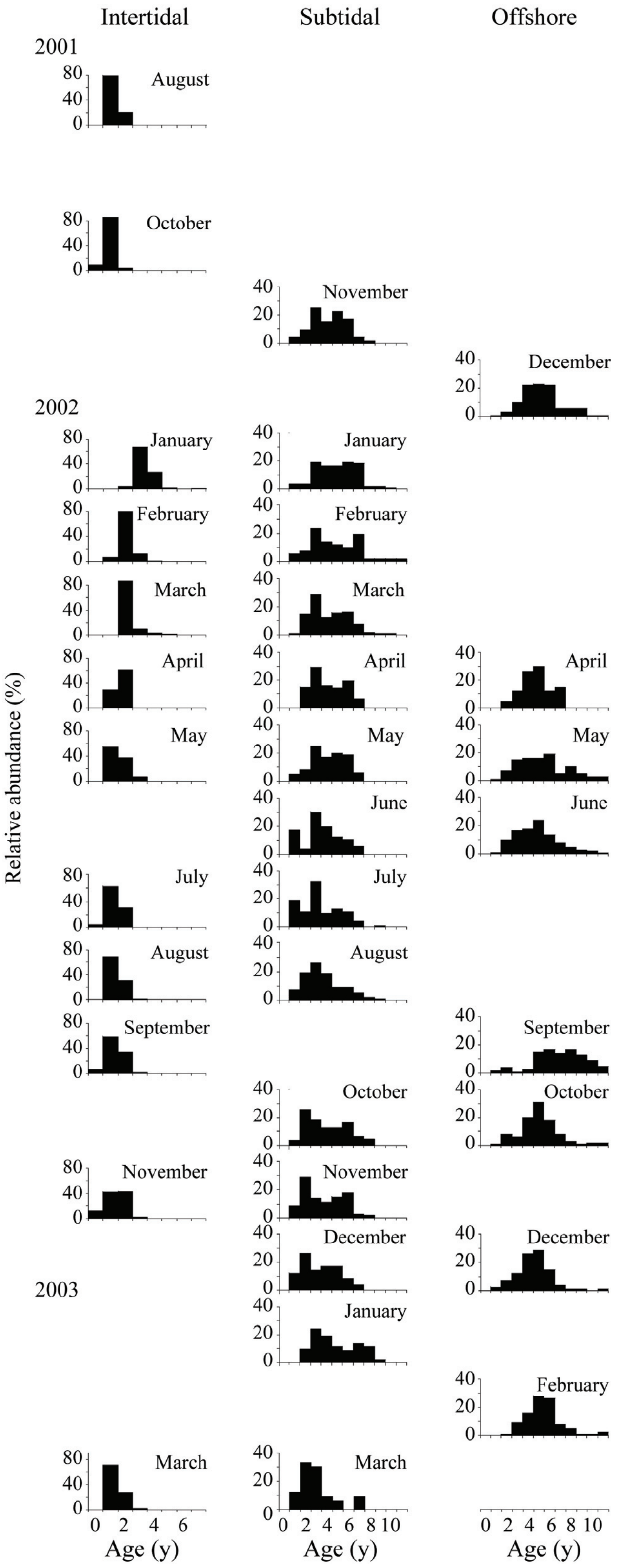

Fig. 4.2. Age composition (y) of intertidal, subtidal and offshore *M. balthica* populations for each sampling date between 2001 and 2003.

Growth

Maximum observed shell length was around 19 mm in the intertidal, 24 mm in the subtidal and 23 mm offshore (Fig. 4.3). Maximum values of somatic mass (mg AFDM) were around 90 mg for the intertidal, 120 mg for the subtidal and 130 mg offshore; and maximum gonadal mass (mg AFDM) was around 14, 28 and 35 mg for intertidal, subtidal and offshore respectively. The estimated maximum length was 15.7 mm for the intertidal, 21.7 mm for the subtidal and 21.0 mm for the offshore location (Table 4.2). Somatic mass-at-age curves were estimated for the month of May 2002 for the intertidal station and June 2002 for the subtidal and offshore stations (during which SMI was maximum, see next section).

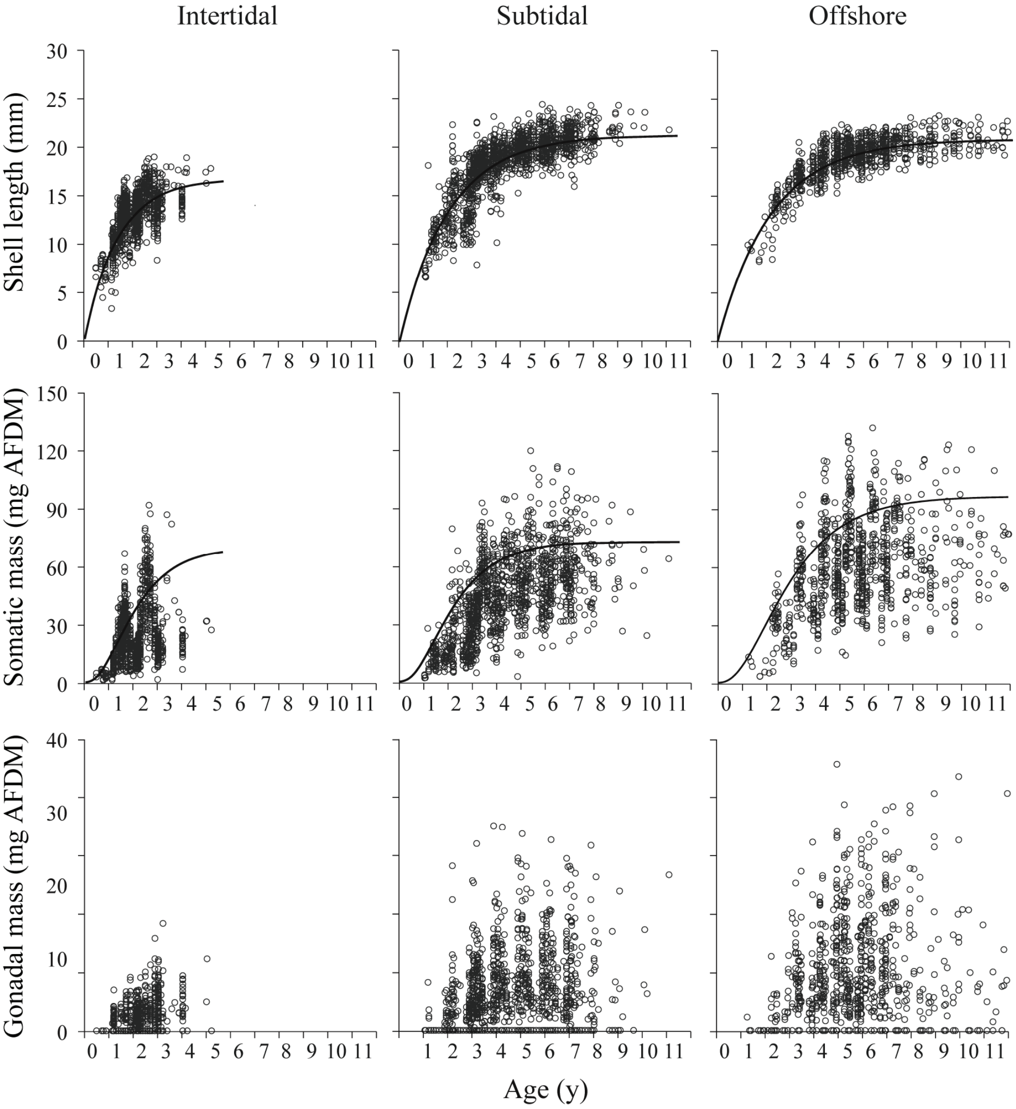

Fig. 4.3. Shell length (mm), somatic mass (mg AFDM) and gonadal mass (mg AFDM) of *M. balthica* plotted against age (y), with fitted Von Bertalanffy growth curves for shell length and somatic mass. The transition between two age groups is considered to be on the first of January.

The estimated maximum somatic mass was 69.4 mg for the intertidal, 72.1 mg for the subtidal and 96.2 mg for the offshore location. Since gonads are emptied during spawning, no growth curve was estimated. Statistically significant differences were found in shell length-at-age curves among locations (F-test, $F_{(2,3347)}$= 55.8, p<0.001), but not in somatic mass-at-age curves (F-test, $F_{(2,319)}$= 1.5, p>0.05). Overall, offshore individuals were the largest in terms of somatic mass and gonadal mass.

Table 4.2. Parameters of the Von Bertalanffy growth curve for maximum shell length (mm) and somatic mass (mg AFDW) of *M. balthica* at the three locations. L_∞ and W_∞ are the maximum asymptotic length (mm) and somatic mass (mg AFDM), k is the growth coefficient (d^{-1}), n is the number of individuals.

Parameter	Intertidal	Subtidal	Offshore
Length-at-age			
L_∞ (mm)	15.72	21.78	21.05
95% C.I.	15.39-16.06	21.56-22.04	20.87-21.23
k (*10^3; d^{-1})	2.503	1.286	1.255
95% C.I.	2.344-2.662	1.241-1.332	1.211-1.300
n	1077	1393	880
r^2	0.48	0.70	0.71
Somatic mass-at-age			
W_∞ (mg)	69.43	72.15	96.20
95% C.I.	51.65-90.94	66.18-78.46	90.64-101.98
k (*10^3; d^{-1})	2.191	2.015	1.525
95% C.I.	1.622- 2.760	1.682-2.348	1.363-1.687
n	48	97	177
r^2	0.58	0.65	0.53

Seasonal patterns in mass

Since no differences in body mass index (BMI), somatic mass index (SMI) and gonadal mass index (GMI) were found between males and females (ANOVA, p > 0.05), sexes were treated together in all analyses. Seasonal cycles of BMI were similar for the three locations (Fig. 4.4) with minimum values between January and March and maximum values around June. In the intertidal and offshore, the amplitude of values along the year was higher than in the subtidal. The latter also presented a lower BMI than the other two locations. BMI did not show much variation with age (Fig. 4.5), which was confirmed by the non-significance of the interaction term Station*Age (Table 4.3). Overall, significant differences in BMI were found between locations (Table 4.3), as reflected by the significant effects and interaction terms Station*Month and Station*Season. SMI had the same seasonal trend as the BMI (Fig. 4.4). The general linear model, showed significant differences in the interaction terms and in the effects of month, season and age separately (Table 4.3). SMI was clearly lower in the subtidal than at the other two stations. Seasonal differences were smallest in the subtidal and strongest in the intertidal. SMI decreased significantly with age (Fig. 4.5) and this decrease was strongest in the intertidal.

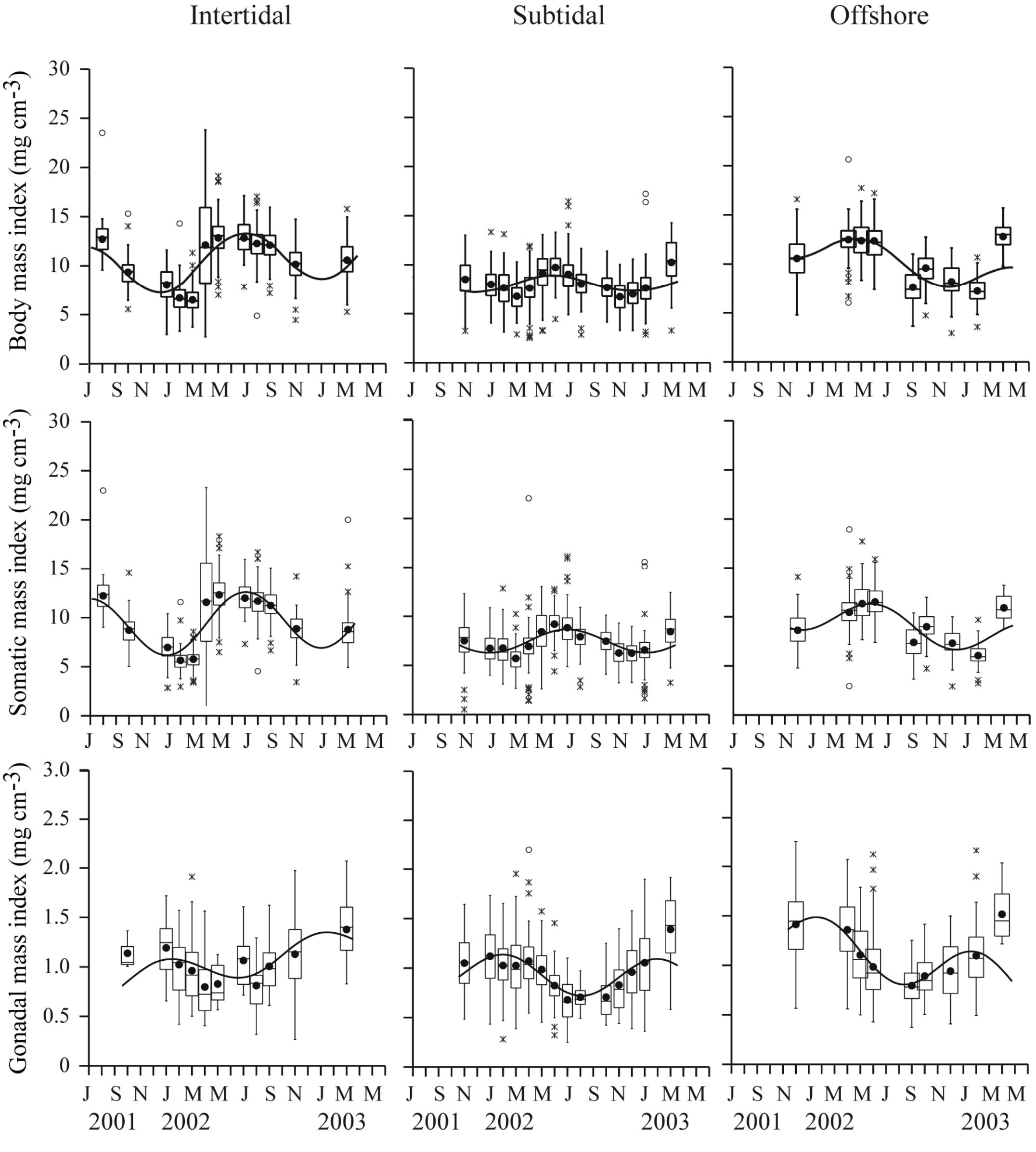

Fig. 4.4. Body mass index (mg cm^{-3}), somatic mass index (mg cm^{-3}) and gonadal mass index (mg cm^{-3}) of *M. balthica* along the year. Curves are model predictions. Data of gonadal mass index are square-root transformed. Full dots and horizontal bars indicate mean and median values, respectively; boxes represent the range within which the central 50% of the values fall; bars represent the data range excluding outliers; outliers and extreme values are, respectively, observations more than 1.5 and 3 times the box range and are represented by asterisks and open circles.

Cycles of GMI were similar for the three locations along the year, with higher values in January-March for 2002 and March-April for 2003 (Fig. 4.4). Lowest values of GMI were found in the months of August and September, during which the number of animals without any gonadal mass was higher (not shown). In the intertidal, this trend was not as obvious because many individuals were juveniles and did not reproduce. Overall, in the intertidal only 40% of the individuals sampled showed developed gonads, in contrast to 63 and 73% in the subtidal and offshore respectively (not shown).

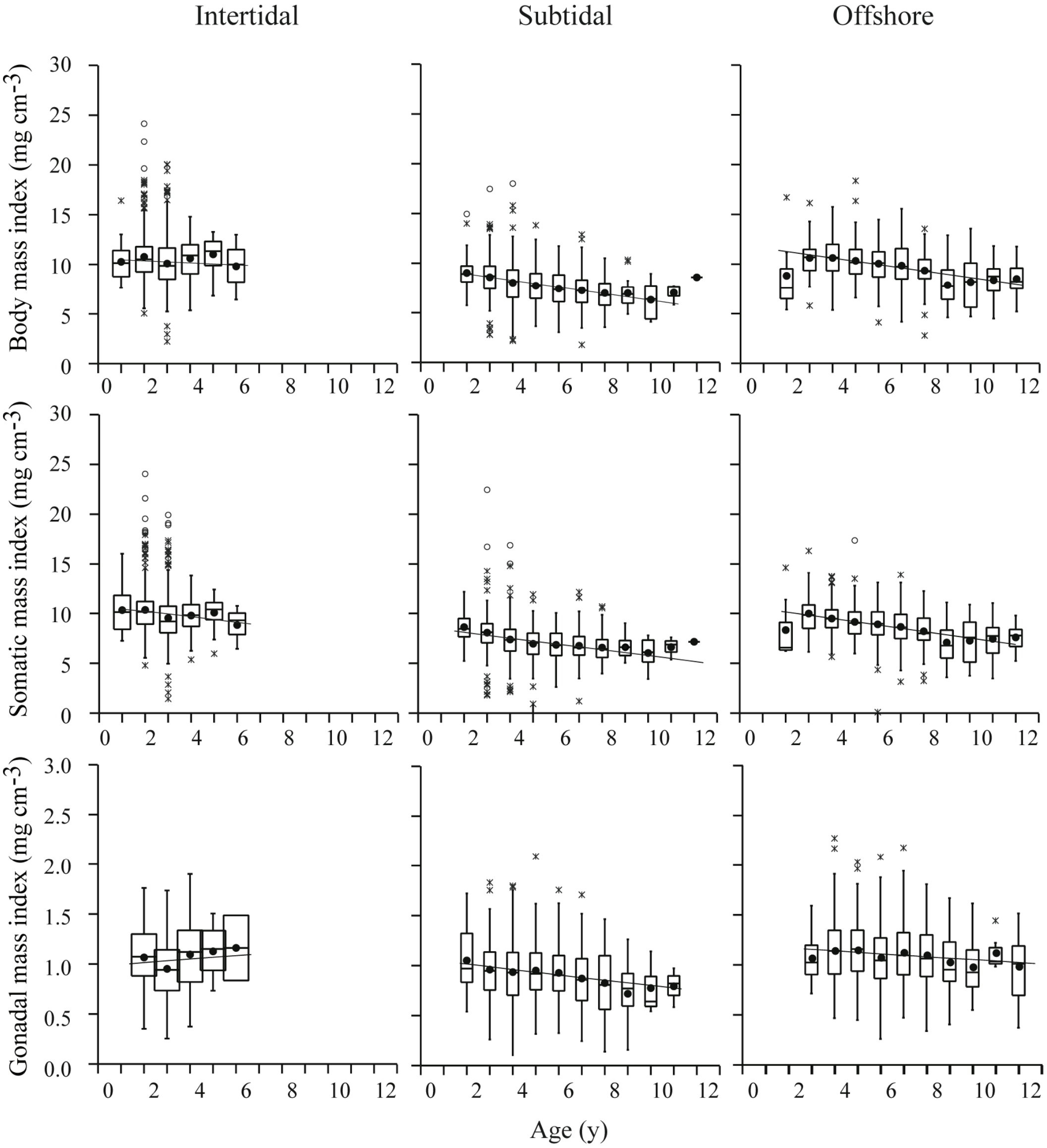

Fig. 4.5. Body mass index (mg cm-3), somatic mass index (mg cm-3) and gonadal mass index (mg cm-3) of *M. balthica* plotted against age (years) for the three stations. Lines are model predictions. Gonadal mass index data are square-root transformed. For more information see legend of Fig. 4.4.

On average, the highest GMI was found offshore. GMI showed significant differences between locations, but did not show a clear relation with age (Table 4.3, Fig. 4.5). In the three ANOVA tests done (for BMI, SMI and GMI), the residuals were normally distributed and there was no trend between residuals and estimates or time.

Table 4.3. Analysis of variance of the body, somatic and gonadal mass indices (mg cm^{-3}) of *M. balthica* from October 2001 to March 2003.

Source	Sum of Squares	df	Mean Square	F	p
Body Mass Index					
Station	979.979	2	489.990	109.795	<0.001
Month	100.925	1	100.925	22.615	<0.001
Season	4233.924	1	4233.924	948.726	<0.001
Age	197.324	1	197.324	44.216	<0.001
Station*Month	1126.317	2	563.158	126.191	<0.001
Station*Season	831.770	2	415.885	93.190	<0.001
Station*Age	17.693	2	8.847	1.982	0.138
Error	13477.501	3020	4.463		
Somatic Mass Index					
Station	673.169	2	336.585	74.113	<0.001
Month	240.548	1	240.548	52.967	<0.001
Season	546.045	1	546.045	120.235	<0.001
Age	2711.151	1	2711.151	596.974	<0.001
Station*Month	712.811	2	356.405	78.478	<0.001
Station*Season	416.902	2	208.451	45.899	<0.001
Station*Age	29.325	2	14.662	3.229	0.040
Error	14064.994	3097	4.541		
Gonadal Mass Index (SQRT-transformation)					
Station	7.693	2	3.847	41.962	<0.001
Month	0.408	1	0.408	4.450	0.035
Season	22.678	1	22.678	247.386	<0.001
Age	0.000	1	0.000	0.000	0.992
Station*Month	12.339	2	6.169	67.299	<0.001
Station*Season	1.622	2	0.811	8.847	0.001
Station*Age	0.722	2	0.361	3.938	0.020
Error	170.232	1857	0.092		

From the decline of GMI in 2002, the spawning periods for the different locations were estimated. In the intertidal, gonadal mass decreased in January-February. In the subtidal and offshore, the decrease occurred later in the season, around April-May (Fig. 4.4). In 2003, sampling stopped before a drop in gonadal mass occurred. The timing of spawning appeared to be earlier for the intertidal (around February) and at lower water temperature (7-8 °C, Cardoso pers. obs.) than at the subtidal and offshore locations (spawning around May at a water temperature of about 11 °C, Cardoso pers. obs.). Gonadosomatic ratio (GSR) was similar between locations (Fig. 4.6). However, in 2002, a clear difference in peaks in GSR could be seen, with highest values in the offshore location and lowest in the intertidal one. Offshore, about 20% of the total body mass consisted of gonads, in contrast with 15% in the subtidal and 10% in the intertidal.

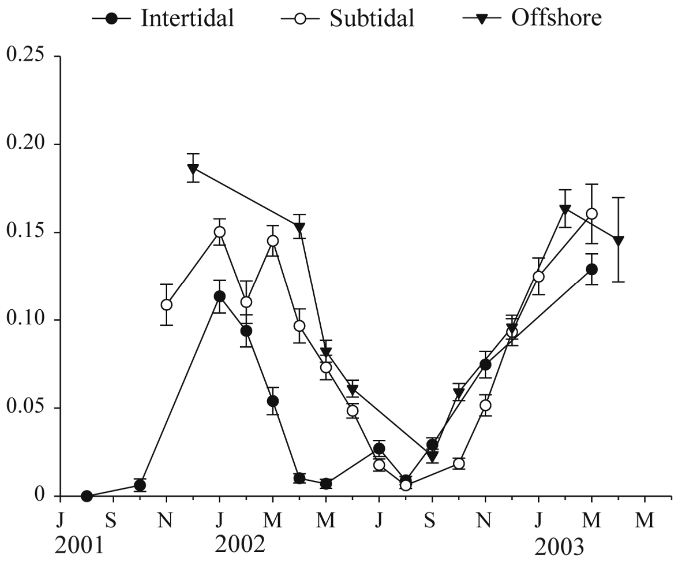

Fig. 4.6. Gonadosomatic ratio (-) of *M. balthica* throughout the year at each location.

To analyse the importance of the different habitats for the persistence of the *M. balthica* population in the western Dutch Wadden, the contribution in terms of gonadal mass (g AFDM m^{-2}) was determined for each habitat in 2002 and 2003. First, the mean gonadal mass per individual was estimated for each age group before spawning (in 2002 for the months of February for intertidal and subtidal locations and December for offshore; in 2003 in the months of March for intertidal and subtidal and April for offshore). Next, a weighted mean gonadal mass per individual (g AFDM ind^{-1}) for the whole population (all age groups) was calculated. Finally, the mean gonadal mass per individual for each area was converted in contribution per m^2 by multiplying with the average density per m^2 (see table 4.1). Results are presented in Table 4.4.

Table 4.4. Estimated contribution in terms of gonadal mass (g AFDM m^{-2}) of each habitat.

Station	Weighted mean gonadal mass (g AFDM ind^{-1})	Contribution per m^2 (g AFDM m^{-2})
2002		
Intertidal	4.3	316.9
Subtidal	9.4	1336.0
Offshore	15.5	868.5
2003		
Intertidal	2.1	159.0
Subtidal	6.3	888.3
Offshore	13.9	778.3

Discussion

Population structure in relation to habitat

Age determination was done by counting the external winter growth marks, a method validated by Lammens (1967). However, in old individuals (> 6 years old) growth marks are closer to each other and more difficult to visualize. Also the earlier rings tend to become less visible (Lammens 1967). This may partially explain some of the observed variability in individual growth within each location. Nevertheless, clear differences in age composition between intertidal and subtidal/offshore locations, illustrate that different life stages prefer different habitats. In this study, 0 years-old *M. balthica* were only found in the intertidal location and not in the subtidal and offshore, confirming that, in general, spat is concentrated in the intertidal (Günther 1991, Beukema 1993, Hiddink and Wolff 2002). This is in agreement with studies on migration patterns of *M. balthica* in the Wadden Sea, reporting settlement in intertidal areas and redistribution to lower intertidal and subtidal areas during the first year of life (Beukema and De Vlas 1989, Beukema 1993, Hiddink and Wolff 2002). The factor triggering initial settlement is unknown.

No individuals older than 5 years were observed in the intertidal, possibly reflecting higher adult mortality in these areas than in subtidal and offshore areas. During submersion, predation by shrimps, crabs and polychaetes (Van der Veer et al. 1998, Hiddink et al. 2002a, Hiddink et al. 2002b) will mainly affect young individuals. In addition, during emersion, adults suffer from predation by birds (Hulscher 1982, Zwarts and Blomert 1992, Dekinga and Piersma 1993). Under the assumption that adult migration is insignificant, the higher instantaneous rate of decrease in the intertidal, might suggest that bird predation is the main factor behind differences in density of adult individuals between habitats. In subtidal and offshore areas, *M. balthica* is not exposed to this type of predation but the existence of predation by large crabs cannot be excluded (Hiddink et al. 2002a).

In bivalves, growth is indeterminate and the asymptotic maximum size is habitat dependent (Sebens 1987). Food availability and other environmental factors affect the physiological costs and influence maximum size. With respect to abiotic conditions, water temperature is by far the most important rate controlling factor. Both the annual temperature patterns as well as mean weekly temperatures were roughly similar for the three locations (Cardoso pers. obs.; http://www.nioz.nl; http://www.waterstaat.nl).

When comparing shell growth between different populations along a latitudinal gradient (Beukema and Meehan 1985, Drent 2004), *M. balthica* from the Wadden Sea showed higher growth than populations from northern and southern locations. This suggests that differences in food conditions between locations may be more important than temperature differences. In other bivalve species, such as scallops (MacDonald and Thompson 1985a, Bricelj et al. 1987, MacDonald and Bourne 1987, Navarro et al. 2000), mussels (Bayne and Worrall 1980, Page and Hubbard 1987, Borrero 1987) and oysters (Paterson et al. 2003), growth rates also seemed to be mainly controlled by food availability. In this respect, feeding conditions for growth of *M. balthica* seem more favourable in the subtidal and offshore: growth of shell is higher at these continuously submerged locations than at the intertidal, also when only similar age classes are considered. Better feeding conditions could be the result of the higher percentage of silt in the water in the subtidal than in the intertidal (Dekker and Waasdorp 2004, Dekker et al. 2002, 2003), leading to higher sedimentation and a potential for better food supply. However, this can not be accessed by the data presented in the present study.

Seasonal patterns in relation to habitat

The seasonal trends in body mass index (BMI), somatic mass index (SMI), and gonadal mass index (GMI) in the intertidal were in accordance with previously described patterns for the Balgzand area (Beukema 1974b, Zwarts 1991, Honkoop and Beukema 1997, Drent 2004). In the subtidal and offshore, these indices followed a similar trend. However, the seasonal variation was different, probably due to differences in food conditions between areas, which could not be accessed by this study. In the intertidal and offshore, the decline in SMI is stronger than in the subtidal, suggesting that either energy uptake is lower or maintenance costs are higher at these locations. In the intertidal, extremely high temperatures during the summer may lead to higher maintenance costs than in the subtidal and offshore locations.

Despite the higher shell growth in the subtidal location than in the intertidal one, GMI is the lowest in the subtidal suggesting poor feeding conditions in this area. In other bivalve species, low food availability has been related to reduced reproductive output (MacDonald and Thompson 1985b, Bayne et al. 1983, MacDonald et al. 1987, Delgado and Camacho 2003). In addition, in *M. balthica*, gonadal development occurs at the expense of body (and somatic mass), since body mass declines with the increase in gonadal mass (De Wilde 1975, Nichols and Thompson 1982, this study). Such patterns have also been observed in other species (MacDonald and Thompson 1986, Lodeiros and Himmelman 1999, Delgado and

Camacho 2003). In this study, the subtidal area had the lowest growth in somatic and gonadal mass.

Importance of different habitats

Selection for higher reproductive output should result in a lower life-span (Calow 1979) and our results seem to support this idea. If subtidal and offshore locations are compared, on average higher reproductive output is observed offshore, but asymptotic size is higher in the subtidal. This evidence is stronger if different latitudinal populations of *M. balthica* are compared (Drent 2004). In comparison to Wadden Sea *M. balthica* populations, populations from Balsfjord (Norway) showed higher reproductive output (about 35% of gonad in relation to total body mass during the peak in gonadosomatic ratio in the Balsfjord vs. 15% in the Wadden Sea) but lower asymptotic length (about 16 mm in the Balsfjord vs. 21 mm in the Wadden Sea). Similar patterns of reduced life-span with increased reproductive output were observed in populations of scallop *Placopecten magellanicus* along its distributional limits in the northwest Atlantic (MacDonald and Thompson 1988).

Intertidal areas are especially important for a relatively short period in the life cycle of *M. balthica,* when spat settles and grows during the first years of life. Although *M. balthica* becomes mature and reproduces at the intertidal, the low percentage of adults with developed gonads and the negative energy balance for most of the year suggest that the contribution in terms of reproductive output might be restricted. The fact that growth and body condition of adults is low in the intertidal, and that most of the individuals do not reach sexual maturation clearly indicates these areas are mainly nursery areas and not good areas for growth and reproduction of adult individuals, at least for *M. balthica*. The subtidal area, with high densities of animals, has the lowest body condition, with low somatic and gonadal mass indices while the offshore location, with the lowest density, shows high BMI, SMI and GMI. Although SMI is similar between intertidal and offshore locations, the main difference lays on the amount of gonads, which is higher offshore. Taking in account that the GMI is low in the subtidal and that somatic and reproductive growth conditions for adult *M. balthica* are not favourable in the intertidal, the amount of spat produced per individual must be higher at the offshore location.

In the western Dutch Wadden Sea, the intertidal, subtidal and offshore areas correspond to about 500 km^2, 1200 km^2 and 450 km^2, respectively (Beukema 1989, Dekker 1989, Holtmann et al. 1996). Inside the Wadden Sea, in intertidal and subtidal areas, densities of *M. balthica* have shown strong variability in the last two decades. In the beginning of the eighties, the contribution of *M. balthica* in terms of total biomass production was higher in the intertidal (Dekker 1989). However, in more recent studies, biomass production was clearly higher in the subtidal areas (Dekker et al. 2002, 2003; Dekker and Waasdorp 2004). Comparing the different habitats and years in terms of contribution in gonadal mass per m^2 (g AFDM m^{-2}), resulted in a ratio of 1:4:3 for intertidal, subtidal and offshore, respectively, in 2002 and in a ratio of 1:6:5 for intertidal, subtidal and offshore in 2003. For an estimation of the total gonadal mass per habitat, the gonadal mass (g AFDM m^{-2}) was multiplied by the area of each habitat (see above), resulting in a ratio of 1:10:2 for the intertidal, subtidal and offshore habitats in 2002 and 1:13:4 for the intertidal, subtidal and offshore habitats in 2003. Despite the fact that offshore animals present higher body condition and higher reproductive output, it is the subtidal area that contributes with most reproductive output in terms of biomass, due to the high densities of *M. balthica* found in these areas. It should be kept in mind that these values are only an indication of the order of magnitude since variability in BMI, SMI and GMI within each habitat was not accounted for. Furthermore, strong year-to-year variability in densities will influence the contribution of each area. These aspects should be part of a future study.

Acknowledgments: We thank the crews of RV 'Navicula' and RV ‘Stern’ for assistance during sampling. Jan Beukema, Wim Wolff, Jaap van der Meer, Jan Drent, Oscar Bos, Pieter Honkoop, Katja Philippart, Pauline Kamermans and two anonymous reviewers provided critical comments on earlier drafts of the paper.

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CHAPTER 5

Spatial variability in growth and reproduction of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) along the west European coast

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Abstract

The Pacific oyster *Crassostrea gigas* was introduced in Europe for commercial purposes in the mid 1960s. It was initially thought that low winter temperatures would restrain this species' reproduction and settlement; however, its present distribution in areas where no introduction has taken place suggests that natural invasion and expansion has occurred. Along the European coast, wild populations of Pacific oysters are already found from northern Germany to southern Portugal. Whether *C. gigas* will continue to further expand through northern waters will depend on its physiological performance. In this study, the performance of wild oyster populations has been studied in terms of growth and reproduction at three stations: La Rochelle (France; 46ºN), Yerseke (Oosterschelde estuary, The Netherlands, 51ºN), and Texel (Wadden Sea estuary, The Netherlands, 53ºN). The French population had the lowest somatic-shell mass ratio, and an increase in maximum shell length, somatic and gonadal mass was observed from France to the Netherlands. In addition, mean oocyte diameter decreased significantly from south to north. The combination of increasing gonadal mass and decreasing oocyte volume suggests an increasing reproductive output in terms of egg numbers from France to The Netherlands. Differences in temperature between locations will at least be partly responsible for the observed patterns; however, other environmental factors (such as food availability, predation pressure, sediment type and/or seston concentration) cannot be excluded. Since smaller eggs (oocytes) are thought to have a longer development time, the environmental conditions along the Dutch coast may result in increased larval dispersal and possibly in further population expansion.

Introduction

Coastal environments have been extensively invaded by exotic (non-indigenous) species, many of them associated with mariculture (for the Wadden Sea see Reise et al. 1998, for The Netherlands see Wolff 2005). Many introductions have failed, but some introduced species have a strong impact on the ecosystem. In the Wadden Sea, some species such as the North American spionid polychaete *Marenzellaria* cf. *wireni* (Essink et al. 1998), the American razor clam *Ensis americanus* (Beukema and Dekker 1995, Armonies 2001), and the Pacific oyster *Crassostrea gigas* (Reise et al. 1998, Dankers et al. 2004, 2006) have even become abundant. The Pacific oyster was introduced for aquaculture purposes in several European coastal waters during the 1960s and 1970s (Meixner 1973, Walne and Helm 1979, Grizel and Héral 1991, Drinkwaard 1999, Wolff and Reise 2002, Dankers et al. 2004, Smaal et al. 2005). It was thought that this species would not be able to reproduce in many of these areas because of its natural distribution in relatively warm waters (Drinkwaard 1999), but its present distribution in northern Europe does not seem to be the result of active introductions only. Natural invasion and expansion are suggested by the recorded new presence, and subsequent increase, in areas where no deliberate introduction has taken place. As a consequence, wild populations are now found along a long stretch of the Atlantic European coast, from northern Germany to southern Portugal (Wehrmann et al. 2000, Dankers et al. 2006, Pouvreau pers. comm. 2005, Peralta pers. comm. 2005, Iglesias pers. comm. 2005).

Pacific oyster larval settlement requires the presence of hard substrates. Thus, in Dutch waters, spat were initially found on dikes (Bruins 1983). Subsequently, invasion and colonisation of Pacific oyster spat in intertidal areas occurred mainly on mussel and cockle beds (Dankers et al. 2004, Diederich et al. 2004). In contrast to other areas (Ren et al. 2003, Diederich et al. 2004), a time lag of about 15-20 years was observed between initial invasion of Pacific oysters at the intertidal Dutch Wadden Sea and population expansion (Dankers pers. comm. 2005). Larval supply may have been the limiting factor for population growth. On the one hand, larval and juvenile survival could have been affected by low winter temperatures because the minimum temperature tolerance of juvenile Pacific oysters is about three weeks at 3 °C in winter (Child and Laing 1998). On the other hand, summer temperatures could have been too low to reach the spawning threshold due to the fact that spawning seems to be induced at around 22 °C (Kobayashi et al. 1997). In corroboration of temperature being a limiting factor in the Wadden Sea, it has recently been noted that invasion and expansion of the Pacific oyster in the German Wadden Sea was accelerated by high late summer water temperatures (Diederich et al. 2004). In summary, water temperature appears to be an important factor for reproduction, survival and further expansion of northern oyster populations. A general latitudinal trend in temperature is observed along the European coast, with average water temperatures decreasing with increasing latitude (http://www.ifremer.fr/, http://www.surf-forecast.com/breaks/, http://www.hmcz.nl, http://

www.bsh.de, http://www.nioz.nl, http://www.dmu.dk, http://www.cefas.co.uk). This decrease in temperature with latitude might potentially determine the ultimate northern limit of the geographical distribution of *C. gigas*.

Further expansion of *C. gigas* will depend on its physiological performance. So far, no information exists on growth and reproduction of wild oyster populations. Therefore, in the present paper, we studied growth and reproduction of three wild oyster populations along a latitudinal gradient, from France (La Rochelle, 46ºN) to the northern part of the Netherlands (western Dutch Wadden Sea, 53ºN). Spatial and temporal variation in growth and reproductive output was assessed by analysing:

(1) age composition and maximum growth rates;
(2) mass allocation to growth and reproduction, by determining amounts of soma and gonads throughout the year;
(3) oocyte size of the various populations to determine the trade-off between egg numbers and egg size.

So far, any analysis of growth in the Pacific oyster has been hampered by the fact that no reliable method was available for age determination. Only recently has a validated method become available based on the seasonal incorporation of manganese in the shell (Langlet 2002, Langlet et al. 2006), and this method will be applied in the present study.

Materials and methods

Identification

Due to the deliberate introduction of Pacific oysters and other *Crassostrea* species from different origins into European waters, it cannot be excluded that along European coasts a mixture of (sub)species was introduced over time and still occurs. Therefore, before starting the sampling program, the presence of *C. gigas* in the sample populations was confirmed by genetic analysis. To that end, the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using primer pair LCO1490-HCO2198 according to Folmer et al. (1994) and resulting sequences were submitted to GenBank (accession codes DQ417690-696).

Sampling

Samples were collected at three intertidal stations (Fig. 5.1) at a similar intertidal level (they were exposed for about 5 h per tidal cycle): La Rochelle (France, 46º N), Yerseke (Oosterschelde estuary, The Netherlands, 51º N) and Texel (Wadden Sea estuary, The Netherlands, 53º N). Salinity conditions were around 33 ppm at La Rochelle and 25-28 ppm at Texel and Yerseke. Around 100 individuals over the whole size range observed were collected randomly in an area of a few km^2, if possible once a month. Sampling took place from June 2003 to May 2004 at La Rochelle and from October 2002 to November 2003 at

Yerseke and Texel. After collection, all samples were stored dry at 5 ºC, transported to the laboratory and processed within the next 48 hours.

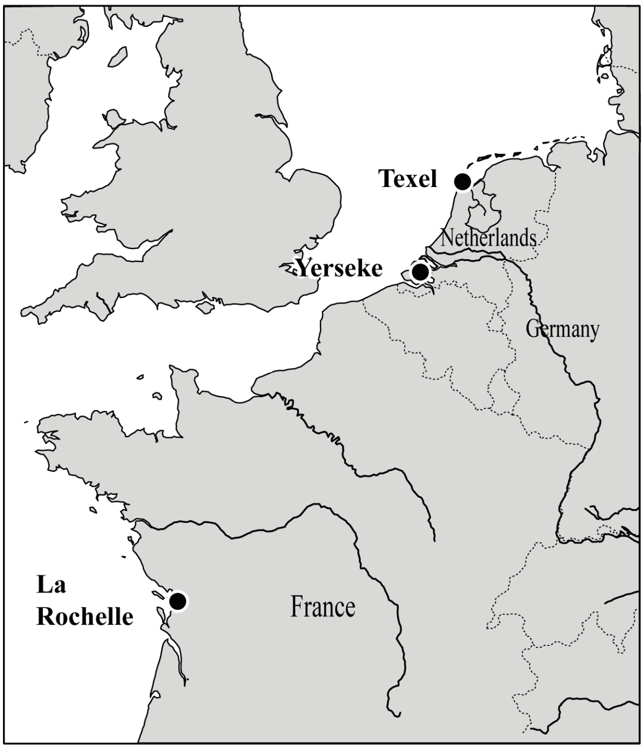

Fig. 5.1. Sampling locations of *C. gigas*: La Rochelle (France), Yerseke (Oosterschelde, The Netherlands) and Texel (Wadden Sea, The Netherlands).

Surface water temperatures were available from long-term data series in subtidal areas in the close vicinity of the sampling stations. Data for La Rochelle were provided by the 'IFREMER, REPHY monitoring network' (via http://www.ifremer.fr/ envlit/region/), data for Yerseke were obtained from the 'Hydro Méteo Centrum Zeeland' (via http://www.hmcz.nl) and data for Texel taken from the 'Royal NIOZ' long-term series (via http://www.nioz.nl, go to Research, Scientific Departments, Physical Oceanography, Ferry and Jerry Observations).

Age determination

Age determination was done by analysing the seasonal incorporation of manganese in the shell according to Langlet (2002) and Langlet et al. (2006). A total of 218 specimens (La Rochelle: 29, Yerseke: 98, Texel: 93) were analysed. Annual growth rings (Fig. 5.2) were determined by analysing the fluctuations in cathodoluminescence of manganese ions on a section of the hinge. For that, shells were cleaned and shell length (defined here as the longest distance from the hinge to the shell edge) of each individual was measured to the nearest 0.01 cm with electronic callipers.

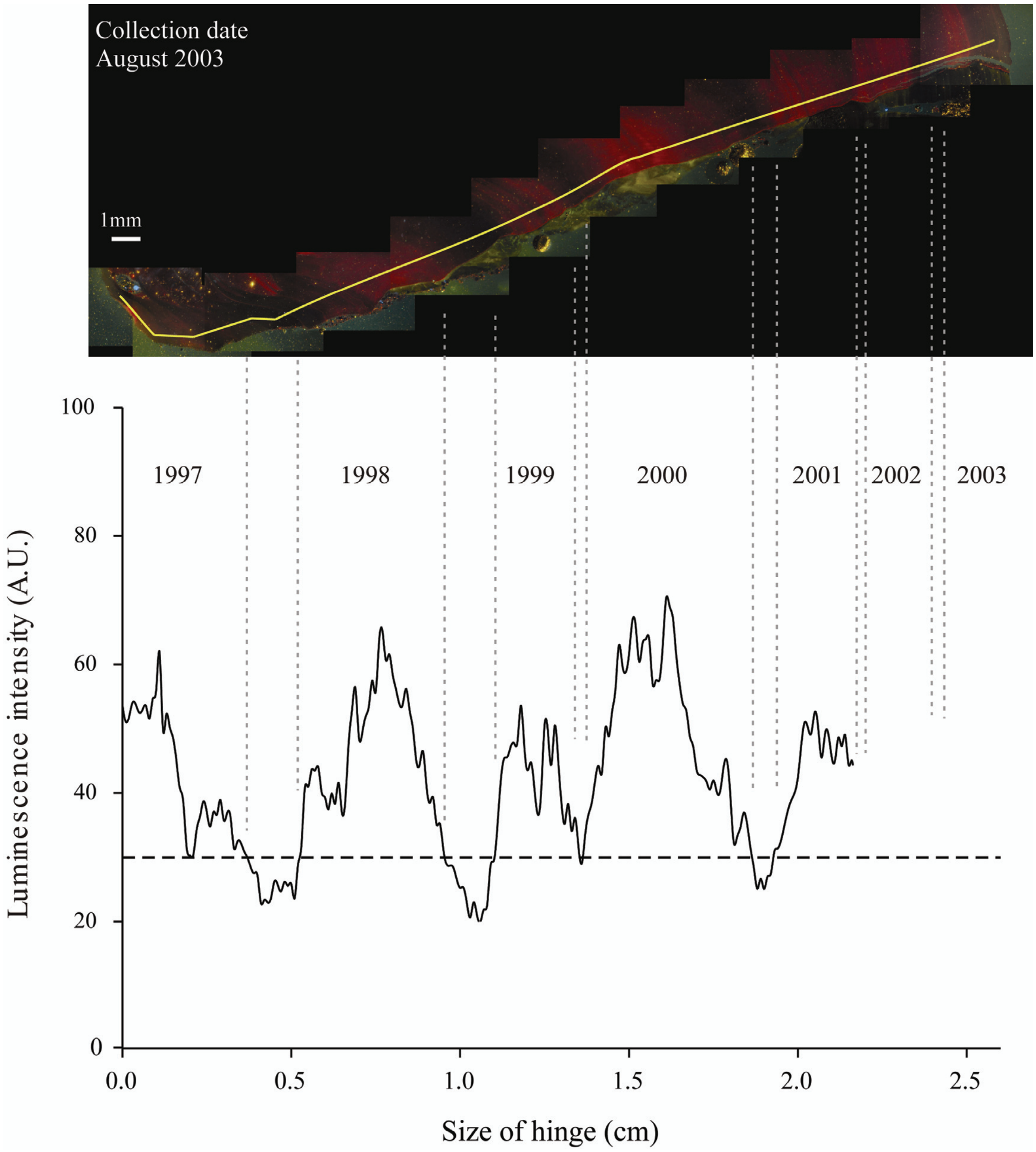

Fig. 5.2. Cathodoluminescence microphotograph (upper panel) of the longitudinal section of the hinge of a 6 year-old *C. gigas* (17.8 cm shell length) from Yerseke, showing successive areas with contrasting natural luminescence; and profile of luminescence intensity (lower panel), measured along the marked line on the photograph, showing 7 peaks corresponding to 7 summer seasons. Horizontal dashed line indicates the threshold under which the luminosity was considered to represent the winter period (see text).

Subsequently, bivalves were opened and all soft parts removed. Shells were left to dry at room temperature for 24 h and weighed to the nearest 0.01 g. After shells were cleaned and dried, the left valves (with hinge) were placed face down in a plastic mould and embedded in epoxy resin (Poly Service, THV-500 epoxyhars and Harder 355), following Ropes (1985). Once hardened, the blocks were sectioned longitudinally through the hinge (Witbaard 1997, Witbaard et al. 1999). The sectioned half valves were then ground flat, wet polished and stuck on a glass slide before being sliced. The facing was ground and wet polished with decreasing grain size polishing suspension (down to 1 µm), leaving a section with a thickness of approximately 500 µm. Digital pictures were then taken with an exposure time of 8 seconds. On the pictures, the variations in luminescence intensity were measured with the ImageJ™ software package (http://rsb.info.nih.gov/ij/) according to the method described by Langlet (2002) and Langlet et al. (2006), whereby the number of peaks of luminosity along a transect through the shell section corresponds with the age of the individual. The intensity of luminescence at the different locations was analysed and a threshold of 30 arbitrary units (A.U.) of luminescence intensity was selected, under which the luminescence was so low that it was considered to correspond to the winter period (for more details see Langlet et al. 2006).

Oocyte size determination

Between June and August 2003, about 50 animals were collected at each station and forced to spawn in the laboratory. After collection, animals were stored one night at 10 ºC. Subsequently, they were placed individually in glass jars (200 to 1000 ml) and a thermal shock was given by adding seawater between 25 and 30 ºC, at a constant salinity of 33 ppm. From each spawned female, a random sample of freshly spawned oocytes was collected, placed on a microscope slide and digital photographs were taken with a Pixera View Finder digital camera fitted to a Zeiss stereo microscope with a final resolution of 1510 pixels per mm. Then sharply focused oocytes were measured using the ImageJ™ software package (http://rsb.info.nih.gov/ ij/). Oocyte size of at least five round eggs per female was determined according to Thorsen and Kjesbu (2001).

Data analysis

Gonads were separated from somatic tissue under a microscope (6.4x). Dry and ash mass of each part were determined to the nearest 0.01 mg, by drying for 4 days at 60 ºC and incinerating for 4 hours at 560 ºC. The difference between dry and ash mass represented the ash-free dry mass (AFDM). Investment in somatic and gonadal mass was analysed by means of the somatic-shell mass ratio (SSM) and gonad-shell mass ratio (GSM) which are defined as the somatic AFDM (mg) divided by shell mass (g) and the gonadal AFDM (mg) divided by shell mass (g), respectively. By dividing AFDM by shell mass, animals of different size could be compared in terms of condition. The relative investment in reproduction was provided by calculation of the gonadosomatic ratio (GSR), described as the gonadal AFDM divided by the total AFDM (soma plus gonads). The extent to which variability in SSM ratio could be accounted for by seasonal variability was examined by ANOVA. Due to an imbalance in the sampling scheme over the year, the effect of time could not be described in terms of differences among all sampling months (that is, by using sampling month as a categorical variable). Instead, we used a linear trend over time in combination with a sinusoidal seasonal effect. The overall time effect was, therefore:

$$\beta 1\ Time + \beta 2 \sin(2\pi ((Month - \beta 3)/12)),$$

in which $\beta 1$, $\beta 2$, and $\beta 3$ are parameters, *Time* is a continuous variable that runs from the first day of observation to the last day, and *Month* is a continuous variable that runs from the first month of observation to the last month. Note that this model is only a linear model when $\beta 3$ is known beforehand. For that, we ran this linear model, which further included the factor site, for all 12 possible values of $\beta 3$ (i.e. the values 1 to 12) and selected the model with the lowest residual mean squares. In order to obtain normality, SSM data were transformed using a squared root transformation.

A similar analysis was used for the analysis of gonadal-shell mass ratio (GSM). However, instead of a sinusoidal function, a block function was used, which divided the year into two periods: a period with a low GSM and one with a high GSM. These periods could differ between sites. Individuals with no gonadal mass were excluded from this analysis. Again, a squared root transformation was used.

For the analysis of growth, shell length (mm), shell mass (g), somatic AFDM (mg) and gonadal AFDM (mg) were plotted against age (years) for each location. Differences in oocyte size (µm) between locations were compared using a two-level nested ANOVA with station and female as categorical factors. All statistical analyses were done using the software package SYSTAT (Wilkinson 1996).

Results

Temperature conditions

Temperature patterns over the last 10 years show that differences between stations occurred mainly in winter (Fig. 5.3a), with milder winters prevailing at La Rochelle. The minimum winter water temperature during this period was 3.5 °C at La Rochelle, while in Yerseke and on Texel it was around 1.7 °C and -0.6 °C, respectively. Summer temperatures were similar for the three stations, although in most years higher maximal temperatures were observed at La Rochelle. During the sampling period, temperatures followed the same trend, with La Rochelle presenting milder winters and slightly warmer summers than the other two locations (Fig. 5.3b). Overall, lowest temperatures occurred between December and February and highest temperatures from June to August. Small differences in temperature between Yerseke and Texel occurred mainly in the summer of 2003, when Yerseke had slightly higher temperatures. On average, water temperatures during the winter period (defined as the period 1 November – 31 March) were around 9 ºC at La Rochelle and 7 ºC at Yerseke and Texel, while in summer (defined as the period 1 April – 31 October) water temperatures were around 19 ºC, 17 ºC and 15 ºC for La Rochelle, Yerseke and Texel, respectively. In 2003, temperatures were higher than in 2002, with higher summer and winter temperatures at the three locations.

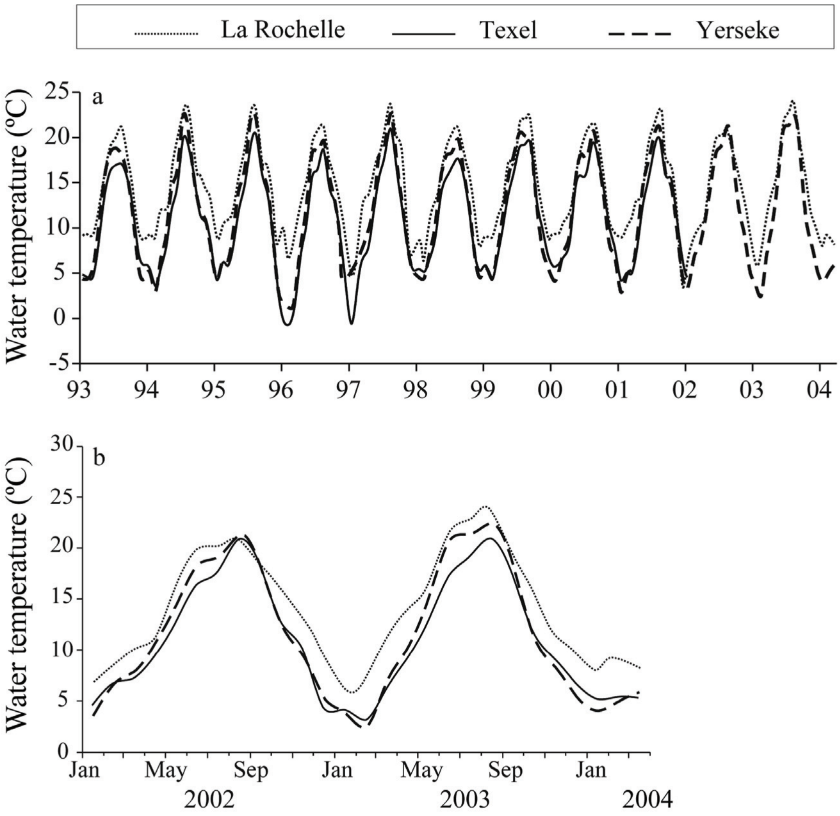

Fig. 5.3. Water temperatures (ºC) at the three locations. (a) monthly mean temperature patterns from the last 10 years; (b) temperature measurements during the sampling period.

Annual growth

Individuals up to 6 years old were found at Texel and La Rochelle, and up to 7 years old at Yerseke. No missing age classes were observed at any of the stations, indicating annual recruitment over the last 7-year period.

At the three locations, shell length, shell mass, somatic mass and gonadal mass showed a large scatter with age (Fig. 5.4). Nevertheless, differences between locations were found. Maximum shell length and mass observed were about 20 cm and 360 g on Texel, 19 cm and 300 g in Yerseke and 10 cm and 48 g in La Rochelle. Maximum somatic and gonadal masses observed followed the same pattern and were around 5.6 and 3.4 g on Texel, 4.5 and 3.1 g in

Yerseke and 0.8 and 0.4 g in La Rochelle. The results suggested an increase in maximum shell length, shell mass and somatic mass from La Rochelle to Texel.

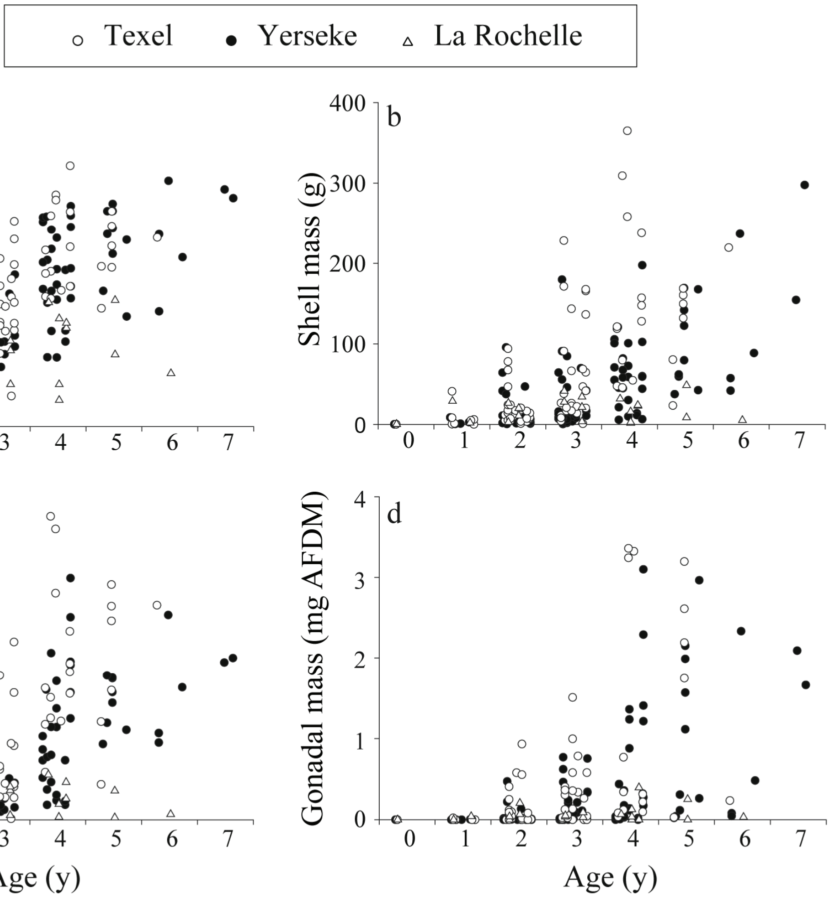

Fig. 5.4. Relationship between age (y) of *C. gigas* and a) shell length (mm); b) shell mass (g); c) somatic mass (mg AFDM) and d) gonadal mass (mg AFDM) (open circles: Texel; full circles: Yerseke; triangles: La Rochelle).

For comparison with previous studies, trials were done to fit Von Bertalanffy Growth (VBG) curves for growth of shell length. Due to a scarcity of individuals >5 y old in the samples, this was only successful for Texel and La Rochelle (Texel: L_∞ = 23.8±6.8 cm, k = 0.001 d^{-1}, n = 93; La Rochelle: L_∞ = 6.8±0.9 cm, k = 0.002 d^{-1}, n = 29). However, the large standard error of the parameter estimates indicates that results were not reliable. Mean values of shell length for each location per age group are presented in Table 5.1 (age groups >4 years old were excluded due to the low number of observations and very large variability).

Significant relationships were found between shell length and total body mass for the three stations (Fig. 5.5a); however, the scatter in the data was considerable. The scatter decreased when shell mass was taken instead of shell length (Fig. 5.5b). Therefore, seasonal growth in somatic and gonadal mass was analysed by standardising for shell mass instead of shell length.

Table 5.1. Mean shell length (cm) per age group of *C. gigas* for Texel, Yerseke and La Rochelle

	Texel		Yerseke		La Rochelle	
Age	Mean±SE	n	Mean±SE	n	Mean±SE	n
0	10.0±1.7	8	9.8±1.3	8	9.1±1.0	9
1	26.9±7.7	13	27.8±11.8	4	42.6±16.2	2
2	70.2±6.4	25	58.2±7.0	16	60.6±9.7	4
3	97.2±7.3	27	76.6±6.3	28	63.3±9.0	5
4	140.0±9.6	13	118.5±6.9	26	65.0±12.6	6

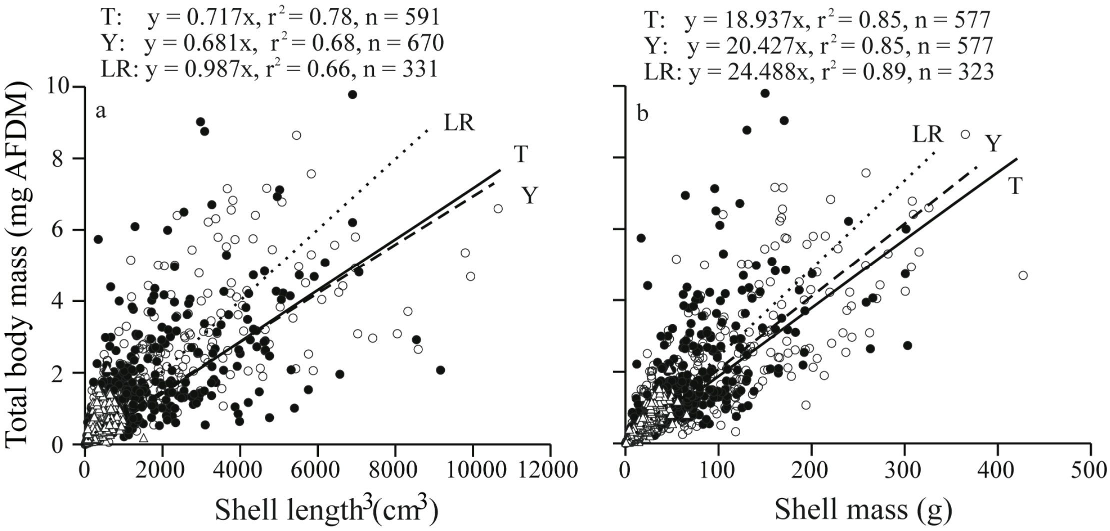

Fig. 5.5. Relationship between total body wet mass (g) of *C. gigas* and a) third power of shell length (cm^3); b) shell mass (g), with weighted regression lines. T: Texel; Y: Yerseke; LR: La Rochelle.

Seasonal patterns

In all analyses, sexes were treated together because no significant differences in somatic- (SSM) and gonadal-shell mass ratios (GSM) were found between sexes (ANOVA, $p > 0.05$). The linear model run to determine the value of β_3 showed the lowest residuals when β_3 was 3 for La Rochelle, 8 for Yerseke and 6 for the Texel. A seasonal pattern in SSM was found in all three locations (Fig. 5.6). An increase in ratio occurred during spring with maximum mean values at Texel in May and at La Rochelle in September. In Yerseke, two peaks in SSM were found, in April/May and in August/September. The decrease in SSM between the two peaks might be due to spawning. Significant differences were found between locations (Table 5.2), whereby the interactions Station*Month and Station*Season (sinusoidal function) were significant. The increase in SSM during the growing season was higher in Yerseke and Texel than in La Rochelle.

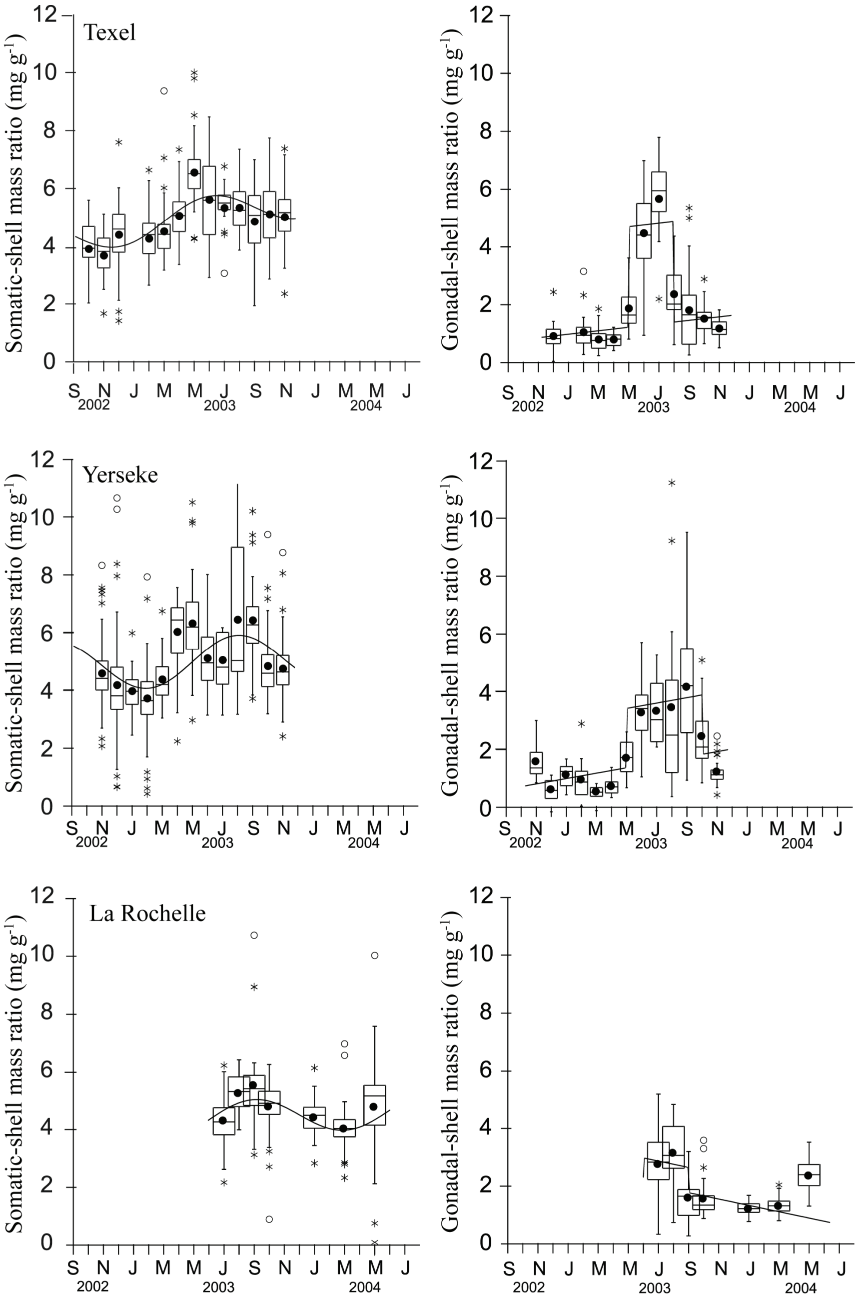

Fig. 5.6. Somatic-shell mass ratio (mg g^{-1}) and gonad-shell mass ratio (mg g^{-1}) of *C. gigas* along the year. Original values were square root transformed. Curves and lines are model predictions. Full dots and horizontal bars indicate respectively mean and median value; boxes represent the range within which the central 50% of the values fall; bars represent the data range excluding outliers; outliers and extreme values are, respectively, observations more than 1.5 and 3 times the box range and are represented by asterisks and open circles.

GSM ratios showed very low values in winter and spring, with peak values in La Rochelle in July/August, in Yerseke from June to September, and on Texel in June/July (Fig. 5.6). Significant differences between locations were found in the interaction term Station*Season (block function) (Table 5.3). These differences were significant between Texel and Yerseke, and Texel and La Rochelle, but not between Yerseke and La Rochelle (Fisher's LSD post-hoc test). In Yerseke and La Rochelle, maximum values were similar but during winter GSM was higher in La Rochelle. At Yerseke, a longer period of high ratios was seen and individuals without gonad were mostly found from November to January. On Texel, most individuals had no gonadal tissue between October and December while at La Rochelle many individuals were empty around March. At this last location, the proportion of animals that spawned completely was lower than at the other locations. Gonadosomatic ratio (used as a measure of reproductive effort) was highest on Texel: about 30% of the total body mass consisted of gonads in July (not shown). If only individuals with developed gonads were considered, this value would even increase to 50%.

Table 5.2. Analysis of variance of the somatic-shell mass ratio (SSM; mg g^{-1}) of *C. gigas*, from October 2002 to April 2004, after square-root transformation.

Source	Sum of Squares	df	Mean Square	F	*p*
Station	26.764	2	13.382	8.543	0.000***
Month	43.635	1	43.635	27.857	0.001***
Season	12.634	1	12.634	8.066	0.005**
Station*Month	21.327	2	10.663	6.808	0.001**
Station*Season	129.450	2	64.725	41.321	0.000***
Error	2437.300	1556	1.566		

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 5.3. Analysis of variance of the gonadal-shell mass ratio (GSM; mg g^{-1}) of *C. gigas*, from October 2002 to April 2004, after square root transformation.

Source	Sum of Squares	df	Mean Square	F	*p*
Station	1.263	2	0.632	0.528	0.590
Month	7.792	1	7.792	6.520	0.011*
Season	214.844	1	214.844	179.774	0.000***
Station*Month	3.912	2	1.956	1.637	0.195
Station* Season	75.488	2	37.744	31.583	0.001***
Error	893.922	748	1.195		

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Oocyte size

Spawning was induced in 56 females and a total of 1619 oocytes were measured. Oocyte size did not differ significantly between months (ANOVA, p>0.05). The mean oocyte diameter of ripe female oysters decreased from south to north, from about 53 µm at La Rochelle, 50.5 µm at Yerseke to 48.5 µm at Texel, corresponding with a volume of 77912 μm^3 at La Rochelle, 67399 μm^3 at Yerseke and 59704 μm^3 at Texel. Variability in oocyte diameter within each location was low, but the mean diameter differed significantly between stations (ANOVA, $F_{(2,55)} = 10.125$, $p < 0.001$).

Discussion

Growth patterns

In this study of the Pacific oyster, large differences were found in the various length-at-age and mass-at-age data between the three areas. The maximum size observed showed a gradient with increasing shell and somatic mass from La Rochelle to Texel and the same pattern was observed for gonadal mass. Oysters from La Rochelle built up relatively small gonads while about 14 times higher values were found at Yerseke and even higher values on Texel. Somatic- and gonadal-shell mass ratios followed the same trend with latitude although differences were not so obvious. On average, somatic-shell mass ratio was lower in La Rochelle than at the other two stations and the peak in gonadosomatic ratio was highest at Texel. Besides, a large part of the individuals from La Rochelle did not spawn completely. Overall, a general trend of increase in terms of growth and reproduction was found from La Rochelle to Texel.

Trends in growth with latitude are generally related to temperature patterns. Along the European coast, mean temperatures vary between around 25 °C in summer and 14 °C in winter in southern Europe, to about 15 °C in summer and 2 °C in winter in northern Denmark and in England (http://www.ifremer.fr/, http://www.surf-forecast. com/breaks/, http://www.hmcz.nl, http://www.bsh.de, http://www.nioz.nl, http://www. dmu.dk, http://www.cefas.co.uk). Nevertheless, north-south trends in growth patterns in bivalve species are inconsistent, suggesting that local environmental conditions are important as well. In the Baltic tellin *Macoma balthica*, for example, growth along the American coast decreases towards the north (Gilbert 1973, Beukema and Meehan 1985) but along the European coast, growth is maximal at intermediate latitude (Beukema and Meehan 1985, Drent, 2004). In populations of *Mya arenaria* from the America coast, no clear pattern in growth was found (Brousseau 1979, Brousseau and Baglivo 1987), and in wild European populations of *C. gigas* maximum shell length in the northern German Wadden Sea was lower (180 mm, Diederich 2006) than on Texel (220 mm, this study). In addition, maximum length of wild *C. gigas* from the south coast of Portugal was observed to be around 155 mm (Peralta pers. comm. 2005) while in La Rochelle it was about 100 mm (this study). Apparently, there is not a clear latitudinal trend in growth in shell length of *C. gigas* along the European coasts.

The seasonal pattern in water temperature indicates a consistent gradient in temperature conditions between the sampled locations. Winter temperatures at La Rochelle are usually higher than at the other stations. Since minimum temperature tolerance of juvenile Pacific oysters is about three weeks at 3 ºC in winter (Child and Laing, 1998), this may be an important factor determining oyster survival during early life. Differences in food conditions could also be responsible for the observed differences in growth. Suboptimal food conditions in Yerseke and La Rochelle could be induced by the high densities in culture plots; however, in both areas, sampling occurred far away from exploited cultures. Sediment type, seston concentration and predation pressure are other factors that could lead to growth differences between populations. These factors have been seen to affect growth of several bivalve species by hampering filtration and digestion processes (Kiørboe et al. 1981, Newell and Hidu 1982, Navarro et al. 1992, Kamermans and Huitema 1994, Gatenby et al. 1996, Iglesias et al. 1996, MacKenzie and McLaughlin 2000, Carmichael et al. 2004). In oysters, such factors seem to play an important role as well. Predation by fish and gastropods has been observed to affect growth of spat and juveniles (Anderson and Connell 1999, Villarroel et al. 2004). Also pollution is known to decrease filtration activity, and lead to inhibition of growth in larvae and adult oysters (Fichet et al. 1998, Encomio and Chu 2000, Nice et al. 2000, Elfwing and Tedengren 2002). Since these factors were not considered in this study, the causes of the observed differences in somatic and gonadal growth between locations cannot be accessed.

In addition to the overall differences between areas, large differences in environmental conditions must also occur within an area, leading to the large variability in individual growth within each location. In fact, most individuals seem to show reduced growth. For bivalve species occurring in dense intertidal beds competition for food seems likely. In the Pacific oyster, food limitation has been observed under field conditions (Fujisawa et al. 1987, Brown 1988, Brown and Hartwick 1988). During suitable temperature conditions, oysters from Canada showed higher growth rates in terms of shell length and body mass when food level was higher (Brown and Hartwick 1988). Similar results have been recorded in other areas for other bivalve species such as mussels (Kamermans 1993, Alunno-Bruscia et al. 2000), cockles (Kamermans 1993, De Montaudouin 1996) and *M. balthica* (Kamermans et al. 1992). The similarity in food source and timing of feeding in the various bivalve species might even suggest that inter- and intra-specific competition for food is a general phenomenon in bivalves. The observed relationship between the primary production of various estuarine systems and its macrofaunal biomass (Herman et al. 1999) supports this suggestion.

Reproductive patterns

In terms of oocyte volume, a significant decrease was observed from La Rochelle to Texel. Along the French Atlantic coast, Lango-Reynoso et al. (2000) found no significant differences between sites. However, these authors measured oocyte size in histological preparations of the gonad where oocytes in different stages of maturation can be found, causing a large variability in the data. During the spawning period, their largest measured oocyte was 61.4 µm and the smallest 19 µm, and the mean population oocyte size reported was of 34.9 µm. Measurements of oocyte size in mature individuals has also been done in other bivalve species and related to body growth. In populations of *Mya arenaria* along the north-eastern American coast, oocyte size was largest in southern populations and a positive relation was found between oocyte size and body growth (Appeldoorn 1995). In the south, the more variable environmental conditions and high temperatures seemed to cause high juvenile mortality, leading to the necessity of investing more energy per egg and the production of larger (and more resistant) but fewer eggs (Appeldoorn 1995). The same trend of growth and oocyte size was seen in the Wadden Sea population of *M. balthica* and *Cerastoderma edule* at different intertidal levels (Honkoop and Van der Meer 1997). In these two species, growth and oocyte size were higher at the lowest tidal level, where submersion time and daily feeding periods were longer. However, in the present study, the opposite trend was observed, with northern populations presenting higher growth but smaller oocytes. Massapina et al. (1999) and Ren et al. (2003) observed a significant positive relationship between the condition of the spawning adults and the oocyte and gonad quality. This could mean that the inverse trend in growth and oocyte volume found between La Rochelle and Texel is a reflection of the fact that under good feeding conditions smaller eggs can be produced without negatively affecting their quality. A definitive answer can only be obtained by an analysis of oocyte quality and composition, in relation to oocyte volume and adult condition.

The potential for future population expansion of *C. gigas*

Conditions for growth and reproduction seem to be more optimal in the northern stations of Texel and Yerseke than in La Rochelle. Development of gonads and reproduction occurred in all areas but was much more successful in the northern stations. This suggests that the process of maturation and reproduction has not been the bottleneck for population expansion neither in the Oosterschelde nor in the Dutch Wadden Sea. Substantial egg and larvae survival necessary to guarantee the next generation may have been the key-factor for population growth in the past. At low abundances, fertilisation may be a problem and only under favourable conditions it will be successful. For instance, the first intensive spatfalls in the Oosterschelde and Wadden Sea were recorded in years with warm summers (Drinkwaard 1999). Also juvenile survival may have been an important factor (Child and Laing 1998) and mild winters may be required to guarantee sufficient juvenile survival to build up the population. The occurrence of successful fertilisation and survival of the planktonic stages may partly explain the observed time lag between introduction and rapid increase in population abundance some years later.

An important point in relation to the expansion of the species seems to be the observed trend in reproductive output. The decrease in oocyte size and increase in gonadal mass from south to north suggests that there is an enormous increase in numbers of eggs spawned, and, therefore, a capacity for further population expansion with latitude. An increase of energy investment per offspring (larger egg volume) results in either large offspring (larvae) or/and shorter egg development time (Kooijman 2000). If within a species, the size (volume) of the hatchling is constant, larger eggs result in shorter incubation (development) time (Kooijman 2000), because more energy for a similar trajectory (till hatching) allows a faster development time. In this way, the larger oocytes (and eggs) produced by oysters at La Rochelle, under relatively poor growth conditions, will develop faster, reducing pelagic stage duration and therefore also restricting larval dispersal. The populations in the Oosterschelde and Wadden Sea area, which are under more optimal conditions, produce larger gonads containing smaller oocytes. The resulting higher egg numbers and longer development time will increase larval dispersal and allow expansion of the population. A consequence may be the pattern observed in the east Frisian German Wadden Sea: introduction of the Pacific oyster occurred around 1980 near Texel and this population is thought to be the source of larval settlement in the 1990s in the East Frisian area (Wehrmann et al. 2000).

The rapid expansion of the Pacific oyster in the Dutch and German Wadden Sea (Wehrmann et al. 2000, Diederich et al. 2005, Dankers et al. 2006) and the observed patterns in growth and reproduction suggest that this species has not reached its eco-physiological limits yet. In fact, the northern distributional limit of *C. gigas* already extends as far as Denmark (Bos et al. 2005, Dankers et al. 2006). Further expansion will depend on environmental conditions, such as food availability, suitable summer temperatures for spawning, presence of hard substrate for initial settling (Bruins 1983) and mild winters, which determine the survival of juvenile oysters (Child and Laing 1998). The Swedish west coast has a seasonal pattern in water temperature similar to that of the Wadden Sea, except that winter temperatures are slightly lower (Pihl and Rosenberg 1982). Together with the extensive presence of hard substrate and the fact that mild winters have been more common during the last years, this suggests that the Pacific oyster may be able to extend its northern distribution.

To obtain more insight, a study of the performance of other wild European Pacific oyster populations over a more marked environmental gradient, not only in terms of temperature but also in terms of food conditions, is required.

Acknowledgements: Thanks are due to M. Alunno-Bruscia and K. Troost for help with sampling in France and Yerseke; to R. Witbaard, F. Lartaud and M. Rafelis for helping with age determination; and to J. van der Meer, J.J. Beukema, W.J. Wolff, N.M.J.A. Dankers, J. Drent, P.J.C. Honkoop and three anonymous referees for their comments on earlier versions of this paper.

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CHAPTER 6

Body-size scaling relationships in bivalve species: a comparison of field data with predictions by the Dynamic Energy Budget (DEB) theory

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Abstract

In this paper, we apply the Dynamic Energy Budget (DEB) theory to bivalve species (1) to provide basic body-size scaling relationships that can be used to predict species characteristics when basic information is lacking, and (2) to analyse the discrepancy between DEB predictions based on energetic constraints and field observations to identify potentially important factors in life history strategy of bivalves. Body-size scaling relationships were identified for size at first reproduction, Von Bertalanffy growth rate, and egg and larval development time in relation to egg and larval volume and temperature conditions. Due to their small egg volume, bivalve species are characterised by a relatively short pelagic larval stage. The main discrepancy between field observations and DEB predictions was in the relationship between egg and larval volume and adult body volume. In bivalves, the characteristics of the early life stages are not related to body size of the species. Since the minimum size of settling larvae is always larger than 125 µm, it is suggested that successful settlement might be the key factor. Settlement size or volume of the fragile larvae must be in balance with the sediment composition, i.e. similar to or larger than that of the sediment grain size.

Introduction

The benthic community of estuarine coastal systems is characterised by a mosaic of species, including a variety of bivalves (for a general overview see: Ketchum 1983, Mathieson and Nienhuis 1991). In the western Dutch Wadden Sea, various bivalve species account for most of the biomass (Beukema 1976). Although the maximum age of the various species is about the same (Tebble 1966), they differ with respect to growth and reproduction. For instance, their maximum length differs by more than a factor of 10 (Tebble 1966, Carriker and Gaffney 1996, Poppe and Goto 1993). Despite these large differences, annual spatfall and subsequent recruitment of all species is successful (Beukema et al. 2001, Van der Meer et al. 2001) indicating that different life history strategies can be adopted successfully in these fluctuating estuarine environments.

Any analysis of life history strategies in terms of the trade-off between growth and reproduction requires a general framework to compare the energetics of the different species in a systematic way. The Dynamic Energy Budget (DEB) theory (Kooijman 1993, 2000) offers this general framework. DEB models can be used in various ways. First of all, various aspects in the life history and energetics of different species can be captured in a single model whereby differences between species are reflected in differences in parameter values only. Van der Veer et al. (2006; Chapter 7) provide estimates of the various DEB parameters and a comparison of sets for a number of abundant bivalve species in Dutch coastal waters. Based on these, Cardoso et al. (2006; Chapter 8) applies the DEB model to analyse the intra- and interspecies differences in energy flow in bivalve species in Dutch coastal waters.

A second application of the DEB theory is the prediction of various body-size scaling relationships. Such relationships have already been under study and under debate for decades since the pioneering work of Kleiber (1932) on the scaling of metabolic rate with body size. Thus, numerous empirical relationships of biological rates as simple functions of body size and other variables have been established (for an overview see Peters 1983). Although this descriptive ecology has proved to be valuable, it has been criticised for seeking post-hoc explanations of observed patterns without experimental tests of hypotheses (Blackburn and Gaston 1999). The DEB theory of Kooijman (1993, 2000), based on surface- and volume-related processes can be considered as a clear response to this criticism. However, the DEB theory has so far not been recognised as such. Despite the fact that it was introduced in the late 1980s, recent literature is still suggesting that we lack a general theory on body-size scaling relationships based on first principles (e.g. Blackburn and Gaston 1999, Brown et al. 2004, Harte 2004). To our knowledge, the DEB theory is the only theory that is based on a set of general assumptions regarding food uptake, storage and utilisation that predicts many types of intra- and interspecific scaling relationships (Kooijman 2000).

In this paper, we develop the predictions of the DEB theory for bivalves with the aims of:
(1) providing basic body-size scaling relationships that can be applied to predict species characteristics in case basic information is lacking;
(2) analysing the discrepancy between DEB predictions based on energetic constraints and field observations to identify potentially important factors in life history strategy of bivalves.

DEB model predictions

A conceptual introduction of the DEB theory can be found in Kooijman (2001), and a full description is given in Kooijman (2000). The DEB theory describes the energy flow through an animal (Fig. 6.1) and also changes therein in environments with varying food densities and temperatures. In the model, three life stages (embryos, which neither feed nor reproduce; juveniles, which feed but do not reproduce; adults which both feed and reproduce) and three main body components (structural biovolume or somatic tissue; stored energy reserves; and gonads and/or stored energy reserves allocated to reproduction) are distinguished. Five equations with a small number of parameters (Table 6.1) can fully determine feeding, growth, survival and reproductive behaviour (see Kooijman 2000).

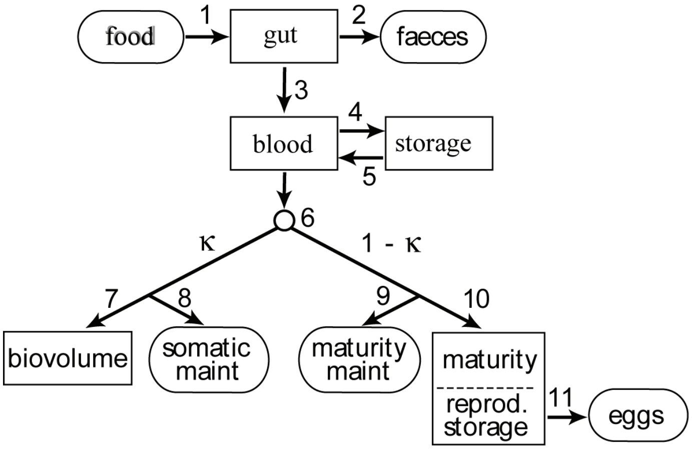

Fig. 6.1. Energy flow through an organism in the DEB model, after Van Haren (1995). Rates: 1 ingestion (uptake), 2 defecation, 3 assimilation, 4 demobilisation of energy into reserves, 5 mobilisation of energy from reserves, 6 utilisation, 7 growth, 8 somatic maintenance, 9 maturation maintenance, 10 maturation, 11 reproduction. The rounded boxes indicate sources or sinks; the rectangles indicate state variables.

The DEB theory implies interspecific body-size scaling relationships via a classification of its parameters into intensive parameters (which do not depend on the body size of the organism) and extensive parameters (which do depend on body size). In the DEB theory, size is measured in terms of volume. However, since literature data on volume is scarce, the volumetric length (i.e. the cubic root of the volume) is used to quantify body size.

Table 6.1. Primary and compound parameters of the DEB model. Notation after Kooijman (2000).

Symbol	Dimension	Interpretation
Primary parameters		
T_A	K	Arrhenius temperature
$\{\dot{J}_{Xm}\}$	J cm^{-2}d^{-1}	Maximum surface area-specific ingestion rate
ρ	—	Losses due to digestion
$\{\dot{p}_{Am}\}$	J cm^{-2}d^{-1}	Maximum surface area-specific assimilation rate
$[\dot{p}_M]$	J cm^{-3}d^{-1}	Volume specific maintenance costs
$[E_m]$	J cm^{-3}	Maximum storage density
$[E_G]$	J cm^{-3}	Volume-specific costs of growth
$[E_V]$	J cm^{-3}	Volume-specific structural energy content
κ	—	Fraction of utilized energy spent on maintenance plus growth
δ_m	—	Shape coefficient
Compound parameters		
$\dot{v} = \{\dot{p}_{Am}\}/[E_m]$	cm d^{-1}	Energy conductance
$\dot{k}_M = [\dot{p}_M]/[E_G]$	d^{-1}	Maintenance rate constant
$g = [E_G]/\kappa[E_m]$	—	Investment ratio
$\dot{r}_B = \left(3/\dot{k}_M + 3fV_m^{1/3}/\dot{v}\right)^{-1}$	y^{-1}	Von Bertalanffy growth rate

Some of the volume- and growth-related relationships are temperature dependent and need to be corrected for temperature differences before a comparison between species can be made. Therefore, we corrected for differences in temperature using the Arrhenius relationship, which is an alternative to the Q_{10} (for details see Kooijman 1993, 2000). The predictions of various body-size scaling relationships are based on Kooijman (1986). There are three types of relationships: (1) primary scaling relationships, which can be derived from the structure of the DEB model and do not involve empirical arguments; (2) secondary scaling relationships, which can be derived from primary relationships and require empirical arguments; and (3) tertiary scaling relationships, which derive from the assumptions of the DEB model but require extra assumptions about individual interactions. While primary (predictions 1 to 3) and secondary (predictions 3 to 6) scaling relationships consider that two species show the same behaviour with respect to energetics as long as food density is constant (the so-called "invariance property"), tertiary scaling relationships (prediction 7) invoke indirect effects via the population level, such as competition for food.

Prediction 1: Maximum theoretical body volume is independent of temperature

According to the DEB theory, under optimal food conditions, the maximum theoretical body volume or length of a species is determined by the resources available and by maintenance requirements, according to Kooijman (2000; page 94):

$$V_{\max}^{1/3} = \kappa\{\dot{p}_{Am}\}/[\dot{p}_M] \qquad [1]$$

wherein:

$V_{\max}^{1/3}$ is the maximum volumetric length (δ*L;m);

κ is the fraction of utilised reserve spent on somatic maintenance plus growth (-)

$\{\dot{p}_{Am}\}$ is the maximum surface area-specific assimilation rate (J cm^{-2} d^{-1}) and

$[\dot{p}_M]$ is the volume-specific cost of maintenance (J cm^{-3} d^{-1}).

Both $\{\dot{p}_{Am}\}$ and $[\dot{p}_M]$ are similarly temperature dependent; hence the ratio is inherently species-specific and not dependent on temperature.

Prediction 2: Egg volume is proportional to maximum theoretical body volume$^{4/3}$

Just after fertilisation, eggs consist almost completely of reserve energy; from then on and until birth, structure develops at the expense of the reserves. Birth is the moment of transition from a non-feeding stage to a feeding stage. Between species, the reserve density at birth, which is the ratio of the amount of reserve and structure, scales with volumetric length because the amount of energy an organism is able to store is dependent on its size (volume). Therefore, the amount of reserve in an egg, and thus the egg volume, will scale with maximum body volume$^{4/3}$. However, there is a large variation in egg size between taxa, thus the tendency of egg size to be proportional to maximum volumetric length only holds for related species (Kooijman 2000), such as bivalves.

Prediction 3: Volume at first reproduction is proportional to maximum theoretical body volume

The κ-rule of the DEB theory states that a fixed fraction κ of the utilised reserve is allocated to somatic maintenance plus growth, the rest to maturity maintenance plus maturation (or reproduction in adults). Juveniles have to mature and become more complex (i.e. develop new organs and regulation systems) while the increase in size (structure) in adults does not include an increase in complexity. The energy spent on development in juveniles is spent on reproduction in adults. In this way, stage transitions (from embryo to juvenile or from juvenile to adult) occur if the cumulative investment in maturation exceeds a specified threshold. In the DEB theory, this threshold is thought to be proportional to maximum structural volume because more complex organisms (with higher structural volume) will take more time to reach maturity. Therefore, volume at first reproduction also scales with maximum body volume. However, this relationship may vary between taxa (Kooijman 2000).

Prediction 4: Volume at hatching and settlement is proportional to the maximum observed body volume

The maximum observed body volume an individual can achieve will depend on the actual prevailing food conditions, according to Kooijman (2000; equation 3.23, page 95):

$$V_\infty^{1/3} = fV_{\max}^{1/3} \qquad [2]$$

where

$V_\infty^{1/3}$ is the ultimate volumetric length (δ*L; m);

f is the scaled functional response (-), which is a measure of food supply (Holling, 1959) and

$V_{\max}^{1/3}$ is the maximum volumetric length (δ*L; m).

Since settlement represents a stage in the development of an individual bivalve, it is natural to scale the size at settlement with that at birth (hatching) and puberty (start of allocation to reproduction). The DEB model is volume structured rather than age structured, and therefore the length of the various life stages is closely related to growth. Species with larger maximum volume will produce larger eggs (see prediction 2), which develop in larger hatchlings because more energy reserves in the egg lead to the production of more structural mass. In this way, larger hatchlings, consisting of more structural mass will develop from larger-sized species. The same arguments hold true for the volume at settlement, because large hatchlings with large structural mass will have a large size (volume) at settlement.

Prediction 5: Egg and larval development time are related to respectively egg and larval volume$^{1/4}$

Egg volume only represents reserve energy for development and hardly contains any structural mass. Therefore the relationship between egg volume and maximum body volume between species is based on the relationship between reserve energy and body volume. Between species, the energy content of an egg is not constant because it scales with body volume$^{1/3}$ (volumetric length). Since hatching volume of a species is proportional to the maximum body volume (Kooijman 2000), total energy content of the egg must scale with body volume$^{4/3}$. Since reserve density of the egg is roughly similar between species, egg volume will scale with adult body volume$^{4/3}$, which also means that body volume scales with egg volume$^{3/4}$ and that volumetric length scales with egg volume$^{1/4}$. Egg development time scales with the energy content of an egg, and this implies that egg development time is related to egg volume$^{1/4}$.

After hatching, feeding starts and uptake processes start to become important. Therefore, the minimum developmental time holds true only at optimal food densities. At optimal food conditions, the length of the larval and juvenile period will, as for egg development, scale linearly with body size (volumetric length) and hence with larval volume$^{1/4}$ (Kooijman 1986). The observed maximum adult size is already reflected in the egg, larval and juvenile size. However, it takes more time to develop and build up a larger adult if the larva is already larger.

Prediction 6: Von Bertalanffy growth rate scales approximately with the maximum observed body volume$^{-1/3}$

Under constant food conditions, or under abundant food conditions in view of the hyperbolic functional response f (Kooijman 1988), the parameter values of the Von Bertalanffy growth curve can be interpreted from an energetic point of view (Kooijman 2000; equation 3.22, page 95) according to:

$$\frac{1}{3\dot{r}_B} = -\frac{1}{\dot{v}} V_{\max}^{1/3} + \frac{1}{\dot{k}_M} \qquad [3]$$

in which

$V_{\max}^{1/3}$ is the maximum length (m)

$\dot{r}_B$ is the Von Bertalanffy growth rate (y^{-1})

$\dot{v}$ is the energy conductance (m d^{-1})

$\dot{k}_M$ is the maintenance rate constant (d^{-1})

Under such conditions, and between species, the Von Bertalanffy growth rate decreases almost linearly with the observed maximum body volume to the power 1/3.

Prediction 7: Maximum observed body volume is positively related to latitude

Energy constraints on distribution patterns of organisms are mainly related to the quantity and quality of the available food. There is some evidence that latitudinal trends in food conditions do exist. Oceanic (=pelagic) primary productivity seems to be higher, on a global scale, in temperate habitats than in arctic and tropical ones (Gross et al. 1988, Behrenfeld and Falkowski 1997, Falkowski et al. 1998). Trends of productivity in coastal areas are more complicated since, in addition to pelagic production, also benthic primary production becomes an important component. Furthermore, coastal areas are under the direct influence of local hydrodynamic and morphological conditions. A comparison of a subarctic, temperate and tropical marine coastal shelf ecosystem suggests on the one hand a trend of increasing planktonic primary production towards the tropics, but on the other hand a more efficient transfer of energy to higher trophic levels at high latitudes (Petersen and Curtis 1980). As an explanation, reduced metabolism and higher conservation of organic matter at low temperatures are suggested (Petersen and Curtis 1980).

In the DEB theory the maximum body volume of a species is determined by three DEB parameters (see Prediction 1) according to:

$$V_{\max}^{1/3} = \kappa\{\dot{p}_{Am}\}/[\dot{p}_M] \qquad [1]$$

Whether a species is able to achieve its maximum volume will depend on prevailing food conditions *f* (see Prediction 4) according to:

$$V_{\infty}^{1/3} = fV_{\max}^{1/3} \qquad [2]$$

Within species, the larger amount of food at higher latitudes, as suggested by the observed trend of more efficient transfer of energy to higher trophic levels at high latitudes, will lead to a larger ultimate size. Hence, a trend of increasing body size/volume with latitude is expected for species covering a wide distributional range. Among species, a size increase towards the poles would be an advantage in surviving long periods of starvation and fluctuating food densities, despite the low temperatures. This is because maximum body volume tends to be proportional to the maximum storage density, which is to the amount of storage material per unit of structural volume (somatic tissue). Small species can only cope with low food levels if these are constant, as it happens in the tropics. Moreover, small body volumes at lower latitudes would help survival because a smaller body volume has lower maintenance costs. Therefore, among species, the DEB theory predicts an increase of body size with latitude.

Methods

Data acquisition

Most of the data presented in this study were assembled from the literature but some unpublished data were added. In all cases, basic data sources of individual references are listed (see Appendix). Most information available consisted of length estimates rather than volumes. Publications by Loosanoff and Davies (1963) on the rearing of bivalve larvae, Tebble (1966) on the British bivalve seashells and Strathmann (1987) on the reproduction and development of marine invertebrates of the Northern Pacific coast were starting points. Information on individual species was compiled by means of literature searches on the Web of Science®. The objective was to gather enough information to be able to test the predictions, and not to collect published information about all species.

Statistical analysis

In order to test whether the different relationships based on field data are in agreement with model predictions, we tested the proportionality of the relationships. Thus, it was assumed that $y = a.x^b$ and that $b = 1$ or -1 (depending on whether the expected relationship is positive or negative), and linear regression models were run after double-log transformation. If the estimated *b* was not significantly different from the predicted *b*, then field data fit with model predictions and the predictions were not falsified.

Results

Since data on the shape coefficient of most of the bivalve species used in this paper were not available, body volume could not be corrected for differences in shape between species and therefore size measures are presented in terms of length.

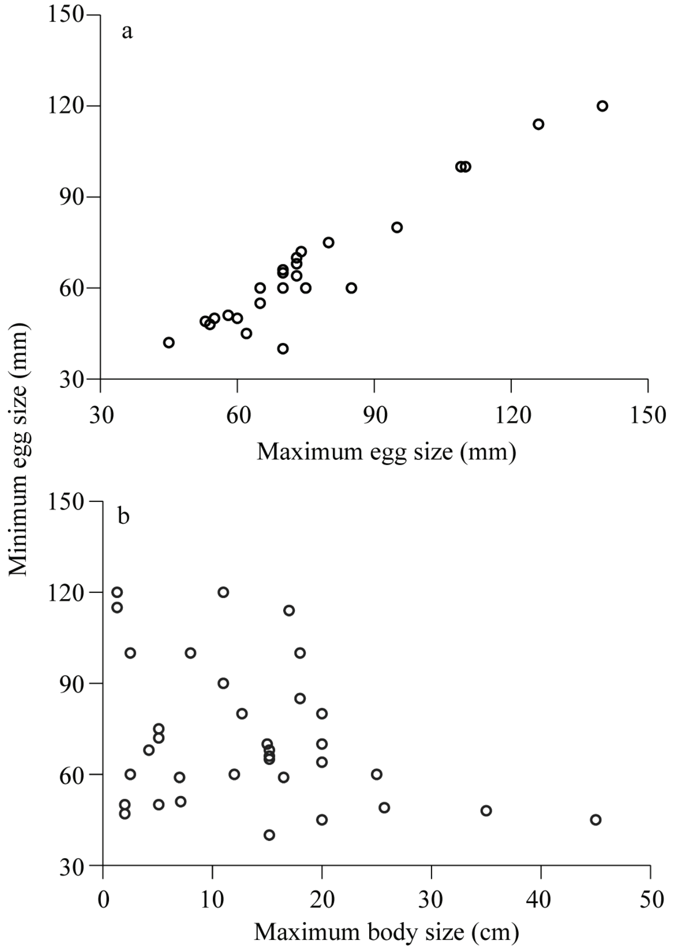

Fig. 6.2. Minimum egg size (um) in various bivalve species in relation to (a) maximum egg size (µm) and (b) maximum body size (cm).

Prediction 1: Maximum theoretical body volume is independent of temperature

To our knowledge no field or laboratory information is available for bivalves to allow a test and falsification of the prediction that maximum volumetric length is independent of temperature.

Prediction 2: Egg volume is proportional to maximum theoretical body volume$^{4/3}$

Since there is no information on the maximum theoretical body volume, maximum body length found in the literature was used. Egg dimensions were mostly presented as egg diameter measurements. For most species, egg diameter (µm) varied between individuals and populations; however between species the range between minimum and maximum egg diameter was similar (Fig. 6.2a). With respect to minimum egg diameter, the range observed varied between about 40 and 120 µm. Minimum egg diameter was not significantly positively related with maximum body size$^{4/3}$(b was significantly different from 1; Fig. 6.2b). In contrast to predictions, this relationship was even slightly negative, although not significantly. This information falsified the prediction.

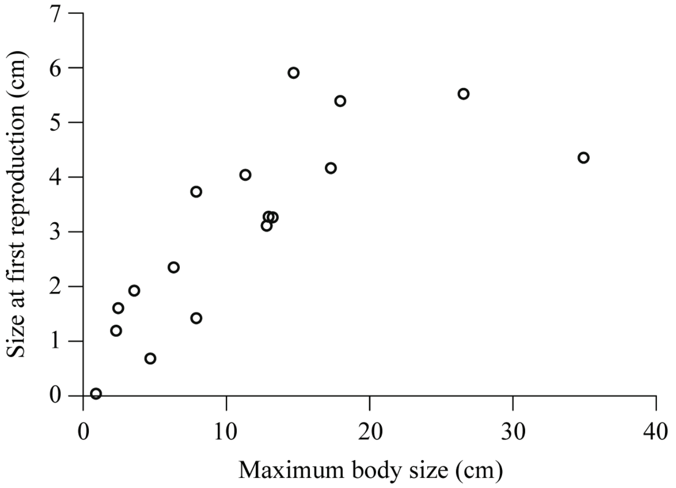

Fig. 6.3. Size at first reproduction (cm) in various bivalve species in relation to maximum body size (cm).

Prediction 3: Volume at first reproduction is proportional to the maximum theoretical body volume

Information on body size at first reproduction was scarce and limited to female bivalves (Fig. 6.3). Size at first reproduction was significantly related to the observed maximum body size. Linear regression revealed that b was not significantly different from 1. Differences in shape between the bivalve families will have been responsible for part of the observed variability.

On average, female bivalves became mature at about 15% of their maximum body size. This information did not falsify the prediction.

Prediction 4: Volume at hatching and settlement is proportional to maximum observed body volume

For larvae, also only length rather than volume measurements were available. For both hatchlings and settling larvae, the minimum and the maximum length were significantly positively related (not shown). Linear regression between minimum egg diameter and minimum length at hatching revealed that *b* was not significantly different from 1 (Fig. 6.4a) but no significant relation was found between minimum egg diameter and minimum length at settlement (Fig. 6.4c). Nevertheless, a positive relationship was observed. Part of the scatter in data might be due to differences in shape. Also minimum length at hatching was not positively related to maximum body length, as revealed by the fact that *b* was significantly different from 1 (Fig. 6.4b). Minimum length at hatching seemed to vary between about 60 and 200 μm irrespectively of maximum body length. The same was observed for the relationship between minimum length at settlement and maximum body length (Fig. 6.4d). The lowest observed length at settlement was about 150 μm while the largest settling larvae were in the range of about 375 μm. This information falsified the prediction.

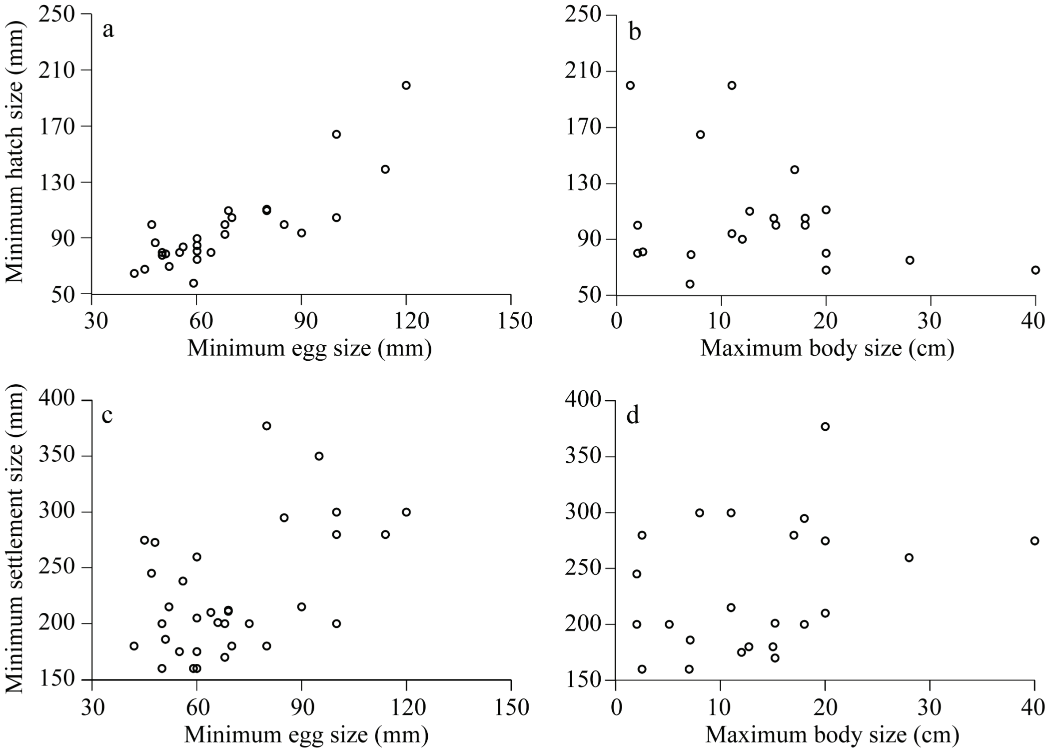

Fig. 6.4. Minimum hatch size (μm) and minimum settlement size (μm) of various bivalve species in relation to (a and c) minimum egg size (μm) and (b and d) maximum body size (cm).

Prediction 5: Egg and larval development time are related to respectively egg and larval volume$^{1/4}$

Information on egg development in relation to water temperature was scarce and observations differed with respect to prevailing water temperature (range 10-24 ºC). In total, information for 14 species could be found. Only for three species, information was available at different temperatures. Observations on larval development were available for 27 species and 35 combinations of water temperature (range 9-33 ºC) and development time. However, information at different temperatures was only available for four species. The relationship between egg/larval development time and egg/larval volume was analysed by the following model:

$$D = a * V^{b} * \exp[-\mathrm{T_A}/\mathrm{T_1} + \mathrm{T_A}/\mathrm{T}] \qquad [5]$$

where D is the egg development time (h) or the larval development time (d), V is the egg or larval volume at hatching (mm^3), a and b are constants, T is experimental temperature (K), $\mathrm{T_1}$ is a chosen reference temperature (K), which has been set in this paper at 293 K (=20°C) and $\mathrm{T_A}$ is the (species-specific) Arrhenius temperature (K). Egg and larval volumes were estimated from minimum egg and larval size and assuming a spherical shape. Two different models were applied (Table 6.2). In model 1, b was fixed at 0.25 according to the predictions of the DEB model, and in model 2 b was set as a parameter. For both egg and larvae, no significant differences were found between the two models (F-test, $F_{(1,13)} = 0.68$, $p = 0.42$ for eggs, $F_{(1,32)} = 0.13$, $p = 0.72$ for larvae), which was confirmed by the fact that the value of $b = 0.25$ fell within the confidence limits of the estimated b (Table 6.2). When only one observation per species was used instead of the whole data set, the same result was found. However, especially in case of eggs, large variability was found in the Arrhenius temperature, suggesting that more data points should be used for a more correct estimate of the relation between egg size and egg development time. Nevertheless, the information above did not falsify the prediction.

Table 6.2. ANOVA of egg and larval development time (Dev; d) in various bivalve species in relation to minimum egg and larval volume at hatch (V; mm^3) and temperature (TEMP, °C) according to the model: Dev = a * V^b * exp[-TA/293 + TA/(273 + TEMP)]. Model 1: b = 0.25; model 2: b estimated.

Model	*a*	*b*	95% CI	TA	95% CI	r^2	n
Eggs							
1	559.8	0.250		7487.8	2979.7-11995.9	0.63	16
2	1792.4	0.390	0.019-0.760	6999.7	2178.4-11820.9	0.65	16
Larvae							
1	124.5	0.250		7779.7	5927.6-9631.9	0.70	35
2	96.7	0.214	0.024-0.405	7656.3	5793.1-9519.4	0.71	35

Prediction 6: Von Bertalanffy growth rate scales approximately with the observed maximum body volume$^{-1/3}$

Data on growth rates and maximum body length were found for 67 species in the Web of Science®. The relationship between the Von Bertalanffy growth rate and maximum length was negative but not significant (*b* was significantly different from -1) (Fig. 6.5a). Part of the scatter might be due to differences in shape between species and the fact that no correction was made for temperature differences. For nine species, however, these corrections could be made, using rough estimates of the mean annual temperature and data on shape coefficients (Bayne and Worral 1980, Rodhouse et al. 1984, Page and Hubbard 1987, Van Haren and Kooijman 1993, Kooijman 1993, 2000).

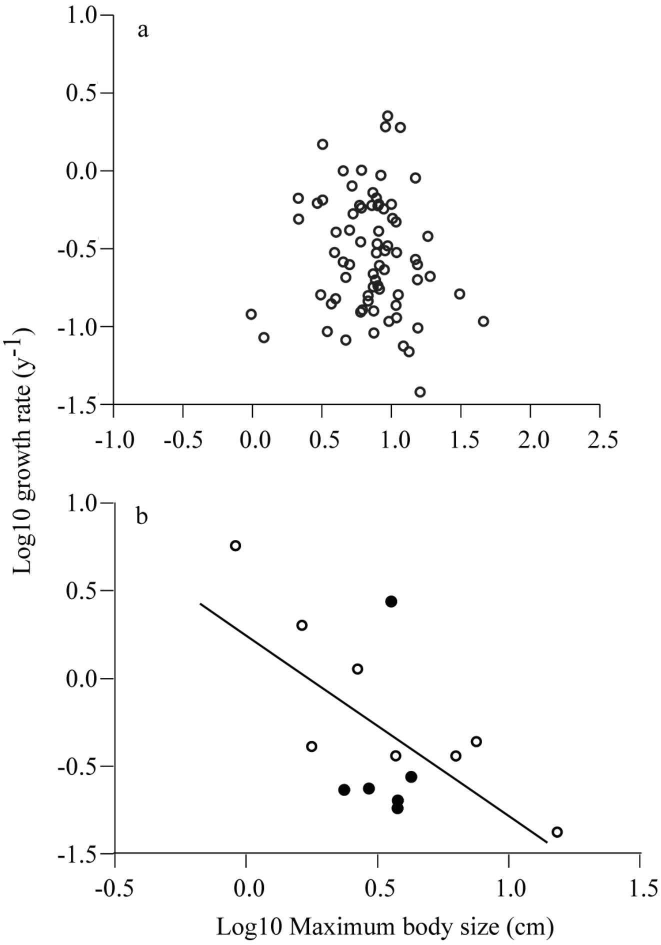

Fig. 6.5. Von Bertalanffy growth rate (y^{-1}) as a function of maximum body size (cm). (a) Complete dataset collected with no correction for temperature and shape differences; (b) dataset corrected for differences in temperature and shape (see text; full circles correspond to data for *Mytilus edulis* only; open circles are data from different species). The line has a slope -1, as expected on the basis of the DEB theory.

By correcting growth rates to a temperature of 20 °C, considering an Arrhenius temperature of 5800 (according to Van der Veer et al. 2006, Chapter 7), the Von Bertalanffy growth rate scaled inversely with maximum length, and *b* was not significantly different from -1 (Fig. 6.5b). In addition, the slope of the regression was close to -1, in accordance with the DEB theory. However, it should be noted that for one of the species, the mussel *Mytilus edulis*, six data points were presented in contrast to one data point for the other species. Intraspecies trends might be interfering with the interspecies comparison. Nevertheless, if the data on mussel were not considered, the significant relationship would still be observed. Therefore, this prediction was not falsified.

Prediction 7: Maximum observed body volume is positively related to latitude

In relation to the interspecific trends, there was hardly any information available on the ultimate volumetric length that different bivalve species achieve. Indirect information was obtained from Tebble (1966), who described the distributional range and maximum observed size for bivalves occurring around Britain. For various species, the maximum size (cm) was combined with an estimate of the midpoint of the distribution of the species. The observed trend between maximum size and latitude suggested that small species occur over the whole range while larger sized species are only observed with increasing latitude towards the pole (Fig. 6.6). Similar information for north-eastern Pacific bivalves suggested no relationship between body size and latitude (Roy and Martien 2001).

Latitudinal trends within species may be affecting the observed trend. Within the temperature limits of a species, food conditions seem to determine the rate of growth, not only over the distributional range of the species (Ansell 1968), but also within local areas (De Montaudouin 1996).

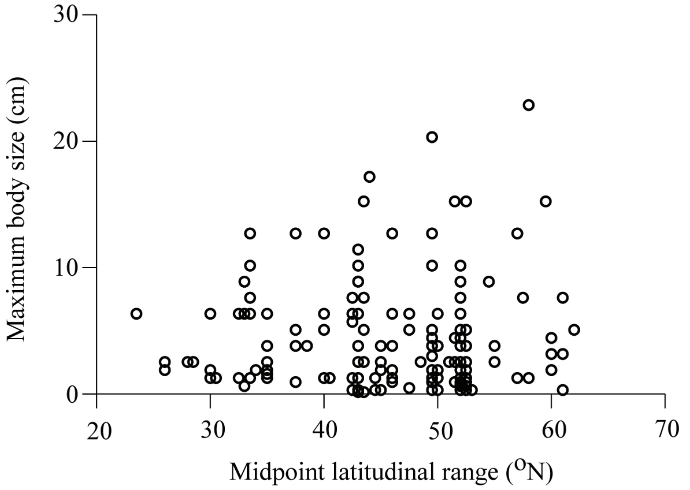

Fig. 6.6. Maximum body size (cm) of various bivalve species in relation to their latitudinal distribution. Data after Tebble (1966), one point per species is represented.

Nevertheless, information on trends in maximum body size/volume with latitude within species is contradictory. In the hard clam *Mercenaria mercenaria*, a positive trend with latitude is suggested (Ansell 1968, Heck et al. 2002). However, in the Baltic clam *Macoma balthica* a negative relationship with latitude was found along the North American Atlantic coast, while no trend was observed along the European Atlantic coast (Beukema and Meehan 1985). The yellow clam *Mesodesma macroides* also showed a negative relationship with latitude (Fiori and Morsán 2004). So it seems that within bivalve species, other factors besides food availability are important as well, influencing the expected latitudinal trend of body size.

Due to the variability in the dataset and information available, we concluded that the data are too variable to allow an interspecies comparison and to support the prediction.

Discussion

Body-size scaling relationships

By focusing on the predictions according to the DEB theory of various scaling relationships between physiological and ecological variables on the one hand and body size on the other hand, we were able to analyse a number of predictions, by comparing them with field data on different bivalve species. For the volume at first reproduction, egg and larval development time and the Von Bertalanffy growth rate, field data did not falsify the predictions by the DEB model. A similar pattern as for flatfish species (Van der Veer et al. 2003) was found in this study (Table 6.3).

Table 6.3. Body-size scaling relationships, comparison between predictions by the DEB theory (Kooijman 1993, 2000) and field data for respectively bivalve (this study) and flatfish species (after Van der Veer et al. 2003).

	DEB prediction	Bivalves	Flatfish
I	Maximum body volume – temperature	?	?
II	Egg volume – maximum body volume$^{4/3}$	-	-
III	Vol. at first reproduction – maximum body volume	+	+
IV	Hatchling/settling volume – ultimate body volume	-	-
V	Egg/larval development – egg/larval volume$^{1/4}$	+	+
VI	Von Bertalanffy parameters	+	
VII	Ultimate body volume – latitude	-	+

?: no data; +: prediction not falsified; -: prediction falsified; blanc: not analysed.

In flatfish, egg development time also scaled with egg volume and temperature (Van der Veer et al. 2003). However, both in flatfish and bivalves, a discrepancy is observed in the relationships of egg and larval volume with maximum body volume. The fact that body length of bivalves was used, instead of body volume, may have added variability to the different relationships, as differences in shape between species were not considered.

The predictions of the DEB model also seem to apply, at least partly, for other groups of invertebrates. A clear relationship between egg and larval size and development time has been reported for various species (Steele 1977, Strathmann 1977, Todd and Doyle 1981, Chester 1996, Marshall and Keough 2003, Miner et al. 2005). The data by Todd and Doyle (1981) on nudibranch molluscs are extensive enough to convincingly illustrate that, irrespectively of development type (planktotrophic, lecithotrophic, direct), egg development is related to egg volume$^{1/4}$, as predicted by the DEB theory. Miner et al. (2005) also showed that egg size in echinoderms was positively correlated to the length of the facultative feeding period, suggesting that larger eggs with higher energy content would allow a longer developmental period. The negative relationship between the Von Bertalanffy growth rate and maximum size has also been demonstrated for several invertebrate groups (Kooijman 1993, 2000).

In contrast to allometric scaling relationships, which are only descriptive and often species-specific, the various body-size relationships predicted by the DEB theory can be used for extrapolations beyond the range of data on which they are based. This holds true especially for the relationship between volume at first reproduction, egg and larval development time in relation to egg and larval volume and temperature, and the Von Bertalanffy growth parameter. For example, model 1 of Table 6.2 can be used to determine development time of eggs and larvae if egg and larvae volume are known. The aspect of the larval stage duration is especially of interest. Due to their small egg size, bivalve species are characterised by a relatively short pelagic larval stage in the order of a few weeks at 10 ºC. For instance, an egg size of 60 µm has a larval stage of 24 d. For a relatively large egg of 120 µm, the difference in pelagic larval stage duration is only about 2 weeks (40 versus 24 d; see Prediction 5). The consequence is that due to their small egg volumes, the period of dispersion of bivalve eggs and larvae will be relatively short in comparison with other marine species.

Besides body-size scaling relationships between species, the DEB model can also be applied to establish body-size relationships within a species, such as for growth, reproduction and respiration in relation to size, but this aspect is the focus of another paper (Cardoso et al. 2006, Chapter 8).

Identification of potentially important factors in life history strategy of bivalves

The main discrepancy between model predictions and field data is related to the relationship between maximum body volume and egg and larval volume. For bivalves, no positive trend between maximum body size and egg size, or larval size at hatching and settlement was observed. In gastropods, such as *Crepidula* spp., egg size and hatch size were also not related to adult body size (Collin 2003). Nevertheless, in flatfish, a positive trend was present; however in absolute values, flatfish invested less energy in individual eggs and larvae than predicted (Van der Veer et al. 2003). Although in fish a significant trend was seen between size at maturity and body size within the same family (Kooijman 2000), no trend was seen in egg size or size at hatching and settlement within bivalves, even within the same family (not shown in the figures).

The consequence is that, in bivalves, the characteristics of the early life stages are not related to body size of the species. Although, in the DEB theory, egg volume is predicted to scale with maximum body size, large variations between taxa are expected to occur in this life stage parameter. If environmental conditions are unstable, small organisms may produce large eggs, which lead to large juveniles that are more adapted to such conditions. Therefore, the relationship between egg size and maximum body size is only expected in related species, with similar tolerance ranges for different environmental variables. Since small species occurred over the whole latitudinal range, any scaling relationship with body size might be biased because a similar body size does not correspond with similar environmental conditions.

The absence of a positive relationship between maximum body size of a bivalve species and larval size at settlement might be related to the sediment characteristics of the settling areas. Successful settlement and survival of bivalve larvae in highly dynamic intertidal environments will include a sequence of events, whereby the dominant factors are the hydrodynamics during settlement and the sediment dynamics in post larval re-suspension (e.g. Bouma et al. 2001). In addition, grain size might be a critical factor for survival of spat after settlement and during re-suspension. Successful settlement might only be possible when settlement size or volume of the fragile larvae is in balance with the sediment composition, which means similar to or larger than that of the sediment grain size. In estuarine and coastal areas such as the western Wadden Sea (Dapper and Van der Veer 1981, Dekker and Waasdorp 2004, Zwarts et al. 2004) and the Swedish west coast (Pihl and Rosenberg 1984) median grain size of intertidal areas varies between 100 and 260 µm and this might explain why the minimum settlement size found in bivalves is above 125 µm. Even small bivalve species must produce settling larvae that are large enough to cope with the sediment characteristics with normal grain sizes of at least about 100 µm. In this respect, an interesting next step would be a comparison of the size of settling bivalve larvae with other benthic invertebrate species, to see whether other groups are also characterised by a minimum settling volume or size.

Acknowledgements: Thanks are due to Wim Wolff, Jaap van der Meer, Jan Beukema and three anonymous referees for their helpful comments on earlier versions of this paper. This research was supported by the project Van Gogh VGP 84-592 from The Netherlands Organization for Scientific Research (NWO).

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Appendix

Literature references used for the estimation of the different relationships (numbers refer to references in footnote).

Species	Size first repr	Min egg diam	Max egg diam	Min hatch size	Max hatch size	Min sett size	Max sett size	Larv dev time	Growth rate
Abra alba	20	20		20	20	20	20	20	34
Abra tenuis	20	20; 1	1	1	1				
Acar transversa				14	14	14	14	14	
Aequipecten operculus									35
Alasmidonta heterodon									36
Anomia simplex		14	14	14	14	14	14		
Arctica islandica	31	15	15	12	12	12	12	12; 17	
Bankia setacea		26		26	26	26		26	
Barnea candida									37
Cerastoderma edule	33	7; 14	7			14	14		
Chlamys hastata								14	
Chlamys varia				16	16	16			
Corbicula japonica									38
Corbula trigona									39
Crassostrea ariakensis	32	32	32						
Crassostrea gigas	9	13; 14	13; 14	14	14				
Crassostrea iridescens									40
Crassostrea madrasensis									41
Crassostrea virginica		14	14	14	14	14	14	14	
Donax denticulatus									42
Donax dentifer									44
Donax incarnatus									45
Donax serra									43
Donax trunculus	28	28							

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Appendix *(continued)*

Species	Size first repr	Min egg diam	Max egg diam	Min hatch size	Max hatch size	Min sett size	Max sett size	Larv dev time	Growth rate
Dosinia anus									46
Ensis directus	8	8; 14	14	14	14	14		14	
Ensis macha									47; 48
Gari solida									49; 50
Haliotis fulgens									51
Haliotis mariae									52; 53
Hiatella artica									54
Katelysia scalarina				10	10	10			
Laevicardium montoni		14	14		14	14	14	14	
Lanternula elliptica									55
Macoma balthica	33	7	7	14		7			
Mactra chinensis									57
Mactra discors									46
Mactra murchisoni									46; 59
Marcia opima				19	19	19			
Mercenaria mercenaria	11; 14	11; 14	11; 14	11; 14	11; 14	11	11	11; 14	
Mesodesma mactroides									60
Modiolus demissus		14		14	14	26	14	14	
Modiolus modiolus		26		26	26	14			
Mya arenaria	3; 2	4; 3; 14; 26	4; 3; 14; 26	26; 14	26; 14	14; 26	14; 26	14	2
Mysella tumida						26	26		
Mytilus edulis	33	7	7	26	26	26	26	25; 26	61; 62; 63; 64
Ostrea angasi									65
Ostrea edulis	9	9	14	14; 9	14; 9	14; 9	14	14	
Ostrea lurida		26	26	26	26	26	26	26	
Panopea abrupta	5	26		26	26	26		26	
Paphies donacina									59

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Appendix *(continued)*

Species	Size first repr	Min egg diam	Max egg diam	Min hatch size	Max hatch size	Min sett size	Max sett size	Larv dev time	Growth rate
Pecten irradians		14	14	14	14	14		14	
Pecten jacobaeus									66
Pecten maximus		21	21			21	21		
Petricola pholadiformis		14	14	14	14	14		14	
Pholas dactylus									37
Pinctada mazatlanica	24								
Pitar morrhuana		14	14	14	14	14	14	14	
Placuna placenta				18	18	18		18	
Polymedosa radiata									67
Protothaca thaca									50
Pteria sterna	24								
Rangia cuneata		27		27	27	27	27	27	
Ruditapes largillierti								10	
Ruditapes philippinarum									68
Semele solida									50
Solemya reidi						26			
Spisula aequilatera									46; 59
Spisula solida									69
Spisula solidissima		30		14	14	14		14	
Spisula solidissima similis		29					26		
Tagelus dombeii									48
Tapes philippinarum	23			26	26	26	14		
Tapes semidecussata		14	14	14	14	14		14	
Teredo navalis		14	14	14	14	14		14	
Tresus capax		26	26	26	26	26		26	
Tridacna derasa									70
Venus antiqua									48
Venus verrucosa									58

References (Appendix):

1 Bachelet, 1989; 2 Brousseau & Baglivo, 1988 ; 3 Brousseau, 1978 ; 4 Brousseau, 1987 ; 5 Campbell & Ming, 2003 ; 6 Casse et al., 1998 ; 7 Honkoop & Van der Meer, 1998 ; 8 Kenchington et al., 1998 ; 9 Kennedy et al., 1996; 10 Kent et al., 1998; 11 Kraeuter & Castagna, 2001; 12 Landers, 1976 ; 13 Cardoso, unpubl. ; 14 Loosanoff & Davies, 1963 ; 15 Loosanoff,1953; 16 Louro et al., 2003; 17 Lutz et al., 1982; 18 Madrones-Ladja, 1997; 19 Muthiah et al., 2002; 20 Nott, 1980; 21 Paulet et al., 1988; 22 Le Pennec et al., 2003; 23 Ponurovsky & Yakovlev, 1992; 24 Saucedo & Monteforte, 1997; 25 Sprung, 1984; 26 Strathman, 1987; 27 Sundberg & Kennedy, 1992; 28 Tirado & Salas, 1998; 29 Walker & Obeirn, 1996; 30 Walker et al., 1996; 31 Witbaard, 1997; 32 Zhou & Allen, 2003; 33 Beukema & Dekker, pers comm.; 34 Bachelet & Cornet, 1981; 35 Heilmayer et al., 2004; 36 Michaelson & Neves, 1995; 37 Pinn et al., 2005; 38 Oshima et al., 2004; 39 Etim et al., 1998; 40 Melchor-Aragon et al., 2002; 41 Alam & Das, 1999; 42 Garcia et al., 2003; 43 Laudien et al., 2003; 44 Riascos & Urban, 2002; 45 Thippeswamy & Joseph, 2001; 46 Cranfield & Michael, 2001; 47 Baron et al., 2004; 48 Urban, 1996; 49 Urban & Tarazona, 1996; 50 Urban & Campos, 1994; 51 Shepherd et al., 1991; 52 Siddeek & Johnson, 1997; 53 Shepherd et al., 1995; 54 Sejr et al., 2002; 55 Urban & Mercuri, 1998; 56 Brey & Hain, 1992; 57 Sakurai, 1993; 58 Arneri et al., 1998; 59 Cranfield et al., 1996; 60 Fiori & Morsan, 2004; 61 Rodhouse et al., 1984; 62 Page & Hubbard, 1987; 63 Bayne & Worral, 1980; 64 Seed, 1969; 65 Mitchell et al., 2000; 66 Peharda et al., 2003; 67 Campos et al., 1998; 68 Goshima et al., 1996; 69 Gaspar et al., 1995; 70 Pearson & Munro, 1991.

CHAPTER 7

The estimation of DEB parameters for various Northeast Atlantic bivalve species

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Abstract

Dynamic energy budgets are used for the description of the energy flow through individual organisms from the assimilation of food to the utilization for maintenance, growth, development and reproduction. In this paper, parameters of Kooijman's Dynamic Energy Budget (DEB) model are estimated for the following Northeast Atlantic bivalve species: the Baltic clam *Macoma balthica* (L.), the soft-shell clam *Mya arenaria* L., the cockle *Cerastoderma edule* (L.), the blue mussel *Mytilus edulis* L. and the Pacific oyster *Crassostrea gigas* (Thunberg). For none of the species a complete set of parameters could be compiled. A special protocol was developed to account for missing values and to achieve consistency between parameters. Species were similar in their optimal temperature range, as reflected in an Arrhenius temperature of 5800 K, which corresponds to a Q_{10} of 2. Differences between species were observed in width of the optimal temperature range. The taxonomic relatedness between species was reflected in similar volume-specific maintenance costs, costs for growth and almost similar maximum storage density of energy. Species differed in their maximum surface area-specific assimilation rate by a factor 6 and in the fraction of energy allocated to reproduction (ranging from 0.15 to 0.50). These differences are reflected in the maximum theoretical total shell length of the species, which varied from about 3 cm in *M. balthica,* 6 cm in *C. edule,* 15 cm in *M. arenaria* and *M. edulis* and 45 cm in *C. gigas*.

Introduction

Dynamic energy budgets (DEB) are used for the description of the energy flow through individual organisms from the assimilation of food to the utilization for maintenance, growth, development and reproduction. In the late 1980's, Kooijman (1986a) published the so-called *k*-rule DEB theory and over the years the DEB model has been successfully applied in describing the energy allocation to growth and reproduction in a variety of species (for an overview see Kooijman 2000). The powerful aspect of Kooijman's DEB theory is that differences between species can be captured in the same model using a different set of parameter values (for a recent overview see Kooijman 2001). However, estimation of these parameters is complicated and can often not be done in a direct way (Van der Meer 2006).

An efficient approach to estimate the various DEB parameters would be an experiment in which growth, reproduction and respiration are determined simultaneously, preferably under varying feeding conditions and food intake (Kooijman 1993, 2000). Especially experiments in which organisms growing under one food condition are transferred to higher or lower food levels are powerful (Kooijman 1986b, Gurney et al. 1990, McCauley et al. 1990). Using weighted non-linear least squares estimation such experiments would result in the basic DEB parameters (Kooijman 1986a). However, such an experiment has not been performed so far and parameter estimates are based on a combination of data sets originating from various sources (Kooijman 1986a,c; Zonneveld and Kooijman 1993, Van Haren and Kooijman 1993, Van der Veer et al. 2001). Van der Meer (2006) illustrated the limitations of this approach by showing that parameter estimates might vary considerably depending on the data set used. For some parameters, the estimates could vary by a factor 10. Therefore, combining data sets and simultaneously estimating the various parameters by means of a weighted non-linear least squares regression was suggested to overcome this problem as much as possible.

In this paper, the approach of Van der Meer (2006) – using literature data through simultaneous regression – is followed as much as possible for the estimation of the various DEB parameters for a number of bivalve species that are abundant in northern European waters, including the Dutch Wadden Sea. However, for none of the species a complete set of parameters could be compiled and in some cases parameter estimates were variable. Hence, a protocol was developed to account for missing values and to achieve consistency between parameters. First, a brief overview is presented on how the various parameters can be estimated and what types of datasets are required. Next, a protocol is introduced on to how to deal with missing estimates in case data sets are lacking and how to achieve consistency between the various estimates. Finally, the protocol is applied to estimate a complete set of DEB parameters for various bivalve species.

Species characteristics

The bivalve species studied belong to the family Tellinidae [the Baltic clam *Macoma balthica* (L.)], Myacidae [the soft-shell clam *Mya arenaria* L.], Cardiidae [the cockle *Cerastoderma edule* (L.)], Mytilidae [the blue mussel *Mytilus edulis* L.] and Ostreidae [the Pacific oyster *Crassostrea gigas* (Thunberg)].

All species can tolerate conditions varying from (sub)tropical to temperate to cold-water (Tebble 1976). Along the European coast, *M. balthica* and *M. arenaria* occur from the White Sea at 70°N to the Gironde estuary at 45°N. *C. edule* can be found from the western region of the Barents Sea to the Iberian Peninsula, and even south along the coast of West Africa as far as Senegal. *M. edulis* is widely distributed in the boreal regions from the western boarder of the Kara Sea south to the Mediterranean but absent from the high Arctic. *C. gigas* was introduced in European waters some decades ago and is now a common species from Portugal to Denmark.

The various species occupy partly different habitats in the Dutch Wadden Sea (Wolff 1983, Dankers and Beukema 1984). *M. balthica* can be found from the upper regions of the intertidal to the outer parts of the tidal inlets and into the coastal zone. *M. arenaria* occurs from the upper regions of the intertidal into the subtidal but in other areas it extends to considerable depths offshore. *C. edule* is present from mid tide to just below the low water mark. *M. edulis* occurs from high in the intertidal zone to below low water in the subtidal and *C. gigas* can be found on hard substrate (stones, mussel and cockle banks) in the intertidal zone.

The maximum age of the various species varies between 8 to 15 years, and they differ also with respect to growth and reproduction. According to the literature, *M. balthica* reaches a maximum shell length of 2.5 cm, *M. arenaria* of about 12-15 cm, *C. edule* of about 5 cm, *M. edulis* of about 15 cm and *C. gigas* of about 40-45 cm (Tebble 1976, Carriker and Gaffney 1996, Poppe and Goto 1993). However, larger individuals have occasionally been found of *M. balthica* (2.8 cm; Dekker pers comm. 2005) and *C. edule* (5.5 cm, Cardoso unpubl. data).

M. balthica becomes mature at a size of 1.0 cm (Honkoop et al. 1998). The corresponding body volume amounts 0.028 cm^3. The average energy content of a *M. balthica* egg with a mean diameter of 107 µm amounts about 5.9 mJ (Honkoop and Van der Meer 1998). For *M. arenaria* the minimum length at maturity is about 2.5 cm or 0.33 cm^3 (Dekker pers. comm. 2005). The mean diameter of an egg in *M. arenaria* is about 70-80 µm (Battle 1932), which is almost the same as in *C. edule* and *M. edulis*. Therefore, similar energy content is assumed of about 1.9 mJ. For *C. edule* the minimum length at maturity is about 1.5 cm, or 0.50 cm^3 and the average energy content of an egg with a mean diameter of 77 µm (excluding the surrounding jelly layer) is about 2 mJ (Honkoop et al. 1999). *M. edulis* becomes mature at a size of about 1.2 cm (Van Haren and Kooijman 1993, Cardoso unpubl. data) which corresponds with a somatic body volume of about 0.06 cm^3. Egg size varies between 70 and

80 µm in diameter (Honkoop and Van der Meer, 1998) and the energy content of such an egg will amount about 1.9 mJ. In *C. gigas,* the minimal size at first reproduction is about 5.0 cm, corresponding with a body volume of 1.4 cm^3 (Kobayashi et al. 1997, Cardoso unpubl. data). The average energy content of an egg with a mean diameter of around 50 µm (Cardoso et al. 2007a, Chapter 5) amounts about 1 mJ. Table 7.1 summarises these life history parameters of the different species.

Table 7.1. Some life cycle parameters for various bivalve species based on field and laboratory data.

Symbol	Dimension	Interpretation	*M. balthica*	*M. arenaria*	*C. edule*	*M. edulis*	*C. gigas*
L_M	cm	Maximum observed length	3	15	6	15	45
$V^{1/3}{}_M$	cm	Maximum observed volumetric length	1.095	4.160	2.286	4.310	7.880
V_P	cm3	Volume at start of reproductive stage	0.028	0.330	0.495	0.060	1.400
L_D	µm	Egg diameter	107	70-80	77	70-80	50
E_0	mJ	Egg energy content	5.9	1.9	2.0	1.9	1.0

Material and methods

Parameter estimation

The various DEB parameters are listed in Table 6.1 (Chapter 6). Compound parameters are built up of primary parameters and can be estimated from these primary parameters; however, in some cases primary parameters are derived from compound parameters. The estimation of the various parameters is based on a compilation of all publications dealing with the subject. Most of the information listed below is taken from Kooijman (2000) and Nisbet et al. (2000). For an overview see Van der Meer (2006). The notation and symbols follow those in Kooijman (2000), whereby the following main rules apply:

1. variables are indicated by symbols and lower case symbols frequently relate to upper case ones via scaling;
2. quantities are expressed per unit of volume with square brackets []; per unit of biosurface area with braces {}; and per unit of mass with angles <>;
3. rates have dots, indicating the dimension per time.

Shape coefficient (δ_m)

The shape coefficient determines how a specific length measurement (total length, standard length, body width) relates to structural body mass, which means that each type of length measurement has its specific shape coefficient. Shape coefficients can be determined from data on length and body mass. In case gonad mass and somatic body mass have been distinguished, data on somatic body mass should be taken, otherwise data on total mass directly after spawning are to prefer. In case only empty shells are present, shape coefficients can be determined by using potato-pulp (assuming that it has specific density of 1 g cm^{-3}) by first measuring the weight of the empty shell, fill it with pulp and close it, remove the excess pulp and weigh it again. In this way, a volume and a length measurement is obtained (Kooijman pers. comm. 2005).

Arrhenius temperature (T_A)

Each species can only tolerate temperature within a specific temperature range. Within part of the temperature tolerance range (i.e. the optimal temperature range), rates increase exponentially with increasing temperature. Outside the optimal temperature range, both at low and high temperatures, rates are reduced. The Arrhenius relationship is an alternative for the Q_{10} and is calculated from experiments whereby a process or rate is determined in relation to temperature and preferably also in relation to body volume/size over the optimal temperature range of a species. Especially oxygen consumption rates seem to be suited for the determination of the Arrhenius temperature (Riisgård and Seerup 2003). For the reduction of rates at both the low and high temperatures, the quantitative formulation by Sharpe and DeMichele (1977) was followed (Kooijman 2000). This formulation is based on the idea that the rate is controlled by an enzyme that has an inactive configuration at low and high temperatures, below and above the optimum temperature, respectively. This means that the reaction rate has to be multiplied by the enzyme fraction that is in its active state. For formulas see Kooijman (2000, page 57). In this way, four extra parameters are needed, T_L and T_H which relate to, respectively, the lower and upper boundary of the tolerance range where 69% (=ln2%) of the enzymes are active, and T_{AL} and T_{AH} which are the Arrhenius temperatures for the rate of decrease at both boundaries.

Maximum surface area-specific ingestion rate $\{\dot{J}_{Xm}\}$

This parameter is calculated from experiments on maximum food intake in relation to body volume/size, preferably at various temperatures over the optimal temperature range of a species.

Maximum surface area-specific assimilation rate $\{\dot{p}_{Am}\}$

The maximum surface area-specific assimilation rate is often estimated in an indirect way from the maximum surface area-specific ingestion rate by assuming a fixed percentage for the losses due to pseudofaeces. A more labour-intensive approach is the determination of the amount of pseudofaeces and/or faeces together with the maximum food intake. Such an experiment results in an estimate of both the maximum surface area-specific assimilation rate and the maximum surface area-specific ingestion rate.

Volume-specific maintenance costs per unit of time $[\dot{p}_M]$

Maintenance costs cannot be estimated directly and precisely from experimental data. Only an indirect approach is possible from changes in energy content by starvation over time, whereby most of the energy is spent on maintenance and only a small part is stored for reproduction. However, the duration of the starvation experiment should not be too long, because otherwise structural mass is broken down as energy source too.

Volume-specific costs for growth $[E_G]$

Volume-specific costs for growth cannot be estimated in a direct way. An approach is the energy content of the structural body mass of an individual after starvation just before mortality occurs multiplied by a conversion factor reflecting growth efficiency.

Maximum storage density $[E_m]$

The maximum storage density can be estimated in an indirect way by comparing the energy content of the structural mass of an individual between well fed and after starvation before mortality occurs. As an indirect estimate, the difference in energy content at the end of the winter and at the end of the growing season can be taken. The difference is considered to represent a minimum estimate of the maximum storage density.

Kappa (κ)

Kappa can only be estimated on the basis of an annual energy budget of an individual whereby the energy flow into growth, reproduction and maintenance is known. However, this requires that estimates of the other DEB parameters are available.

Energy conductance ($\dot{v}$)

The energy conductance can be calculated from the relationship between the von Bertalanffy growth rate and the maximum volumetric length at a standardized temperature (Kooijman 1988, 2000). Another approach is by experiments on oxygen consumption during starvation, since, during starvation, energy reserves decrease and growth ceases so that after a while the animal spends energy only on maintenance and reproduction (see Evers and Kooijman 1989).

Maintenance rate constant ($\dot{k}_M$)

The maintenance rate constant can be estimated from data on mass (energy) loss during starvation and from data on respiration ontogeny during the embryonic period. Also data on the survival probability as a function of age can be used. The maintenance rate constant can be estimated indirectly as the ratio of energy conductance and maintenance rate coefficient from experiments on oxygen consumption rate at constant food densities, but only if energy reserves can be split up from structural mass.

Investment ratio (g)

There is no experimental set up that can result in a direct estimate of the investment ratio. For various relationships between g and other primary and compound parameters see Kooijman (2000).

Protocol for missing primary parameters and parameter consistency

Previous analyses (Van Haren and Kooijman 1993, Van der Veer et al. 2001, Cardoso et al. 2001, Van der Meer 2006) resulted in large variability in parameter estimates and also showed that in general information is lacking for the estimation of a complete set of primary parameters. Therefore, the following protocol was adopted to deal with missing parameters and to establish parameter consistency. The procedure involves the following steps:

Determine the Arrhenius temperature

The DEB theory assumes that within an individual all physiological rates are affected by temperature in the same way, but this would also apply in related species (Kooijman 2000). Therefore, missing values and consistency between estimates can be obtained from the assumption that a similar Arrhenius temperature is expected in related bivalve species, whereby species-specific differences are only reflected in differences in optimal temperature, temperature tolerance boundaries of the tolerance range and in the Arrhenius temperatures of the rates of decrease at both boundaries.

Determine the maximum storage density $[E_m]$, the volume-specific maintenance costs $[\dot{p}_M]$ and the volume-specific costs for growth $[E_G]$

Missing values and consistency between estimates of the maximum storage density $[E_m]$ can be achieved from the basic assumption of the DEB theory that the maximum storage density between related species is proportional to the volumetric maximum length (Kooijman 2000).

With respect to the volume-specific maintenance costs $[\dot{p}_M]$, the basic assumption is that the maintenance of cells is considered to be the major part of the maintenance of the whole individual. Furthermore, the DEB theory assumes that the maintenance costs of a cell depend on the complexity of the cell (Kooijman 2000). The membrane pacemaker theory illustrates that this assumption may well be a realistic one (Hulbert and Else 2005). The assumption implies that in related species, the volume-specific maintenance costs per unit of time should be similar. However, this only holds true at the same temperature within their optimal range, i.e. around 20 °C.

As for the volume-specific costs of maintenance, the volume-specific costs for growth $[E_G]$ of a cell are considered to depend on the complexity of the cell (Kooijman 2000) implying that in related species, the volume-specific costs for growth and the volume-specific energy content will be similar. The volume-specific costs for growth can be computed from the volume-specific energy content of the organisms and the costs for material synthesis. Information on the energetic efficiencies of synthesising material is mainly restricted to endotherms, whereby in general for protein and fat synthesis costs in the order of 20% are listed (e.g. Aoyagi et al. 1988, Roberts and Young 1988). Under the assumption that the costs for material synthesis for other components are in the same order of magnitude and that a similar amount is required for establishing and completing the cellular metabolism, this would mean total energy overhead costs in the order of 40%.

Determine κ

After $[E_G]$, $[E_m]$ and $[\dot{p}_M]$ are determined, simulation with the DEB model can be run to determine the value of κ whereby the ratio between somatic mass and gonad mass just before spawning corresponds with field observations. Since the estimate of κ is based on the ratio between somatic mass and gonad mass, this estimate is not dependent on the parameter value of the maximum surface area-specific assimilation rate $\{\dot{p}_{Am}\}$.

Determine the maximum surface area-specific assimilation rate $\{\dot{p}_{Am}\}$

Finally, once κ and $[\dot{p}_M]$ are known, the formula for maximum volumetric length under optimal food conditions (f=1) is applied to estimate $\{\dot{p}_{Am}\}$ according to:

$$V_m^{\frac{1}{3}} = \kappa * \frac{\{\dot{p}_{Am}\}}{[\dot{p}_m]}$$

In this way a complete and consistent set of DEB parameters can be obtained even when there are some missing values.

Data acquisition and analysis

In principle, published data sets were used for estimating the different parameters. Information published in graphical form was scanned and digitalized by means of GrabIt®. Next, the data were tabulated and stored for further analysis. All statistical analyses were done in R (R Development Core Team 2004).

Only in case of lack of information was additional unpublished information used. If species-specific information was lacking, the following factors were used: 1 g wet mass = 0.12 g ash-free dry mass (AFDM); 1 g AFDM = 0.4 g C; 1 g AFDM = 23 kJ and 1 cal = 4.2 J (Beukema 1977, Brey et al. 1988, Cardoso unpubl. data).

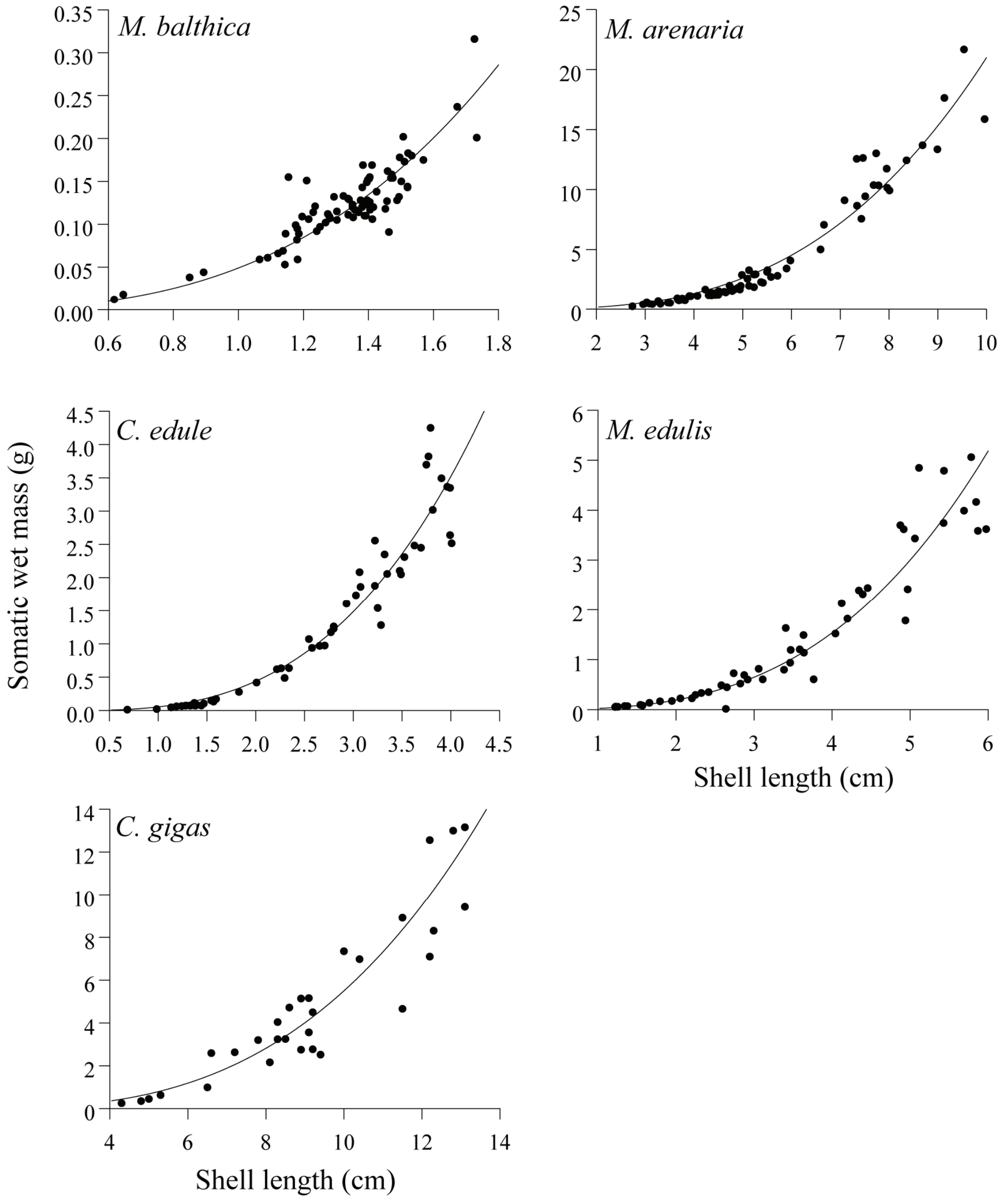

Fig. 7.1. Total length (cm) – somatic wet mass (g) relationships for various bivalve species. Mean shape coefficient (±S.E.): *M. balthica* 0.365±0.10 (n=80; r^2=0.97); *M. arenaria*: 0.277±0.08 (n=75; r^2=0.97); *C. edule*: 0.381±0.11 (n=57; r^2=0.96); *M. edulis*: 0.287±0.09 (n=51; r^2=0.95); *C. gigas*: 0.175±0.08 (n=30; r^2=0.95).

Results

Parameter estimation

Shape coefficient (δ_m)

The estimates of the shape coefficient for the various species were based on total length-somatic wet mass data from the western Dutch Wadden Sea (Cardoso et al. 2007a, 2007b, in press; Chapters 2, 3, 4 and 5). The various estimates (±S.E.) by means of weighted regression were highly significant (Fig. 7.1). *M. balthica* (0.365±0.098) and *C. edule* (0.381±0.114) had the highest shape coefficient, followed by *M. edulis* (0.287 ±0.090) and *M. arenaria* (0.277±0.077). *C. gigas* had the lowest value (0.175±0.060).

Arrhenius temperature (T_A)

The estimates of the Arrhenius temperature and of the lower and upper boundary of the tolerance range were based on experiments on oxygen consumption as function of temperature. For *M. balthica* results from De Wilde (1975) and from Wilson and Elkaim (1991) were used. For *M. arenaria* data published by Kennedy and Mihursky (1972) and from Lewis and Cerrato (1997) were taken. For *C. edule* data from Newell and Bayne (1980) were analysed and in *C. gigas* experimental data from Gerdes (1983) and from Bougrier et al. (1995) were used. In *M. edulis*, information on change in heart rate in relation to temperature by Widdows (1973) was analysed. The various estimates of the Arrhenius temperature (±S.E.) differed between 5290±1108 K in *C. edule*, 5672±522 K in *M. balthica*, 5722±229 K in *C. gigas*, 7022±551 K in *M. edulis* and 7051±453 K in *M. arenaria* (Fig. 7.2). These Arrhenius temperatures corresponded with Q_{10} values of respectively 1.98, 2.00, 2.00, 2.47 and 2.49. An analysis for all species combined resulted in an estimate of 5826 ±195 K.

The lower and upper boundary of the tolerance range where 69% (=ln2 %) of the enzymes are active and T_{AL} and T_{AH}, the Arrhenius temperatures for the rate of decrease at both boundaries are presented for the various species in Fig. 7.3 and Table 7.2. *M. balthica* and *M. edulis* had the lowest optimal temperatures, followed by *C. gigas*, *M. arenaria* and *C. edule*. Especially *C. gigas, M. arenaria* and *C. edule* are able to tolerate high temperatures in the upper 20s °C. The Arrhenius temperature of the rate of decrease at the lower temperature boundary was more different between species (45430 – 1300000 K) than the Arrhenius temperature of rate of decrease at the upper boundary (30000 – 49368 K).

Table 7.2. Temperature tolerance for various bivalve species based on field and laboratory data.

Symbol	Dimension	Interpretation	*M. balthica*	*M. arenaria*	*C. edule*	*M. edulis*	*C. gigas*
T_{OPT}	K	Optimum temperature	287	295	301	289	293
T_L	K	Lower boundary of tolerance range	273	278	278	275	281
T_H	K	Upper boundary of tolerance range	290	306	306	296	305
T_{AL}	K	Arrhenius temperature for rate of decrease at lower boundary	6700000	1300000	51154	45430	75000
T_{AH}	K	Arrhenius temperature for rate of decrease at upper boundary	49368	30177	47126	31376	30000

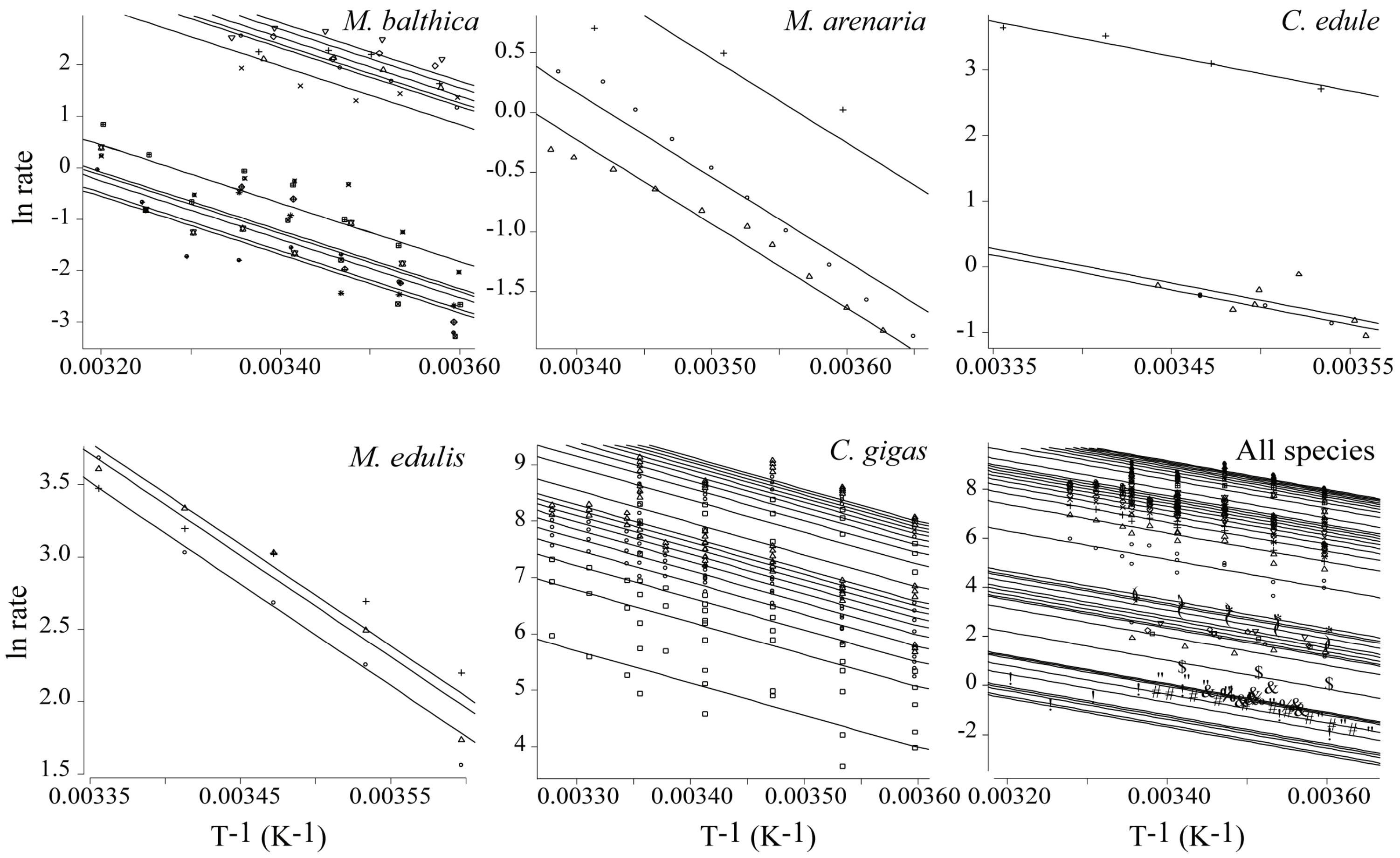

Fig. 7.2. Arrhenius plot (ln rate versus 1/T) for the oxygen consumption data for various bivalve species. Arrhenius temperature (±S.E.): *M. balthica:* 5672 ± 522 K (n=57; r^2=0.94); *M. arenaria*: 7051±453 K (n=30; r^2=0.95); *C. edule:* 5290 ±1107 K (n=11; r^2=0.98); *M. edulis:* 7022±552 K (n=11; r^2=0.94); *C. gigas*: 5722±229 K (n=179; r^2=0.92); all species together: 5826±195 K (n=281; r^2=0.99).

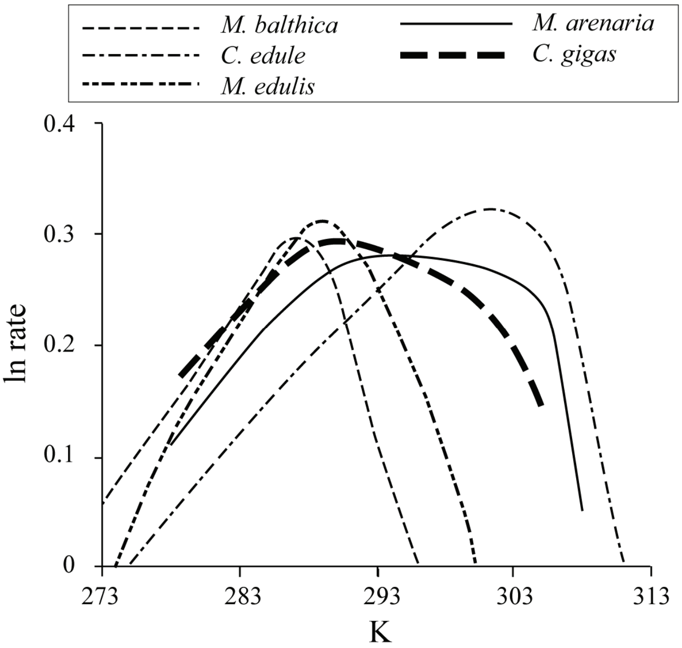

Fig. 7.3. Relative temperature tolerance for *M. balthica*, *M. arenaria*, *C. edule, M. edulis* and *C. gigas*. Lines indicate predicted values based on estimates of T_L, T_H and rates of decrease at lower (T_{AL}) and upper (T_{AH}) boundary from Table 7.2. Data for *M. balthica* after Hummel (1985); for *C. edule* after Kingston (1974); for *M. arenaria* after Lewis and Cerrato (1997); for *M. edulis* after Schulte (1975); and for *C. gigas* after Bougrier et al. (1995).

Maximum surface area-specific ingestion $\{\dot{J}_{X m}\}$ *and assimilation rate* $\{\dot{p}_{Am}\}$.

The only substantial data set on the maximum surface area-specific ingestion rate was on clearance rate and on filtration rate in *C. gigas* (Bougrier and Bourles unpubl.). The combined data set resulted in estimates with a large standard error, perhaps because filtering rate was measured by the change in algal densities, while it is also dependent on the algal density. The maximum surface area-specific filtration rate at 20 °C amounted to 7.65±14.3 dm^3 cm^{-2} h^{-1}, and a saturation coefficient of 8.89±19.2 10^6 cells dm^{-3} (Fig. 7.4a). The relationship between food concentration and filtration rate resulted in an estimate of the maximum surface area-specific filtration rate at 20 °C of about 50 J cm^{-2} h^{-1} or 1.2 kJ cm^{-2} d^{-1} (Fig. 7.4b). The maximum surface area-specific assimilation rate was derived from the maximum surface area-specific ingestion rate using an assimilation efficiency of 0.75 after Gerdes (1983). The estimate of the maximum surface area-specific assimilation rate $\{\dot{p}_{Am}\}$ amounted to 0.9 kJ cm^{-2} h^{-1} at 20 °C, again with a large variability.

Volume-specific maintenance costs per unit of time $[\dot{p}_M]$.

In *M. balthica,* the volume-specific maintenance rate could be estimated from a starvation experiment at various temperatures (Drent and Honkoop 2004). Gonad mass remained stable at 9 ºC indicating that at this temperature the mass decline [0.057(±0.016) mg ADFM d^{-1}, which corresponds with 1.311 J d^{-1} Beukema 1997] must roughly have equalled the maintenance demands.

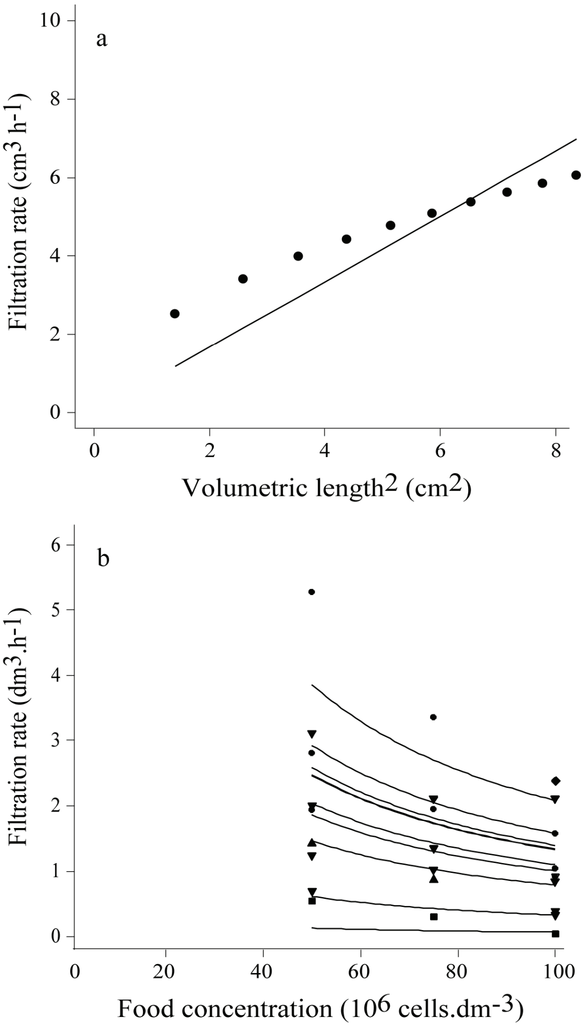

Fig. 7.4. Maximum surface area-specific ingestion and assimilation rate for *C. gigas,* based on (a) relationship between filtration rate (cm^3 h^{-1}) and volumetric length (cm^2) at 20 °C. Estimate of maximum surface area-specific ingestion rate: 7.65±14.3 dm^3 cm^{-2} h^{-1}; saturation coefficient of 8.89±19.2 10^6 cells dm^{-3}. (b) relationship between filtration rate (cm^3 h^{-1}) and food concentration (10^6 cells cm^3) for different body wet masses (cm^3) at 20 °C. Estimate of the maximum surface area-specific ingestion rate: 50 J cm^{-2} h^{-1} at 20 °C.

This volume-specific maintenance rate was used by a structural body mass at the start of the experiment of 21 mg AFDW, which corresponds with 175 mg or 0.175 cm^3 wet mass. This implies a volume-specific maintenance rate of 7.49 J cm^{-3} d^{-1} at 9 ºC, or 16.04(±4.50) J $cm^{-3}d^{-1}$ at 20 °C. In another approach, Honkoop and Beukema (1997) estimated a loss due to maintenance during winter in *M. balthica* of 0.29 body mass index (BMI) corresponding with

12.18 J per ºC deviation of the average winter temperature (4 ºC). At an Arrhenius temperature of 5672 K this difference of 1 ºC represented 5% of the volume-specific maintenance rate costs at 20 ºC. At the start of the winter period (90 days) initial BMI was at a maximum of 13.8 mg AFDM, which corresponded with 116.66 mg wet mass. This means a volume-specific maintenance rate of 23.2 J cm^{-3} d^{-1} at 20 ºC.

In *M. arenaria*, the volume-specific maintenance costs were estimated from the growth experiment at various temperatures under excess of food (Lewis and Cerrato 1997). Total tissue mass remained stable at 12 ºC at an ingestion rate of about 80 µg C d^{-1}. Since losses due to digestion will be low in this type of experiments with algal cultures, ingestion rate will more or less reflect assimilation rate. Part of the energy will be used for building up reproductive material. Therefore, the fact that total tissue mass did not increase implied that assimilation did not compensate for maintenance costs and that the individuals were starving. Applying the general conversions, and a shape coefficient of 0.277, it implies that an assimilation of 37 J d^{-1} did not meet the maintenance requirements of a 6-7 cm *M. arenaria* representing between 4.5 and 7 g somatic mass. This means that the volume specific maintenance costs were higher than 5–8 J cm^{-3} d^{-1} at 12 ºC or 10–16 J cm^{-3} d^{-1} at 20 ºC under the assumption of an Arrhenius temperature of 5826 K. Another indirect estimate of the volume-specific maintenance costs for *M. arenaria* was obtained from the field data of Zwarts (1991). Immature individuals lost 2.72 mg AFDM over a period of 60 days between October and December. At the end of December their wet mass was 0.099 g, which means an energy loss of 10.49 J cm^{-3} wet mass at an average temperature of about 9 ºC, corresponding with less than about 26.7 J cm^{-3} d^{-1} wet mass at 20 ºC, since a small part of this energy will reflect the flow to reproduction.

For *C. edule*, an indirect estimate of the volume specific maintenance rate could be obtained from the field data of Zwarts (1991). Immature individuals lost 1.42 mg AFDM over a period of 60 days between October and December. At the end of December their wet mass was 0.046 g, which means an energy loss of 11.7 J cm^{-3} wet mass at an average temperature of about 9 ºC, corresponding with maximum about 23.5 J cm^{-3} d^{-1} wet mass at 20 ºC, since part of this energy reflects the flow to reproduction.

In *M. edulis*, the volume specific maintenance rate was estimated in an indirect way from the data on oxygen consumption in starved mussels at 15 ˚C (Bayne and Thompson 1970). Oxygen consumption rate declined and stabilized after 60 days at 0.07 ml O_2 h^{-1} or 0.095 ml O_2 h^{-1} (1 mg=31.25 mM and 1 ml=41.19 mM at 15 ˚C), which corresponds with 33.35 J d^{-1} for a mussel of 4.5 cm or 2.15 g somatic wet mass. This results in an estimate of 15.5 J cm^{-3} d^{-1} at 15 ˚C or maximal 23.5 J cm^{-3} d^{-1} at 20 ˚C, since a small part of this energy flow will be related to maturity maintenance.

In *C. gigas,* the volume-specific maintenance rate was estimated from the loss of energy content during starvation (Whyte et al. 1990). At a constant temperature of 10 °C, the energy content decreased from 14.19 kcal into 7.19 kcal over a period of 150 days, at a structural volume of 15 g wet mass at the end of the experiment. This results in an estimate of 13 J $cm^{-3}d^{-1}$ at 10 °C, which corresponds with maximal 26 J $cm^{-3}d^{-1}$ at 20 °C.

Volume-specific costs for growth $[E_G]$

In all species, there were no data to estimate the volume-specific costs for growth. Only the energy content of structural mass could be determined for the various species based on the seasonal pattern in somatic mass in the Dutch Wadden Sea (Fig. 7.5), whereby it was assumed that the minimum in somatic mass reflected an individual consisting of structural mass only with hardly any reserves left.

In *M. balthica*, the lowest somatic mass index corresponded with about 3 mg AFDM cm^{-3}. In combination with the shape coefficient of 0.365, this implied an energy density of 63 J per 0.048 cm^3 wet mass or 1.294 kJ cm^{-3} wet mass. In *M. arenaria*, the lowest somatic mass index observed in the field corresponded with about 1.36 mg AFDM cm^{-3}. In combination with the shape coefficient of 0.277, this meant an energy density of 28.6 J per 0.021 cm^3 wet mass or 1.342 kJ cm^{-3} wet mass. In *C. edule*, the lowest somatic mass index observed corresponded with about 3.5 mg AFDM cm^{-3}. In combination with the shape coefficient of 0.381, this meant an energy density of 73.5 J per 0.055 cm^3 wet mass or 1.328 kJ cm^{-3} wet mass. In *M. edulis*, the lowest somatic mass index occurred in January (Zwarts 1991). From the seasonal pattern in somatic mass index it could be estimated that this minimum would be in the order of about 2 mg AFDM cm^{-3}. In combination with the shape coefficient of 0.287, this meant an energy density of 42 J per 0.024 cm^3 wet mass or1.770 kJ cm^{-3} wet mass. In *C. gigas*, the minimum energy content occurred at a somatic mass index of 3.3 in November, which corresponds with 229 J. In combination with the shell mass – wet mass relationship (Cardoso et al. 2007a, Chapter 5), this implied a volume specific energy content of 1.125 kJ cm^{-3}

There are no estimates available in bivalves for the energy costs of growth to build up somatic mass.

Maximum storage density $[E_m]$

In all species, estimates of the maximum storage density were based from the difference in average somatic mass index of the population at the end of the winter when stores are low and at the end of the growing season when stores are high (Fig. 7.5). Data used originated from the intertidal in the western Dutch Wadden Sea (Cardoso et al. 2007a, 2007b, in press; Chapters 2, 3, 4 and 5). In *M. balthica*, somatic mass index showed a maximum of about 11 mg AFDM cm^{-3} in August and a minimum of about 6 mg AFDM cm^{-3} in February, which meant a storage density of about 5 mg AFDM cm^{-3}. With a shape coefficient of 0.365, this implied a storage density of 5 mg AFDM or 105 J per 0.049 cm^3 wet mass or 2.16 kJ cm^{-3} wet mass. In *M. arenaria*, the difference in somatic mass index in the intertidal at the end of the winter (4 mg AFDM cm^{-3}) and at the end of the growing season (6 mg AFDM cm^{-3}) amounted about 2 mg AFDM cm^{-3}.

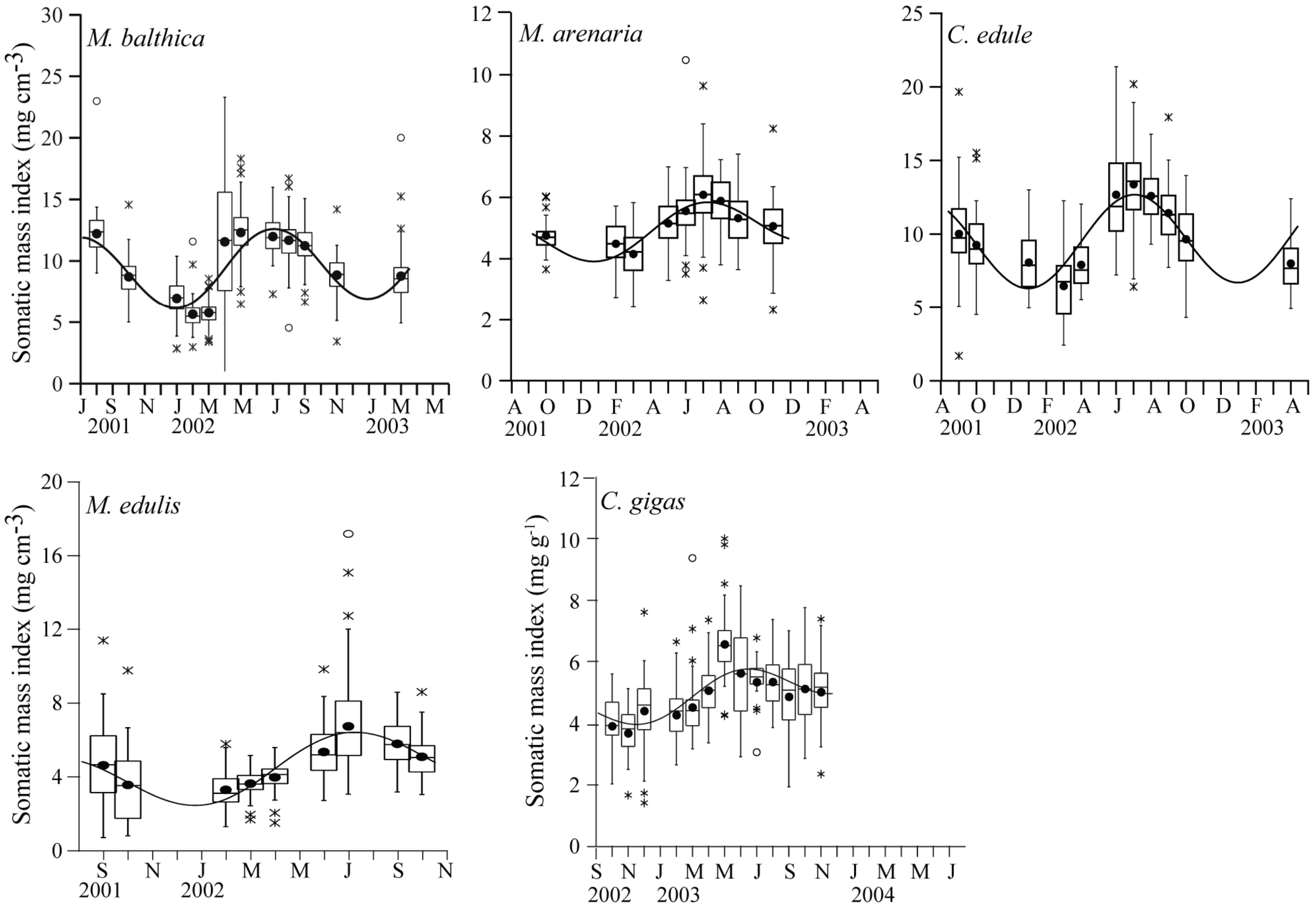

Fig. 7.5. Seasonal pattern in somatic mass index (SMI) for various bivalve species in the intertidal (Balgzand) of the western Dutch Wadden Sea. For *C. gigas* SMI is defined as somatic mass divided by shell mass (mg AFDM g^{-1}), for the other species SMI is defined as somatic mass divided by shell length^3 (mg AFDM cm^{-3}). Data after Cardoso et al. (2007a, 2007b, in press; Chapters 2, 3, 4 and 5). Blocks indicate lower 25% and upper 75% of data. Lines show 95% confidence limits.

In combination with a shape coefficient of 0.277, this meant a storage density of 2 mg AFDM or 42 J per 0.02 cm^3 wet mass or 2.1 kJ cm^{-3} wet mass. In *C. edule*, the difference in somatic mass index at the end of the winter (6.5 mg AFDM cm^{-3}) and at the end of the growing season (12 mg AFDM cm^{-3}) amounted in the intertidal about 5.5 mg AFDM cm^{-3}. In combination with a shape coefficient of 0.381, this meant a storage density of 5.5 mg AFDM or 42 J per 0.055 cm^3 wet mass or 2.08 kJ cm^{-3} wet mass. In *M. edulis*, the difference in somatic mass index occurred between August and January-February and amounted about 2.5 mg AFDM cm^{-3}. In combination with the shape coefficient of 0.287, this meant an energy density of 63 J per 0.024 cm^3 wet mass or 2.19 kJ cm^{-3} wet mass. In *C. gigas*, the difference in mass at the end of the winter and at the end of the growing season amounted about 20 mg AFDM per gram shell mass, which corresponded with 460 J per gram shell mass. In combination with the shell mass – wet mass relationship (Cardoso et al. 2007a, Chapter 5), this implied a maximum storage of 2.34 kJ cm^{-3} wet mass.

Protocol for missing primary parameters and parameter consistency

The parameter estimation for the various species did not result in a complete set for any of the species (Table 7.3). A protocol was applied to obtain estimates for missing values and achieve consistency between parameters.

Determining the Arrhenius temperature

The various estimates of the Arrhenius temperature (±S.E.) for the various species differed between 5290±1108 K and 7051±453 K, but with relatively large standard deviations. The analysis for all species combined resulted in an estimate of 5826±195 K (n = 281; $r^2 = 0.99$), and a value of 5800 K was adopted for all species.

Determining $[E_m]$, $[\dot{p}_M]$ *and* $[E_G]$

The estimates of the maximum storage density $[E_m]$ in the various species ranged from 2.080 in *C. edule* to 2.340 kJ cm^{-3} in *C. gigas*, and, as predicted by the DEB model, they showed a positive relationship with maximum volumetric length (n = 5; $r^2 = 0.63$). The values for the various species were adopted in line with the observed relationship, which resulted in slight changes, i.e. from 2.085 in *M. balthica* to 2.295 kJ cm^{-3} in *C. gigas*.

The various estimates of the volume-specific maintenance rate $[\dot{p}_M]$ at 20 ºC indicated a range between a minimum of 21 and a maximum of 26 J cm^{-3} d^{-1}. Following the DEB assumption that in related species the volume-specific maintenance costs per unit of time should be similar at the same temperature within their optimal range, i.e. around 20 ºC, an average value of 24 J cm^{-3} d^{-1} was taken for all species.

The volume-specific costs of maintenance are estimated in two steps. First, the volume-specific structural energy content was determined, and next this value was increased by the costs for material synthesis.

Table 7.3. Estimates (±S.E.) of DEB primary parameters for the various bivalve species at 293 K (20 °C) based on field and laboratory data.

Symbol	Dimension	Interpretation	*M. balthica*	*M. arenaria*	*C. edule*	*M. edulis*	*C. gigas*
T_A	K	Arrhenius temperature	5672±522	7051±453	5290±1108	7022±551	5722±229
$\{\dot{J}_{X\,m}\}$	J $cm^{-2}d^{-1}$	Maximum surface area-specific ingestion rate					1200
ρ	—	Losses due to digestion					0.25
$\{\dot{p}_{Am}\}$	J $cm^{-2}d^{-1}$	Maximum surface area-specific assimilation rate					900
$[\dot{p}_M]$	J $cm^{-3}d^{-1}$	Volume specific maintenance costs	(1) 16±4.5 (2) 23.2	(1) 10-16 (2) <26.7	<23.5	<23.5	<26
$[E_m]$	J cm^{-3}	Maximum storage density	2160	2100	2080	2190	2340
$[E_G]$	J cm^{-3}	Volume-specific costs of growth					
$[E_V]$	J cm^{-3}	Volume-specific structural energy content	1294	1342	1328	1770	1125
κ	—	Fraction of utilized energy spent on maintenance plus growth					
δ_m	—	Shape coefficient	0.365±0.098	0.277±0.077	0.381±0.114	0.287±0.090	0.175±0.060

According to the DEB theory, the volume-specific energy content and the costs for growth will be similar for related bivalve species.

The various estimates of the volume-specific structural energy content were all in the same range between 1.125 and 1.360 kJ, except for *M. edulis* (1.770 kJ). Based on these values, an average of 1.35 kJ cm^{-3} was taken for all species. The costs for material synthesis are thought to be in the order of 40% (see above). This would result in an estimate of the volume-specific costs for growth $[E_G]$ of about 1.9 kJ cm^{-3}, built up of 1.35 kJ material costs and 0.55 kJ energy overhead costs. These costs for biosynthesis are in the same order as various estimates for avian postnatal growth (summarized by Weathers 1996).

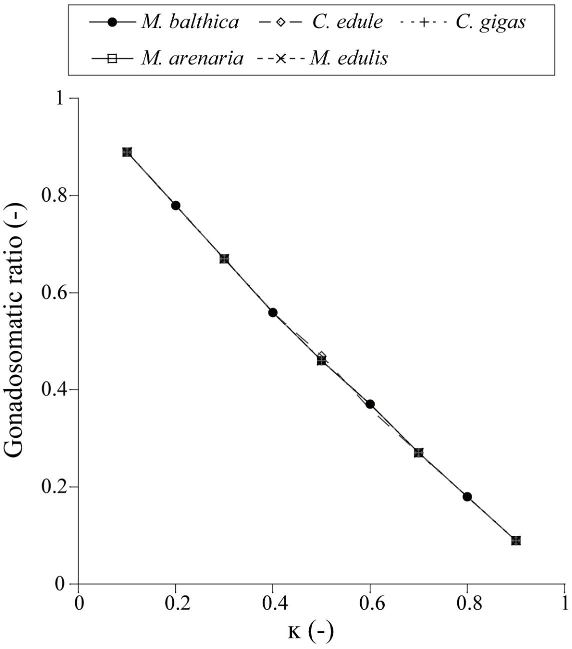

Fig. 7.6. Relationship between κ (-) and gonadosomatic ratio (-) just before spawning for the bivalves *M. balthica*, *M. arenaria*, *M. edulis*, *C. edule* and *C. gigas* based on simulations of the DEB model with species-specific parameter values for $[E_G]$, $[E_m]$ *and* $[\dot{p}_M]$. For values see Table 7.4.

Determining κ

κ was determined by model simulations whereby the DEB model structure (DEB model version 1 in Stella$^{®}$) was used as published by Van der Veer et al. (2001). The listing of the model structure in Van der Veer et al. (2001) was corrected with respect to the definition of the utilization rate: the term of the volume-specific costs of maintenance needs to be divided by the costs for growth. For all species, simulation runs were made whereby κ varied between 0.1 and 0.9. The maximum surface area-specific assimilation rate $\{\dot{p}_{Am}\}$ was kept at 500 J cm^{-2} d^{-1} (20 ºC), since the estimate of κ is not dependent on the parameter value of $\{\dot{p}_{Am}\}$. Parameters of the various species differed with respect to maximum storage density. Figure

7.6 presents the relationship between the gonadosomatic ratio (gonad mass divided by somatic mass+ gonad mass) just before spawning and κ. For all species, the relationship was about the same. Slight differences could be observed which were caused by the differences in maximum storage density between species. κ was determined based on information on the gonadosomatic ratio just before spawning for the various species in the western Dutch Wadden Sea (Cardoso et al. 2007a, 2007b, in press; Chapters 2, 3, 4 and 5), for those individuals containing gonad mass (Fig. 7.7).

In *M. balthica*, the gonadosomatic ratio just before spawning amounted about 0.18, which corresponded with a value for κ of about 0.80. In *M. arenaria*, the gonadosomatic ratio just before spawning was higher, about 0.20-0.24. Such a value was generated with a κ of about 0.75. In *C. edule*, the gonadosomatic ratio was low (about 0.16), and as a consequence the estimate of κ was higher, about 0.80. In *M. edulis*, the gonadosomatic ratio was about 0.28, which corresponded with and estimate of κ of 0.70. In *C. gigas*, gonadosomatic ratio was very high, more than 0.50, which means that κ should be in the order of 0.45.

Determining $\{\dot{p}_{Am}\}$

The surface area-specific assimilation rate $\{\dot{p}_{Am}\}$ for the various species was estimated by the relationship with maximum volumetric length, κ and the volume-specific maintenance costs $[\dot{p}_M]$, according to:

$$V_m^{1/3} = \kappa * \frac{\{\dot{p}_{Am}\}}{[\dot{p}_M]}$$

Volumetric length of the various species was calculated by multiplying the shape coefficient with the observed maximum length for *M. balthica*, *M. arenaria*, *C. edule, M. edulis* and *C. gigas*: 3, 25, 6, 15 and 45 cm, respectively (see above). *M. balthica* had the smallest surface area-specific assimilation rate of 32.9 J cm^{-2} d^{-1} at 20 °C, followed by *C. edule* (68.6), *M. arenaria* (133), *M. edulis* (148) and *C. gigas* (420). The estimate for *C. gigas* was lower than the one based on the data set by Bougrier and Bourles (unpubl. data), but it fell within the large variability range. Therefore, the estimate of 420 J cm^{-2} d^{-1} was adopted to ensure consistency between parameters.

Estimation of compound parameters

The complete set of primary parameters (Table 7.4) allows the estimation of various compound parameters.

The energy conductance $\dot{v}$ stands for the ratio of the maximum surface area-specific assimilation rate to the maximum volume-specific reserve energy density ($\{\dot{p}_{Am}\}/[E_m]$) and it can be considered as measure of conductance (cm d^{-1}). The values differed by more than a factor 10, whereby the lowest values were observed in *M. balthica* (0.016) and in *C. edule* (0.032).

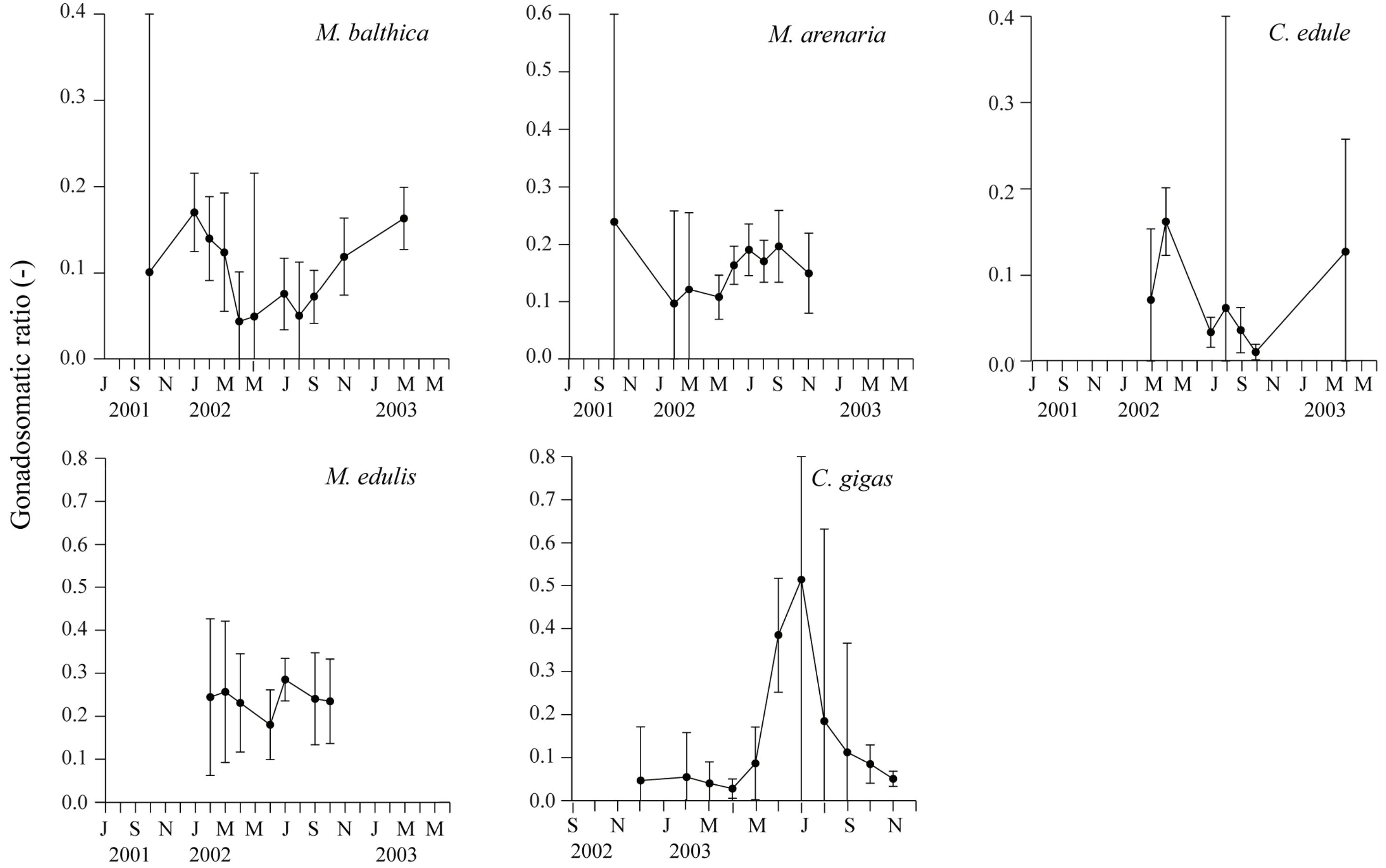

Fig. 7.7. Seasonal pattern in gonadosomatic ratio for various bivalve species in the intertidal (Balgzand) in the western Dutch Wadden Sea. Data after Cardoso et al. (2007; in press-a,b; Chapters 2, 3, 4 and 5) using only data from individuals with gonad mass.

M. arenaria and *M. edulis* had a higher energy conductance (0.061 and 0.067, respectively), but the highest value was observed in *C. gigas* (0.183).

The maintenance rate constant $\dot{k}_M$ stands for the ratio between the volume-specific maintenance costs and the volume-specific costs for growth ($[\dot{p}_M]/[E_G]$) and the dimension is d^{-1}. Both the volume-specific maintenance costs and the volume-specific costs for growth are similar for all bivalve species. Hence for all species the maintenance rate constant was 0.013 d^{-1}.

The investment ratio *g* is the ratio between the costs of new biovolume and the maximum potentially available energy for growth plus body maintenance ($[E_G]/\kappa[E_m]$). *M. balthica, M. arenaria* and *C. edule* had almost the same values (1.123 - 1.162), followed by *M. edulis* (1.239) and *C. gigas* (1.840).

Table 7.4. Parameter set of primary and compound parameters for various bivalve species at 293 K (20 °C), determined by a combination of direct estimates based on field and laboratory data (see table 7.3) and results of the estimation protocol for missing parameters.

Symbol	Dimension	Interpretation	*M. balthica*	*M. arenaria*	*C. edule*	*M. edulis*	*C. gigas*
T_A	K	Arrhenius temperature	5800	5800	5800	5800	5800
$\{\dot{J}_{Xm}\}$	$J\ cm^{-2}\ d^{-1}$	Maximum surface area-specific ingestion rate	43.9	177.3	91.5	196.8	560.0
ρ	—	Losses due to digestion	0.25	0.25	0.25	0.25	0.25
$\{\dot{p}_{Am}\}$	$J\ cm^{-2}\ d^{-1}$	Maximum surface area-specific assimilation rate	32.9	133.0	68.6	147.6	420.0
$[\dot{p}_M]$	$J\ cm^{-3}\ d^{-1}$	Volume-specific maintenance costs	24	24	24	24	24
$[E_m]$	$J\ cm^{-3}$	Maximum storage density	2085	2180	2115	2190	2295
$[E_G]$	$J\ cm^{-3}$	Volume-specific costs of growth	1900	1900	1900	1900	1900
$[E_V]$	$J\ cm^{-3}$	Volume-specific structural energy content	1350	1350	1350	1350	1350
κ	—	Fraction of utilized energy spent on maintenance plus growth	0.80	0.75	0.80	0.70	0.45
δ_m	—	Shape coefficient	0.365	0.277	0.381	0.287	0.175
$\dot{v}$	$cm\ d^{-1}$	Energy conductance	0.016	0.061	0.032	0.067	0.183
$\dot{k}_M$	d^{-1}	Maintenance rate constant	0.013	0.013	0.013	0.013	0.013
g	—	Investment ratio	1.139	1.162	1.123	1.239	1.840
$\dot{r}_B$	y^{-1}	Von Bertalanffy growth rate	1.097	0.730	1.150	0.848	0.730

The von Bertalanffy growth rate $\dot{r}_B$ can be estimated from the maximum length ($V_m^{1/3}$), the energy conductance ($\dot{v}$) and the maintenance rate constant ($\dot{k}_M$) (Kooijman 1988), according to:

$$\frac{1}{3\dot{r}_B} = -\frac{1}{\dot{v}} V_m^{\ 1/3} + \frac{1}{\dot{k}_M}$$

M. arenaria and C. gigas had the lowest values (0.730 y^{-1}) followed by *M. edulis* (0.848 y^{-1}) and *M. balthica* (1.097 y^{-1}). *C. edule* had the highest value (1.150 y^{-1}). In accordance with predictions of the DEB theory (Kooijman 1988), the von Bertalanffy growth rate scales with maximum volume to the power –1/3 (n = 5; r^2 = 0. 55).

Discussion

Ideally, each theory should allow predictions that can be falsified or supported by evidence. In case of the DEB theory, supporting evidence has been substantial since its first publication in 1986 (Kooijman 1986a,b,c), starting with the prediction of body-size relationships (Kooijman 1986a) and numerous applications, such as egg development (Kooijman 1986c), growth and reproduction of *Daphnia* spec. (Evers and Kooijman 1989), embryo development (Zonneveld and Kooijman 1993). Applications in the marine environment were restricted so far to the blue mussel *M. edulis* (Van Haren and Kooijman 1993), the Pacific oyster *C. gigas* (Ren and Ross 2001), the greenshell mussel *Perna canaliculus* (Ren and Ross 2005) and more recently to some flatfish species (Van der Veer et al. 2001, 2003) and copepod reproduction (Kuijper et al. 2004).

Despite the extensive evidence in support of the DEB theory, relative less attention has been paid to one of the most powerful and attractive aspects of the DEB theory, the ability to compare species with respect to aspects of their life history and energetics by capturing them into the DEB parameters and comparing differences in parameter values. A preliminary analysis for various bivalve (Cardoso et al. 2001) and flatfish species (Van der Veer et al. 2001) has illustrated the attraction of such a comparison. One of the reasons might be that the direct link between DEB parameters and experimental and field data is rather complicated. Ideal would be an experiment in which simultaneously food conditions, ingestion, somatic growth and respiration are measured (Kooijman 2000, Van der Meer 2006). However, such an experiment should be carried out for each species, which would seriously hamper the applicability of the DEB model for the comparison of species. Therefore, as a second best approach, we rely on existing information, whereby we try to estimate the DEB primary parameters according to a more or less standard procedure (see also Van der Meer 2006).

Estimation procedure

The standard procedure for estimating individual parameters is combining data sets as much as possible and estimating parameters by means of simultaneous regression following; this was worked out and illustrated for *M. edulis* by Van der Meer (2006). The improvement is the fact that instead of various estimates of a parameter based on single data sets; only one estimate is generated as best fit for all data sets. However, existing data sets are often too limited to allow simultaneously estimation of all parameters (see Van der Meer 2006). For some primary parameters, lack of data can be overcome by calculating the value from relationships with other parameters or from relationships between related species (Kooijman 2000). Finally, there are species-specific problems. In bivalves, estimates of maximum ingestion and assimilation rate are difficult to determine due to the complicated food intake mechanisms and the dependence on seston load in the water (Kooijman 2006). The estimate of the maximum assimilation rate for *C. gigas* based on the relationship to other DEB parameters was much too lower but within the range of the estimate based on laboratory experiments. This might be a typical problem in bivalves; in other groups such as flatfish species, maximum assimilation rates can be calculated much more reliably from laboratory observations (Fonds et al. 1992, Van der Veer et al. 2001).

Some of the primary parameters can only be calculated from relationships with other parameters, whereby the followed sequence is: based on maintenance costs, costs for growth and maximum storage density, κ is determined by means of model simulation and finally the maximum assimilation rate is calculated from the formula of maximum volumetric length under optimal food conditions. A weak link in this approach is clearly the estimate of the costs for growth. These costs are based on information on the energy efficiency in endotherms and the conversion used is only a reflection of the expected order of magnitude. The conversion used might be realistic since protein synthesis takes up a large fraction of the energy cost of the cell. However, it is clear that more information is needed about the energy efficiency of material synthesis in ectotherms. Once the parameter values for maintenance costs, costs for growth and maximum storage density have been determined, the estimation of κ seems rather robust since it is based on the gonadosomatic index. In principle, this index is a reliable estimate based on extensive data sets, however two aspects might have biased the estimates. First of all, in all species individuals were present without any gonad mass at all (Cardoso et al. 2007a, 2007b, in press; Chapters 2, 3, 4 and 5) and they were excluded. Furthermore, another assumption made is that food conditions were more or less constant since otherwise periods of starvation shortly before reproduction might have affected the gonadosomatic index. Another source of bias might be the maximum length of the species. On the one hand, this is based on the maximum observed length in numerous records and therefore rather robust. On the other hand, the maximum length of the species refers to the theoretical situation of optimal food conditions during the life time of the species, while numerous reports indicate that competition for food and food limitation is a normal phenomena in bivalves (a.o. Beukema et al. 1977, Fréchette and Bourget 1985, Thompson and Nichols 1988, Kamermans 1992, Kamermans 1993, Smaal 1997). An underestimation in the estimate of maximum length translates directly into the estimate of the surface area-specific assimilation rate. It can only be concluded that the precise value of the maximum length is unknown but that the estimate will be in the correct order of magnitude.

The energy conductance is a parameter that needs further attention. It is considered to be an intrinsic parameter, which means that the parameter should be similar in related taxonomic species and between species independent of volumetric length (Kooijman 2000). However, in contrast to the predictions of the DEB theory, the various estimates of the energy conductance differed by a factor of 5, which was caused by the fact that the volume-specific assimilation rate and the maximum energy density differed in their relationship with volumetric length. Correcting the volume-specific assimilation rate or the maximum energy density in such a way that the energy conductance becomes similar between species results in large discrepancies between model simulations and field data. Furthermore, data on the energy content of various bivalve species (Beukema 1997) suggest a weak relationship with volumetric length, but in the same order of magnitude as the estimate of the maximum storage density in this paper (Fig. 7.8). The present information suggests that within bivalves, energy conductance might be dependent on volumetric length.

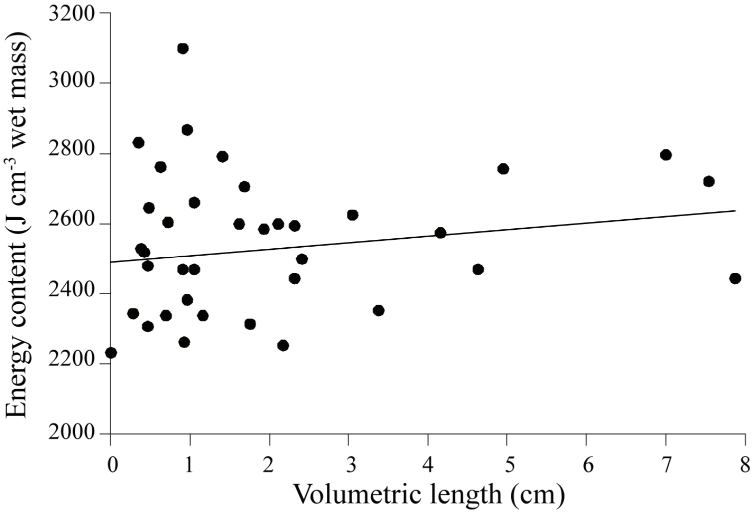

Fig. 7.8. Energy content of various bivalve species in relation to volumetric length. Energy contents were taken from Beukema (1997). Volumetric length was estimated from data on maximum length (Tebble 1976) in combination with shape coefficients from Table 7.4.

Accuracy of the parameter estimates

Although the estimation procedure might be rather robust, the accuracy of the various parameter estimates is not clear at the moment. For instance, the shape coefficient is used in the calculation of the volume-specific energy content, storage density, and maintenance costs.

However, for all species, the order of magnitude of the standard error was relatively large, about 30% of the estimate. As a consequence, a bias might have occurred in the estimates of volume-specific energy content, storage density, and maintenance costs. The solution is simple, to increase the number of observations to reduce the standard error of the shape coefficient of the various species.

Another factor is the gonadosomatic index to estimate κ. In this paper the mean gonadosomatic index is used. The 95% confidence interval illustrates that there is variability between individuals and even individuals without gonad mass are found just before spawning. Lack of gonadal mass might result from infections by parasites (Zwarts 1991), however at present it is unclear whether variability between individuals originates from differences in food conditions affecting the ratio between gonadal mass and somatic mass or whether it reflects differences in κ between individuals. In general, the impact of parasites is an increase of κ whereby the host grows to a larger size and the parasites yield the flux to development and reproduction. So the host 'rejuvenates' and ceases reproduction (Kooijman 2000). Different degrees or stages of infection will, therefore, also mean differences in κ.

Estimates of assimilation and ingestion rate are also assumed to be constant and similar between individuals; however, morphological dynamics of the foraging apparatus of bivalves is well known (a.o. Theisen 1982, Mettan 1993, Payne and Miller 1995, Barillé et al. 2000, Drent et al. 2004). Flexibility in gill-palp ratios is the result of changes in either gill mass or in palp mass or in both (Drent et al. 2004). Particles are captured by the gills and, therefore, gill mass is directly related to maximum ingestion rate. Hence, differences in gill mass might affect the maximum ingestion rate. On the inner surface of the palp, selection of particles takes place and palp mass is therefore directly related to the selection efficiency, which determines the maximum assimilation rate. In addition, environmental conditions might induce a shift of *M. balthica* from deposit- to filter-feeding (Kamermans 1992) and affect both maximum assimilation and ingestion rate. Again, controlled laboratory experiments are required to get more insight in intraspecific variability in assimilation and ingestion in the various bivalve species. Furthermore, the description and analysis of the process of feeding is restricted to the parameters of ingestion rate and assimilation rate. However, in bivalves, other processes might also regulate and control filtration and ingestion, as has been described in detail in the light of the DEB theory by Ren and Ross (2005)

Comparison of species

The taxonomic relationship between bivalve species is reflected in the similarity of various DEB parameters. Despite differences in distributional and temperature tolerance range, the temperature dependence in the optimal range as indicated by the Arrhenius temperature appears to be rather similar for the various species, around 5800 K. This Arrhenius temperature is in the same range as those observed for various North Atlantic flatfish species (Van der Veer et al. 2001). Differences between species are reflected in differences in optimal range and width of the range, whereby the tolerance range between lower and upper boundary in *M. balthica* is relatively narrow (17 K) compared with the range in the other species (24 – 28 K). The volume-specific cost for growth is the only other parameter that is similar for the various bivalves. The volume-specific structural energy content in bivalves of 1.3 J cm^{-3} is much lower than the estimate for various flatfish species (Van der Veer et al. 2001), which might be considered as a reflection of the differences in cell complexity. Maximum storage density is not similar between species but is related to volumetric length. Nevertheless, maximum difference between species is only 10%: from 2.09 J. cm^{-3} in *M. balthica* to 2.29 J cm^{-3} in *C. gigas*.

Species differences are especially present in differences in the maximum surface area-specific assimilation rate and in κ. Maximum assimilation rate differs by a factor of 13 between *M. balthica* (32.9 J cm^{-2} d^{-1}) on the one hand and *C. gigas* (420 J cm^{-2} d^{-1}) on the other hand. The observed differences in κ are also substantial from 0.45 in *C. gigas* to even 0.80 in *M. balthica* and *C. Edule*, but the differences in energy stored in reproduction between *C. edule* and *C. gigas* in absolute sense are even much higher when also differences in egg diameter are taken into account. The range of κ observed in bivalves corresponds with that found in flatfish, except for the very low value of 0.45 for *C. gigas*.

The estimates in this paper are a step forwards compared with previous preliminary analyses (Cardoso et al. 2001), but it is clear that the various parameter estimations require some further fine-tuning. Such a fine-tuning might result from specially designed experiments, but an alternative might be expanding the present information with estimates for other related bivalve species. In this paper the focus has been especially on interspecific differences. At present, it remains unclear whether intraspecific variability in parameter values occurs and, if so, whether it can be related to variability in environmental conditions, genetic variability or phenotypical plasticity.

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CHAPTER 8

Intra- and interspecies comparison of energy flow in bivalve species in Dutch coastal waters by means of the Dynamic Energy Budget (DEB) theory

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Abstract

The energy flow in various bivalve species (*Macoma balthica*, *Mya arenaria*, *Cerastoderma edule*, *Mytilus edulis* and *Crassostrea gigas*) in different habitats (intertidal, subtidal and offshore areas) of Dutch coastal waters was analysed by comparing growth observations in the field with model simulations by means of the Dynamic Energy Budget (DEB) theory. The reconstruction of the seasonal food conditions suggested that with respect to food consumption, *M. balthica* and *C. edule* are the most successful species, followed by *M. arenaria*, and *M. edulis* and *C. gigas*. Positive growth was correlated with the beginning of the spring bloom in primary production and food limitation between species was lowest during this period. After the spring bloom, severe food limitation during the summer period was found. As a result, negative body growth occurred during most of year. DEB model simulations both on an annual and a seasonal basis also showed that, at the prevailing water temperatures, growth of the different species was not maximal. It remains uncertain whether the suboptimal food conditions were the consequence of food limitation or also of reduced filtration efficiency due to the high sediment load in the water.

Introduction

The fact that the macrobenthic biomass in temperate estuaries can be predicted from primary production data (Herman et al. 1999) suggests that food limitation is a common phenomenon. Long-term studies in the western Dutch Wadden Sea, where changes in phytoplankton were associated with parallel changes in zoobenthic biomass (Beukema et al. 2002) support this view. Also on a local scale, competition for food has been reported from stomach content analysis of filter-feeding bivalves (Hummel 1985b, Kamermans et al. 1992, Kamermans 1993, Cognie et al. 2001) and from the observations that bivalve filtration indeed results in particle depletion of the overlying water mass (Asmus et al. 1992, Jonsson et al. 2005). This evidence suggests that food may be the prime limiting factor for benthic biomass in estuarine systems and it points to strong intra- and interspecies interactions.

Environmental changes in temperature and food conditions will affect the energy available for the different physiological processes and determine rates of growth and reproduction. Therefore, any analysis on the role of intra- and interspecific interactions on the population dynamics of macrozoobenthos requires as a starting point a comparative study into the physiology of the various species. Ideally, such a comparative bioenergetic study should be based on a general framework built on first principles. The Dynamic Energy Budget (DEB) theory (Kooijman 1988, 2000) offers such a framework for a quantitative description of the energy flow through an individual and the allocation of energy over growth and reproduction in relation to environmental conditions and food intake. The same model can be applied to different species, whereby intraspecific variability in growth and reproduction is caused by differences in environmental conditions (temperature, food), and interspecific variability is caused by differences in parameter values, in combination with differences in environmental conditions (Kooijman 2000).

The elegance of the DEB model is two-fold; (1) it is based on first principles and (2) only a few equations and parameters fully determine and describe feeding, growth and reproduction. Once these species-specific parameters are known, growth and reproduction can be determined if the fluctuations in temperature and food are known. The DEB model can also be applied the other way round for the reconstruction of environmental conditions from observed growth and reproduction patterns in the field. This innovative approach to reconstruct food conditions for various species under field conditions provides insight into the presence of food limitation and competition among species.

In this paper, we apply the DEB model for the reconstruction of food conditions for macrobenthic species in Dutch coastal waters. The focus is restricted to the most common bivalve species, not only because they are abundant and have similar food sources, but also because their age and hence growth can be determined from the analysis of annual shell growth marks. Only a few bivalve species account for most of the biomass: the Baltic tellin *Macoma balthica* (L.), the edible cockle *Cerastoderma edule* (L.), the blue mussel *Mytilus* *edulis* L., the soft-shell clam *Mya arenaria* L. and the recently introduced Pacific oyster *Crassostrea gigas* (Thunberg) (Beukema 1976, 1979; Dankers and Beukema 1983, Dekker 1989, Holtmann et al. 1999, Cardoso et al. 2007a). In the Dutch Wadden Sea and adjacent coastal waters, these species occupy different habitats (Wolff 1983). *M. balthica* can be found from the upper regions of the intertidal and the subtidal to the outer parts of the tidal inlets in the coastal zone. *M. arenaria* occurs mainly from the upper regions of the intertidal to the subtidal but in other areas it extends to considerable depths offshore. *C. edule* is present from mid tide to just below the low water mark and *M. edulis* occurs from high in the intertidal zone to below low water in the subtidal. *C. gigas* can be found on hard substrate (stones, mussel and cockle banks) in the intertidal. *M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* are suspension-feeding species, while *M. balthica* is a deposit-feeder also capable of suspension-feeding (Hummel 1985).

This paper is built on a number of contributions. Extensive field data on seasonal patterns in growth and reproduction of the various species in relation to habitat (intertidal, subtidal, offshore) have been collected by regularly sampling over the years 2001 – 2003 (Cardoso et al. 2007a, 2007b, in press; Chapters 2, 3, 4 and 5). At the same time, the complete set of DEB parameters for the various bivalve species was determined in a systematic way based on various field and laboratory datasets (Van der Veer et al. 2006). The final objective of this paper is to reconstruct food conditions for the various species and habitats by applying the DEB model (c.f. Kooijman 2000, Van der Meer 2006).

Materials and methods

Field data

Field data on growth and reproduction of the various bivalves in Dutch coastal waters was collected during a monthly sampling programme of about 1.5 years between 2001 and 2003 (details can be found in Chapters 2, 3, 4 and 5). In short, about 100 individuals of each species were sampled, if possible, every month, and water temperature was also measured at each sampling date. For each collected bivalve, age, shell length, ash-free dry mass of body and reproductive organs were determined. In this way, it was possible during each sampling to split up the sample into age groups and to estimate the mean somatic mass (total body mass excluding gonads) for each species and habitat. Next, these monthly samples allowed determining the seasonal variation in somatic mass split up per age group for each species and location.

The following analyses were made for each species and location:

(1) growth of shell length;

(2) seasonal growth patterns of somatic mass for each age group;

(3) seasonal pattern of the mean instantaneous growth rate (G; d^{-1}) per age group according to:

$$G = \frac{\ln \overline{SM_{i+1}} - \ln \overline{SM_i}}{\Delta t}$$

whereby:

G is the instantaneous growth rate per sampling date

$\overline{SM_i}$ is the mean somatic mass at sampling period *i*;

Δt is the time interval in days between sampling *i* and sampling *i+1*.

DEB model simulations

DEB model

The DEB model describes the energy flow through an animal (see Fig. 6.1, Chapter 6) and also changes therein in environments with varying food densities and temperatures. A conceptual introduction to the Dynamic Energy Budget theory can be found in Kooijman (2001) and in Van der Meer (2006), a full description is given in Kooijman (2000). The DEB model has been translated into Stella® (see Van der Veer et al. 2001). The listing of the model structure in Van der Veer et al. (2001) was corrected with respect to the definition of the utilization rate: the term of the volume-specific costs of maintenance needs to be divided by the costs for growth.

Applying the DEB model requires a complete set of DEB parameters for each species, as well as temperature and food conditions as input variables. In this study the parameter values published by Van der Veer et al. (2006, Chapter 7) were taken. For temperature data at each location, measurements during sampling were taken. The observed water temperatures in the field were converted into weekly averages and used as input table in the DEB model. The link between food conditions in the field and ingestion rate ($\dot{J}_X$) as a function of food density is described in the DEB model by a hyperbolic functional response (Kooijman 2000):

$$\dot{J}_X = \{\dot{J}_{Xm}\} f V^{2/3}$$

where $\{\dot{J}_{Xm}\}$ is the maximum ingestion rate per unit of surface area; $V^{2/3}$ is the body surface area and f is the scaled functional response. Values of the scaled functional response vary from 0 (starvation) to 1 (optimal, maximal food conditions). This response curve corresponds with the type II response curve as proposed by Holling (1959). The relation between filtration rate in bivalves and the amount of food is well described by this type of curve (Kooijman 2000, 2006). Filtration rate decreases with increasing food concentration, leading to a constant ingestion rate (Fig. 8.1), since at a certain moment in time, ingestion rate cannot increase further due to the time necessary for food digestion. The output of all DEB simulations is energy measured in Joules. They were converted from energy (J) into ash-free dry mass by applying the conversion factors listed in Van der Veer et al. (2006, Chapter 7) and subsequently into shell length by applying the shape parameter. For procedure see Kooijman (2000) and Van der Meer (2006).

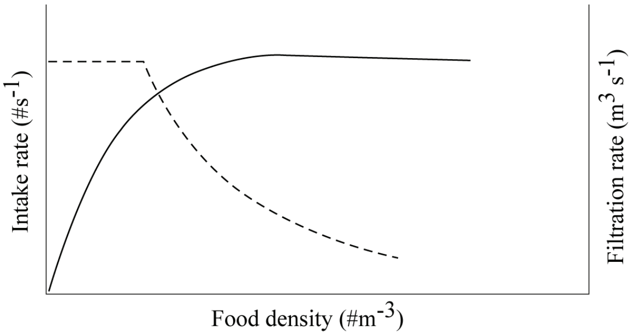

Fig. 8.1. Schematic representation of the process of food intake (solid line) and filtration (dashed line) in relation to food density.

Model simulations

DEB simulations were restricted to the reconstruction of food conditions for the various species in the various locations under prevailing water temperature conditions. Two different types of simulations were done:

(1) reconstruction of the average annual food conditions based on the field data of the growth curves in shell length;

(2) reconstruction of the seasonal pattern in food conditions based on the field data of the seasonal pattern in mass and the instantaneous growth rate.

The reconstruction of the food conditions in the field was done by manually changing the value of the scaled functional response *f* over the year in the simulation model until the simulated values of shell length and instantaneous growth rate were similar to the patterns observed in the field.

To determine the accuracy of the seasonal pattern in reconstructed food conditions, linear regressions in the form of Y = a*X without intercept were calculated between the observed instantaneous growth rates in the field and the reconstructed ones by means of the DEB model simulations. These regressions were made for all species and locations and for each age group separately.

Results

The DEB parameter set for the various species is presented in Tables 7.1, 7.2 and 7.4 (Chapter 7). Water temperatures in the field are presented for each location in Fig. 8.2. At the intertidal and subtidal, small spatial differences in water temperature were found for the various species.

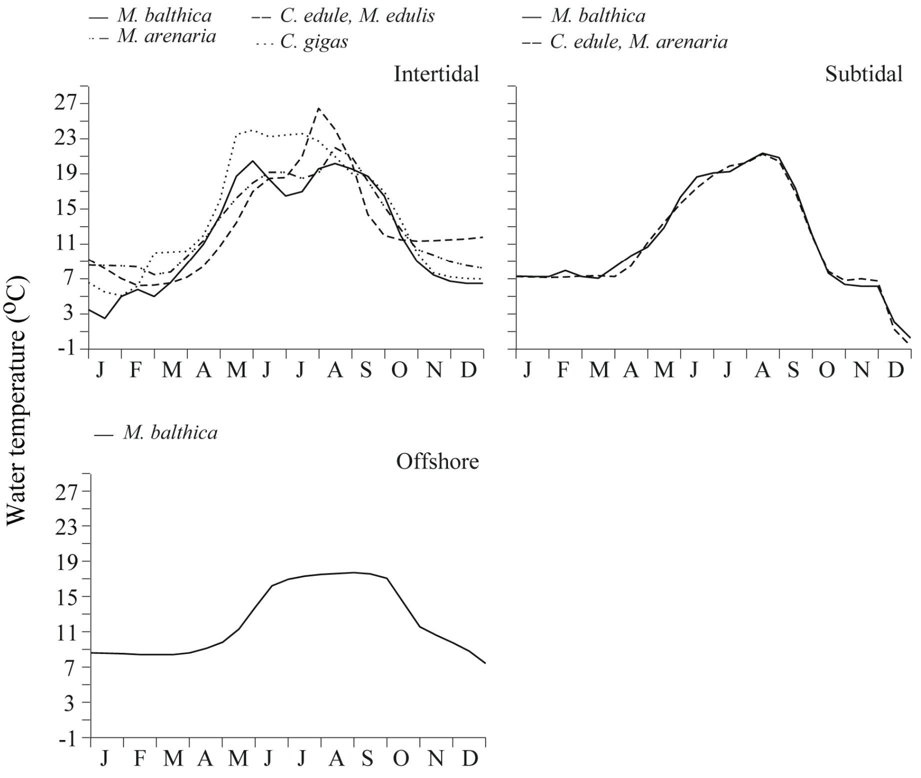

Fig. 8.2. Mean pattern in sea surface water temperature (°C) in the various habitats where *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* were sampled in 2002 (Cardoso et al. 2007a, Chapter 5, Cardoso pers. obs.).

Reconstruction of the average annual food conditions

Field data

The maximum observed growth (in shell length) in the field showed some differences between locations (Fig. 8.3). For *M. balthica*, the maximum observed size in the intertidal location was around 2.0 cm, while in the subtidal and offshore, the maximum size was around 2.4 and 2.3, respectively. In contrast, *M. arenaria* and *C. edule* presented higher growth in intertidal areas, with a maximum observed size of about 10.5 and 4.5 cm in the intertidal and 10.0 and 4.0 cm in the subtidal. *M. edulis* showed a maximum size of 7.0 cm, while the largest *C. gigas* found was around 20.0 cm.

DEB simulations

Simulated growth curves of shell length for fast and slow growing individuals are shown in Fig. 8.3. In *M. balthica*, growth in the intertidal and subtidal was faster than maximum growth under optimal food conditions ($f = 1$) predicted by the DEB model. For the other species and locations, observed patterns in the field could be simulated by the DEB model; however, the average scaled functional response that corresponded with the simulated growth curves differed between species. Although there were small differences in maximum growth between habitats, it hardly influenced the estimate of the reconstructed food conditions in terms of *f*. The estimated averaged scaled annual functional response in the field ranged from 0.6 to 1.0 for *M. balthica*, between 0.5 and 0.8 for *M. arenaria*, between 0.7 and 1.0 for *C. edule*, between 0.3 and 0.5 for *M. edulis* and between 0.2 and 0.5 for *C. gigas*. For *M. balthica*, *M. arenaria*, and *C. edule*, *f* hardly differed between habitats.

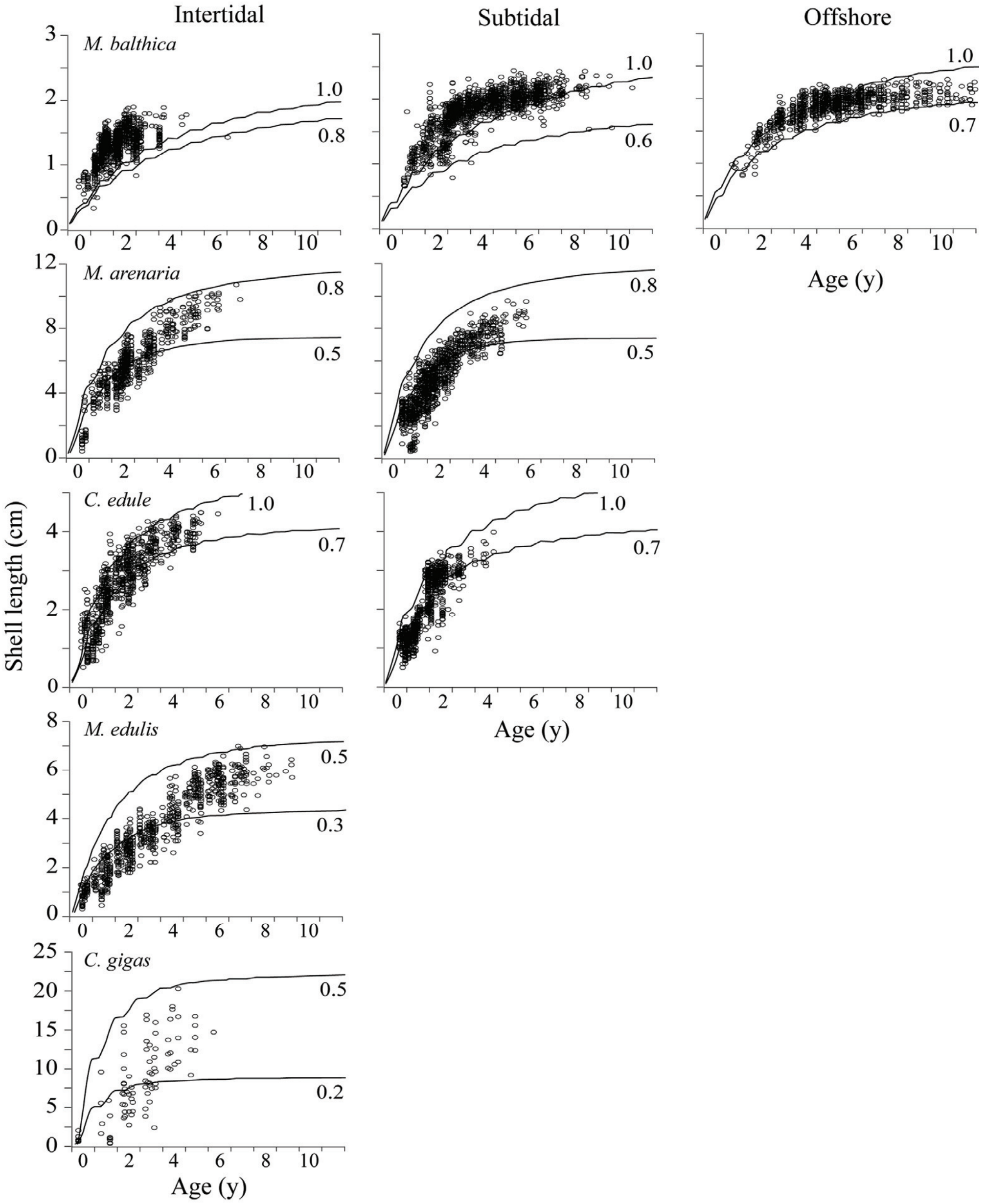

Fig. 8.3. Observed shell length (cm) with age (y) of *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* in the various habitats. Simulated growth curves were added for fast- and slow-growing individuals. Values indicate the average scaled functional response *f* used to simulate the curves under prevailing water temperature conditions. Field data from Cardoso et al. (2007a, 2007b, in press) and Chapters 2, 3, 4 and 5.

Reconstruction of the seasonal pattern in food conditions

Field data

For all age classes of the various species and habitats, the seasonal patterns of somatic mass showed a similar pattern of increase in mass during the growing season in spring/summer and a reduction during the winter season (Fig. 8.4). However, there were some differences between species with respect to timing of the growing season; for details see Cardoso et al. (2007a, 2007b, in press) and Chapters 2, 3, 4 and 5.

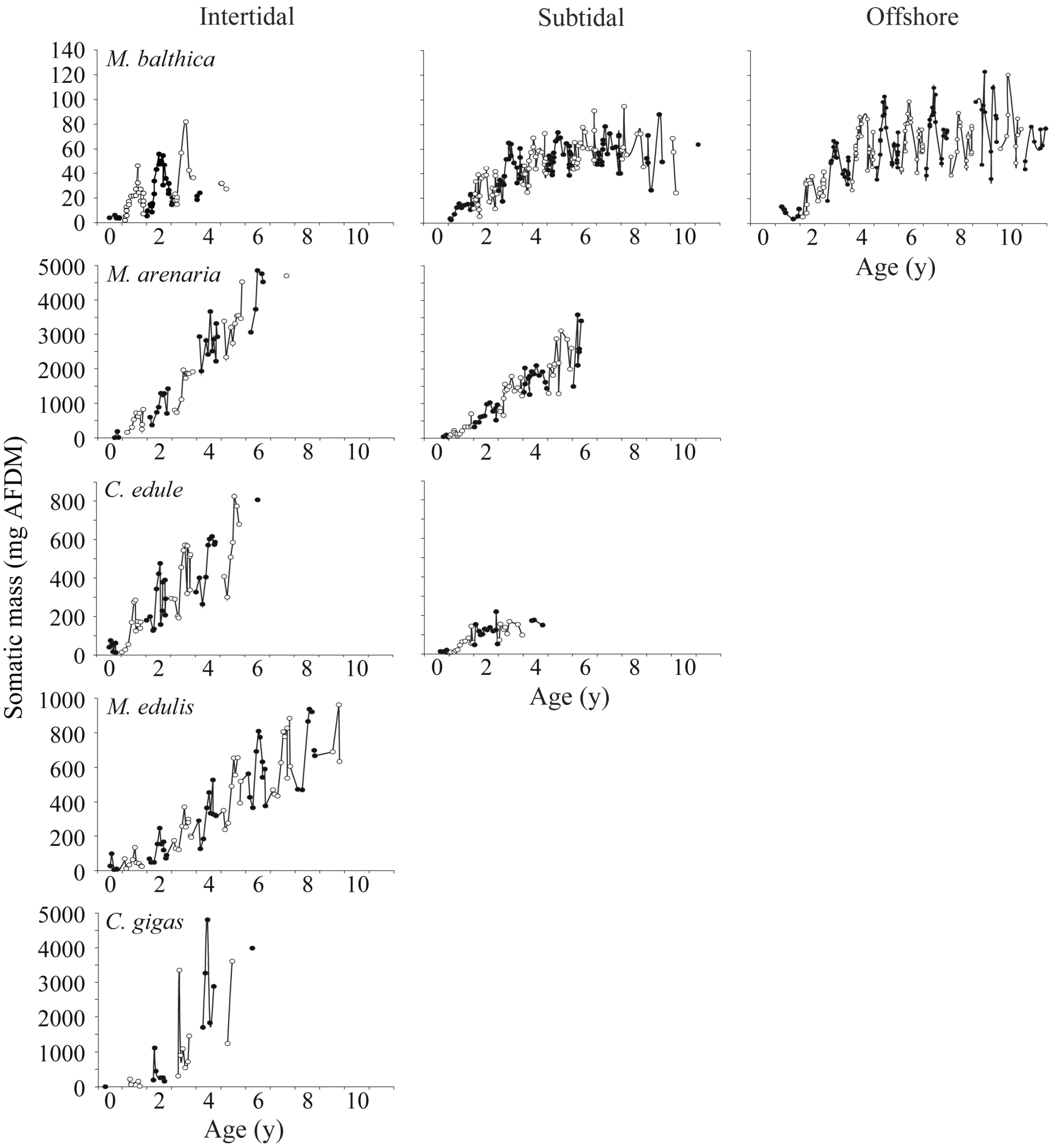

Fig. 8.4. Seasonal patterns in mean somatic body mass (without gonad mass) with age (y) of *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* in the various habitats. Various age classes are indicated alternatively with open and closed symbols. Field data from Cardoso et al. (2007a, 2007b, in press) and Chapters 2, 3, 4 and 5.

Within species, not only differences between age groups but also differences between habitats were observed. Somatic mass increase was, overall, higher in the intertidal than in subtidal areas. In this species, the offshore location showed a higher annual increase in somatic mass than at the subtidal but variability was also higher.

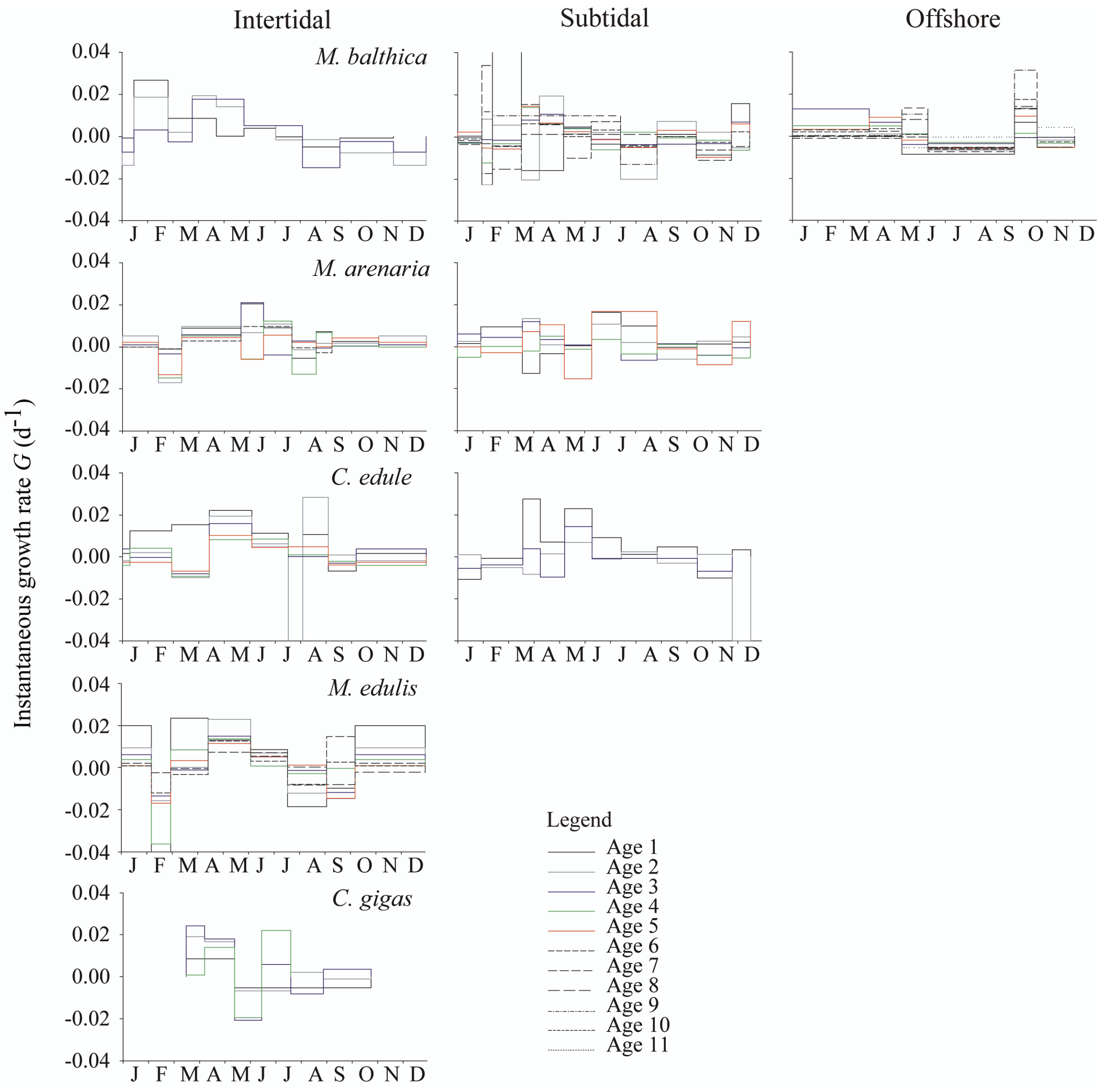

Fig. 8.5. Seasonal pattern in instantaneous growth rate of somatic mass (G, d^{-1}) of the different age classes of *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* in the various habitats. Data based on Fig. 8.4.

From these seasonal patterns in somatic mass with age, the seasonal patterns in instantaneous growth rate (G, d^{-1}) per age class were calculated for each species and habitat

(Fig. 8.5). The patterns in instantaneous growth rate reflected the patterns in somatic mass: positive values (indicating growth) during the growing season in spring- summer and negative values during the winter season. No clear trends in the annual variability in *G* with age could be observed, except for *M. edulis* where the variability in *G* decreased with age.

Low numbers of individuals were responsible for part of the variability in *G* in some age classes. In most species and for most habitats the mean instantaneous growth rate decreased with age, which reflects the shift in energy flow from growth to maintenance with increasing body size/volume (Fig. 8.6), except for *M. balthica* offshore and *C. gigas* in the intertidal. However, in *C. gigas* the standard errors of the estimates were very large.

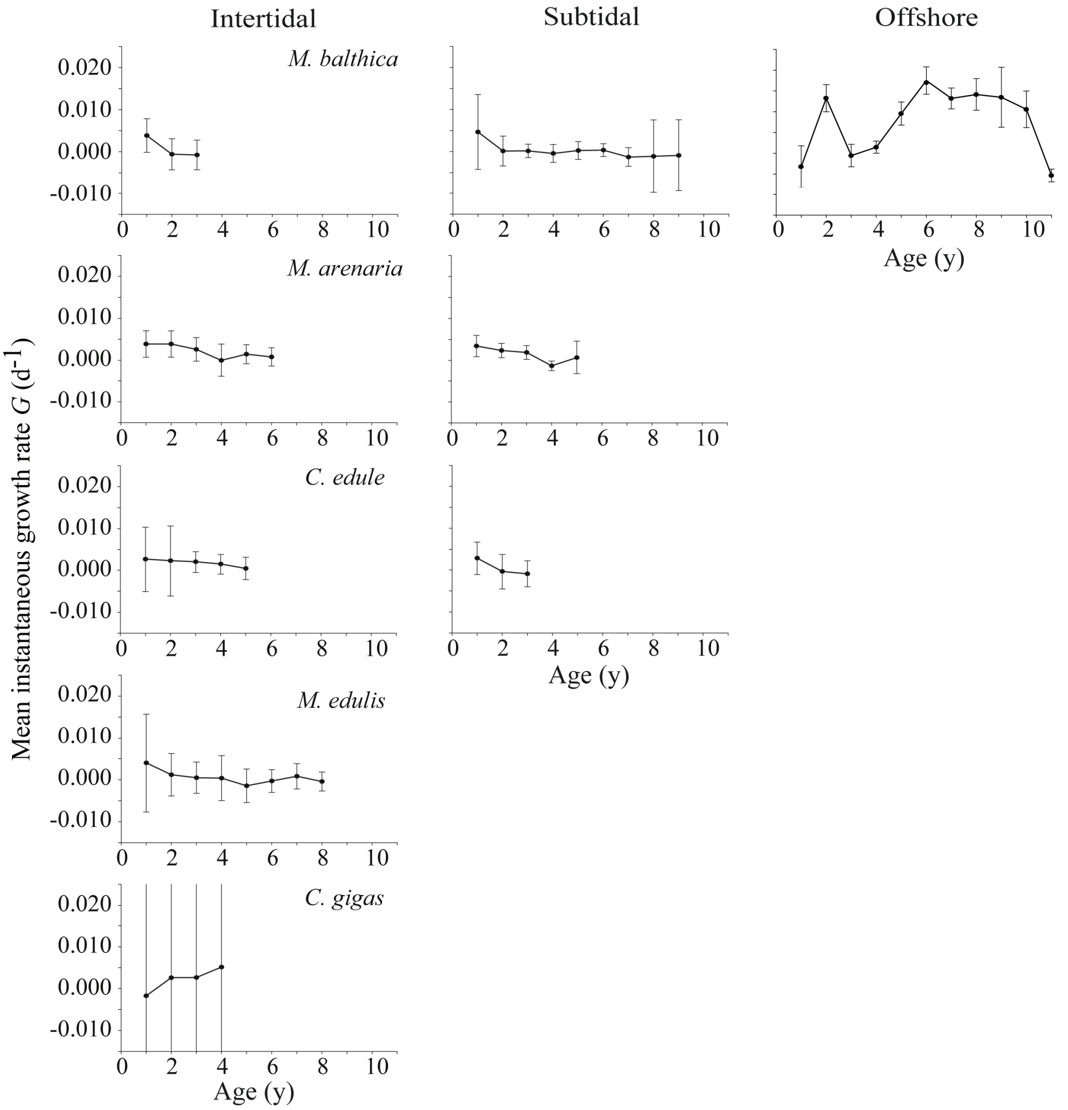

Fig. 8.6. Mean annual instantaneous growth rate of body mass (*G*±SE, d^{-1}) in *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* with age (y) in the various habitats based on data of Fig. 8.5. Data from Cardoso et al. (2007a, 2007b, in press) and Chapters 2, 3, 4 and 5.

DEB simulations

The seasonal pattern in food conditions could be reconstructed for all species and all locations in an acceptable way as is illustrated by the fact that for most species and locations the relationship between observed and reconstructed instantaneous growth rate was statistically significant (Table 8.1). The results of the simulation of the seasonal pattern in instantaneous growth rate indicated for all species a seasonal pattern in food level (Fig. 8.7).

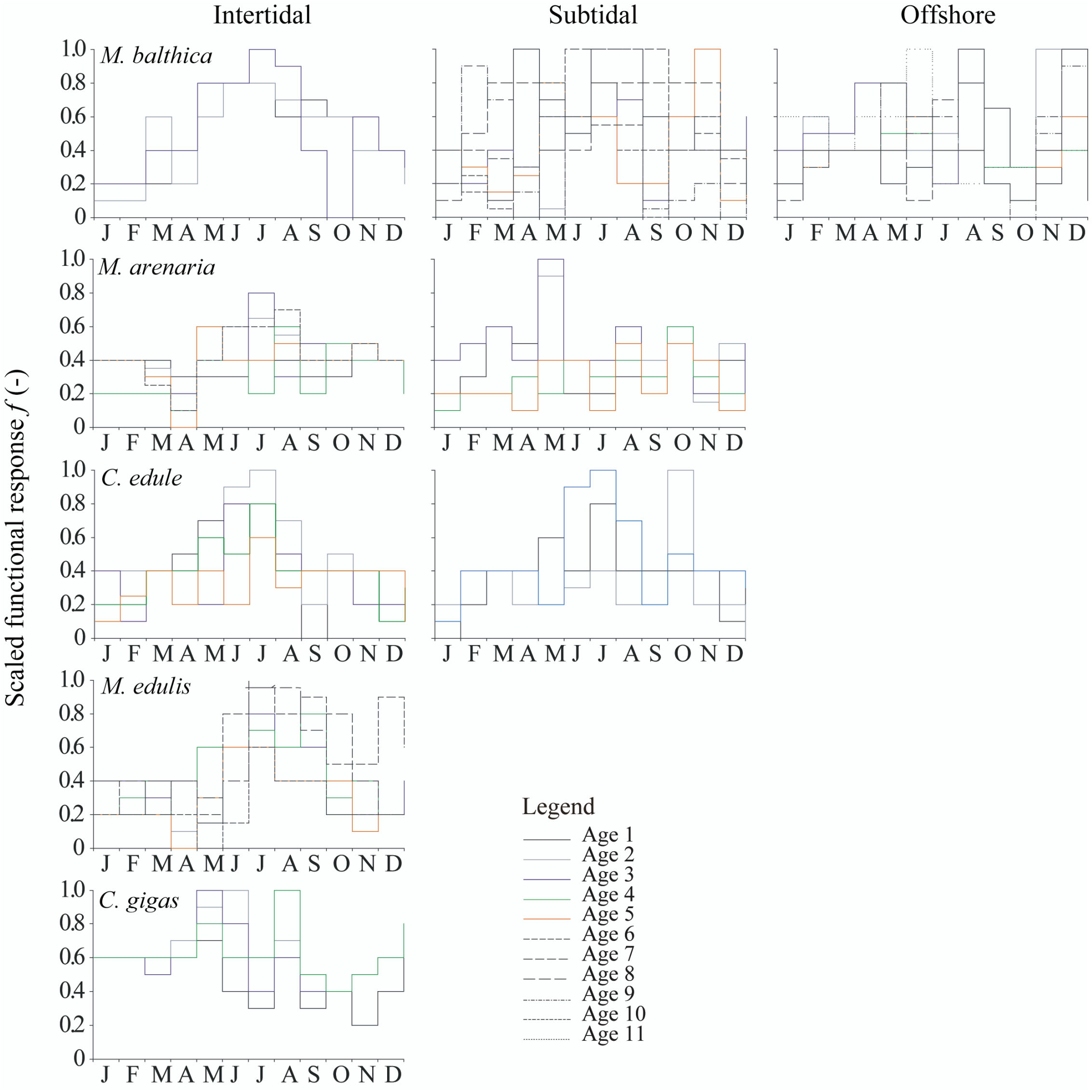

Fig. 8.7. Reconstructed seasonal pattern of food conditions, expressed as scaled functional response (-) for different age classes of *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* in the various habitats by means of DEB model simulations of the seasonal pattern in instantaneous growth rate of body mass (G, d^{-1}) in the field (data of Fig. 8.5).

Table 8.1. Relationship between observed seasonal pattern in instantaneous growth rate in the field (Y) and reconstructed instantaneous growth rate according to DEB model simulations (X) for each age group of *M. balthica, M. arenaria, C. edule, M. edulis and C. gigas* in different habitats, according to Y = *a**X.

	Intertidal				Subtidal				Offshore			
Species	Age class	*a*	n	r^2	Age class	*a*	n	r^2	Age class	*a*	n	r^2
M. balthica	1	0.795	7	0.86	1	1.183	9	0.89	1	1.130	3	0.94
	2	1.182	9	0.94	2	0.845	11	0.72	2	0.765	5	0.89
	3	1.938	7	0.82	3	1.088	11	0.76	3	1.400	6	0.96
					4	0.973	11	0.77	4	0.921	6	0.94
					5	1.039	11	0.64	5	0.998	6	0.91
					6	0.739	9	0.62	6	1.175	6	0.84
					7	0.814	11	0.67	7	0.959	6	0.86
					8	4.110	5	0.38	8	1.096	5	0.85
					9	1.077	3	0.61	9	3.544	5	0.81
									10	1.549	4	0.93
									11	1.114	5	0.82
M. arenaria	1	0.815	7	0.82	1	0.802	10	0.90				
	2	1.037	8	0.84	2	1.138	10	0.84				
	3	0.974	8	0.82	3	0.894	10	0.80				
	4	1.028	7	0.82	4	0.655	10	0.63				
	5	1.038	8	0.81	5	0.852	8	0.57				
	6	0.919	5	0.83								
C. edule	1	0.825	8	0.83	1	0.617	10	0.84				
	2	1.599	8	0.78	2	0.409	10	0.40				
	3	0.930	7	0.87	3	0.982	7	0.81				
	4	0.957	7	0.95								
	5	0.824	6	0.82								
M. edulis	1	0.984	7	0.77								
	2	1.313	7	0.92								
	3	0.932	7	0.91								
	4	1.785	7	0.61								
	5	1.123	7	0.89								
	6	0.740	7	0.67								
	7	2.010	7	0.61								
	8	1.737	5	0.80								
C. gigas	1	1.005	3	0.98								
	2	1.109	5	0.84								
	3	1.673	6	0.82								
	4	1.450	4	0.89								

n is the number of observations during the year; due to missing sampling dates or missing age classes during a sampling, n is lower than 12. *a* is the regression coefficient.

Although differences could be found between species, age classes and locations, the general pattern was similar with highest values during the growing season in spring/summer and lowest values during the winter season. In some cases, the scaled functional response was for a short period equal to 1, indicating optimal food conditions. But maximum values during the growing season in spring/summer were often lower than 1 (between 0.6 and 0.8). During the winter period, values dropped to around 0.2 and sometimes even to 0. The variability in the reconstructed seasonal pattern in food conditions between age classes was caused by variability in *G* in the field (see section above).

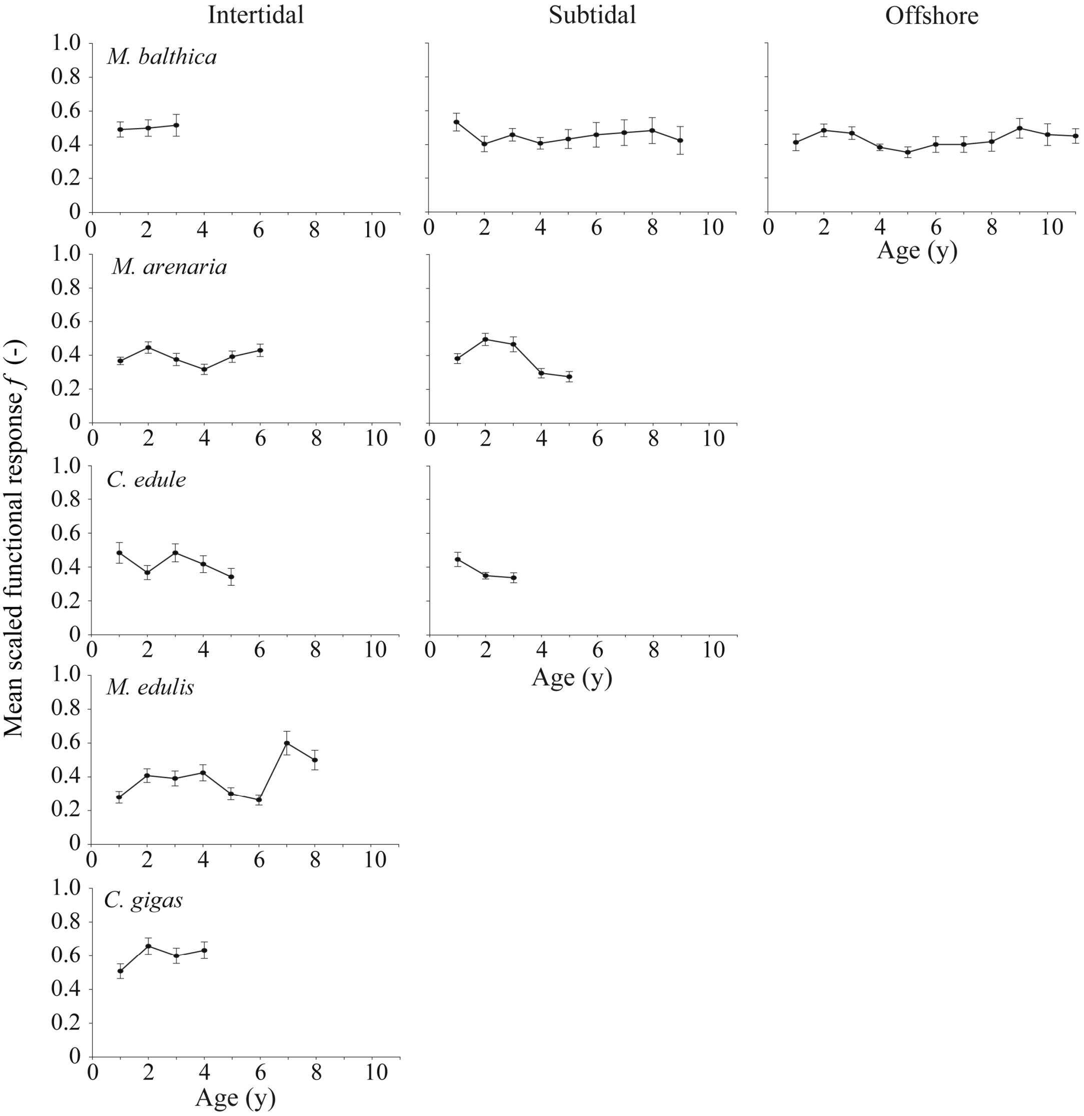

Fig. 8.8. Mean annual value of reconstructed seasonal pattern of food conditions, expressed as scaled functional response (±SE, -) for *M. balthica, M. arenaria*, *C. edule*, *M. edulis* and *C. gigas* in the various habitats by means of DEB model simulations of the seasonal pattern in instantaneous growth rate of body mass (G, d^{-1}) in the field, based on Fig.8.6. For original data see Fig. 8.4.

Considering all age classes together, the mean annual scaled functional response was about the same for all species between 0.4 and 0.6 (Fig. 8.8). With respect to trends in mean annual scaled functional response with age, the various species showed a more variable pattern. In *M. balthica* at all locations and in *M. arenaria* in the intertidal no clear pattern was found. In *C. edule* and in *M. arenaria* in the subtidal, there was a suggestion of a decrease in the mean annual scaled functional response with increasing age. Finally, in *M. edulis* and in *C. gigas* the mean annual scaled functional response showed some increase with increasing age.

Discussion

Accuracy of presented dataset

Some precaution in analysing the results is required because some factors might have affected the simulation exercises or/and the accuracy of the field data. Food reconstruction by means of DEB model simulation is only possible if DEB parameters, water temperature data and field data are available. At least for one species (*M. balthica* in the intertidal and subtidal) there is a mismatch between field data (growth in terms of shell length) and DEB simulations.

The DEB parameters were systematically estimated by Van der Veer et al. (2006), but not all parameters could be identified and a protocol had to be adopted to fill in missing values and to achieve parameter consistency. For some of the DEB parameters parts of the same dataset were used. For instance, the estimate of the maximum storage density $[E_m]$ for the various species is partly based on their seasonal pattern in somatic mass index, the somatic mass per cubic shell length (see Van der Veer et al. 2006). Furthermore, parameter values are kept the same for individuals of the same species, although some variability is expected (Kooijman 2000). This stresses the importance of well-designed experiments in which food conditions, ingestion, somatic growth and respiration are controlled and measured for individual bivalves (c.f. Van der Meer 2006).

Accurate water temperature registrations are also critical. In this study, single measurements were taken during each sampling and extrapolations were done for each week, in comparison with information from nearby registration stations. For details see Cardoso et al. (2007a, 2007b, in press) and Chapters 2, 3, 4 and 5. Continuous measurements preferably on an hourly basis by means of temperature loggers, both in the water column and the upper sediment layer would have been more optimal and more precise.

The quality of the field data is also essential. The reconstruction of the average annual food conditions was based on age-shell length growth curves, where accurate age determination is a crucial point. In *M. balthica* and *C. edule* external shell marks can be easily used to determine age. However, in old individuals, growth marks are very close to each other and more difficult to visualise. In addition, disturbance marks are not always visually distinguishable from real growth marks and can be counted as such. Both *M. balthica* and *C.*

edule generally show clear marks, easy to identify, which can be used with relative accuracy for age determination (Lammens 1967, Seed and Brown 1978, Iglesias and Navarro 1990). However, it cannot be excluded that the discrepancy between model simulations and field data in *M. balthica* in the intertidal may result from incorrect age determination. In *M. arenaria* and *M. edulis*, age was estimated in a similar way as for *M. balthica* and *C. edule*. In both species, structural marks on the shell surface were used to determine annual growth patterns in many areas (Newcombe 1936, Brousseau 1979, Brêthes and Desrosiers 1981, Roseberry et al. 1991, Littorin and Gilek 1999, Maximovich and Guerassimova 2003, Zotin and Ozernyuk 2004). However, the identification of shell marks as year rings is not so obvious as for *M. balthica* and *C. edule*, especially for individuals over 2-3 years old. In relation to *C. gigas*, an innovative validated method based on the cathodoluminescence of manganese ions on a section of the hinge was applied for age determination and seemed to be reliable (Cardoso et al. 2007a). The fact that for all species the variability in age-shell length growth curves was about the same for all age classes may suggest that the observed variability is simply caused by bias in age determination. The large variability in age-somatic mass relationships also suggests bias in age determination, especially in older individuals. Furthermore, the growth curves for the various species were estimated by combining different age classes, and thus different cohorts. Ideally, a long-term series of at least 12 years in which a single cohort is followed through time should have been used but such information was not available for the different habitats sampled in this study and it was impossible within the timeframe of the present study (4 years). Therefore, some of the discrepancy between field observations and DEB model estimates may also be due to bias in the field data.

Does food limitation imply food competition?

As stated by Herman et al. (1999), the mixing and flushing of water masses in highly dynamic estuarine systems create a continuous redistribution of the pelagic food sources, which a priori suggests competition between suspension feeding bivalves. Food limitation is also suggested in this study from the reconstruction of the food conditions for the various species in the different habitats.

The overlap in food sources between bivalve species (Hummel 1985b, Kamermans et al. 1992, Kamermans 1993, Cognie et al. 2001) and the large filtering capacity of suspension-feeding bivalves, in combination with their high abundance, are strong arguments which support the idea that the observed food limitation is caused by competition for food. For this to happen, bivalves must be able to deplete their food source in the overlying water mass, which was indeed observed in previous studies (Asmus et al. 1992, Jonsson et al. 2005). Verwey (1952) provided indirect evidence for the Dutch Wadden Sea, by calculating that filter-feeders could filter the volume of the area within days, suggesting that food competition is likely to occur. Stronger evidence was the direct response of the biomass of filter-feeders in the western Wadden Sea to a near doubling of the primary production between the 1970s and 1980s (Beukema and Cadée 1997).

However, this picture may be more complex, because Beukema and Cadée (1997) also indicated that this direct response of filter-feeders' biomass to the increase of primary production did not occur in all areas but was restricted to areas with favourable environmental conditions. In a recent study on Pacific oyster, Pouvreau et al. (2006) found different response curves of food consumption, depending on the amount of chlorophyll-a and suspended matter in the water. The suggestion that silt and other particles could affect the half saturation constant and, hence, the functional response, has been worked out by Kooijman (2006).

In temperate areas, such as the Wadden Sea, both primary production and suspended matter show a clear seasonal pattern with a peak production in spring followed by a lower second peak in autumn (Baretta and Ruardy 1988, Cadée and Hegeman 2002, Colijn and Cadée 2003). It is expected that the seasonality in food conditions will be reflected in the scaled functional response and hence food intake of the bivalves. The reconstruction of the functional response by DEB supports this idea. Overall, food limitation for bivalves is most likely a combination of competition for food (filtration rate), during some periods of the year, in combination with food selection (handling time) due to high sediment loads in the water mass.

Intraspecific comparison

For all species the variability in growth among sites is small compared with the variability among individuals within sites, but this may partly be caused by bias in age determinations. Since temperature conditions are in the optimal range for all species, except for a short period in the summer for *M. balthica* (Van der Veer et al. 2006), it is unlikely that differences in temperature conditions are causing this variability. Variability in food conditions is more likely. Especially *M. edulis* and *C. gigas* grow in high densities in beds (Beukema 1976, Dankers et al. 2004, Diederich 2006) and this may cause differences in food conditions between individuals. For the other species, the observed differences in individual growth are more difficult to understand because individuals are not very close to each other (at a spatial scale from 1 m to 100 m). *M. arenaria* and *C. edule* showed higher maximum growth in shell length and somatic mass in intertidal than in subtidal areas. In *C. edule*, although growth in shell length is in the same order of magnitude in both locations, a large discrepancy was observed between somatic mass growth between intertidal and subtidal areas. This suggests that *C. edule* in the subtidal may be starving and may also imply that *C. edule* in the subtidal immigrate from the intertidal and subsequently starve. *M. balthica* showed higher growth in length in the subtidal and offshore than in the intertidal, but for the first years of life, growth in terms of somatic mass seemed to be better in the intertidal. The fact that large *M. balthica* are only found in subtidal and offshore areas and that settlement occurs mainly in the intertidal suggests that intertidal areas may be more favourable for growth of young individuals (Cardoso et al. in press; Chapter 4).

The seasonal pattern in instantaneous growth rate is roughly similar between age classes and habitats with positive growth in spring. For some species, also positive growth is observed in autumn but, with exception of *M. balthica* offshore, variability is large. There is a suggestion for a second period of growth in bivalve populations offshore, in contrast to observed patterns at intertidal areas of the Dutch Wadden Sea (Beukema et al. 1985, Beukema and Desprez 1986). For some age groups, the low r^2 between observed and simulated instantaneous growth rate, led to large variability in scaled functional response. This mainly happened in older age classes, due to a low number of individuals in the samples.

These differences in growth between locations may also indicate that there is intraspecies variability in DEB parameters. Part of the variability will be caused by the fact that the model results in terms of energy (J) and mass (g) were converted into length. At least for *M. balthica*, also differences in shape coefficient between areas (Luttikhuizen et al. 2003) may have been responsible for part of the discrepancy. To find out which DEB parameter is the most likely candidate to vary among individuals requires more laboratory experiments. However, the fact that bivalves are able to adjust their gill-palp ratio to changing environmental conditions in sediment load (Honkoop et al. 2003) indicates that at least some variability in the surface area-specific assimilation rate can be expected.

Interspecies comparison

The seasonal pattern in somatic mass illustrates that for all species positive growth is correlated with the beginning of the spring bloom in primary production (Cadée and Hegeman 2002). At this time, functional response values are highest for all species. However, after the growing season, low values of functional response are found, indicating that strong food limitation must have occurred during the summer period, at relatively high temperatures. As a result, negative body growth is observed during the rest of the year, as shown by the decrease in somatic mass and the negative instantaneous growth rate.

A comparison of the range of scaled functional response for the various bivalve species, as indicated by their growth curves in the field, suggests that *M. balthica* and *C. edule* are the most successful species with f values between 0.7 and 1.0. *M. arenaria* showed average functional response between 0.5 and 0.8, *M. edulis* between 0.3 and 0.5 and *C. gigas* between 0.2 and 0.5. At present, it is difficult to disentangle which factors are responsible for this pattern. *M. balthica* may be able to shift between deposit- and suspension-feeding, and therefore also able to feed during periods when the tidal flats are emerged. However, the seasonal pattern of the functional response between the various habitats does not stress the importance of this factor.

Overall, the reconstruction of food conditions based both on the annual growth in shell length and on the seasonal pattern in somatic mass with age, showed that, at the prevailing water temperatures, growth of the different species is not maximal. It remains unresolved whether the suboptimal food consumption is the consequence of food limitation only or also of a reduced filtration efficiency due to the high sediment load in the water (c.f. Kooijman 2006).

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CHAPTER 9

Synthesis

Introduction

In this thesis, intra- and interspecific interactions between bivalve species of the Wadden Sea were analysed. The Dynamic Energy Budget (DEB) theory (Kooijman 1988, 2000) was selected as framework to quantify interactions among bivalve species. Since the DEB theory is based on energy flows in organisms, inter- and intraspecies interactions, such as food competition, can be analysed. After a general introduction (Chapter 1), the basic information on various bivalves was collected by studying growth and reproductive output of the different species in relation to environmental conditions (Chapters 2-5). An introduction to the DEB model was presented and its assumptions regarding body-size scaling relationships were tested (Chapter 6), followed by the estimation of the required parameters for the various bivalve species (Chapter 7). The DEB model was then applied to simulate bivalve growth under water temperature conditions observed in the field and various food levels. By comparing the simulation results with field data, intra- and interspecies competition in terms of food was determined and the seasonal pattern in food conditions for the various species and habitats was reconstructed (Chapter 8). This approach provided insight into the presence of food limitation and competition among species. In the present chapter, the main conclusions from the previous chapters are summarized. Limitations to using the DEB approach and suggestions for further research are discussed.

Field studies

Field data on growth and reproductive output of *Macoma balthica*, *Mya arenaria*, *Cerastoderma edule*, *Mytilus edulis* and *Crassostrea gigas* were collected with the final aim to test the predictions of DEB model simulations with field data. However, such descriptive studies also gave insight into the physiology of these species in the western Wadden Sea.

In chapter 2, the reproductive investment of the blue mussel *M. edulis* was studied with the aim to test whether reproductive failure could be the cause for the low recruitment of *M. edulis* observed in the western Dutch Wadden Sea. Results showed that, despite the increase in mean winter temperature during the last decades, no significant relationship was found between body condition in late winter and mean winter temperature. Hence, reproductive failure due to a negative effect of increasing temperature on body condition and reproductive output will not have been a cause for the low recruitment in recent years. Nevertheless, reproductive investment by the mussel population studied was not optimal since complete spawning hardly occurred. Resorption of gonadal tissue due to unfavourable environmental conditions (too high temperature and/or food limitation during summer) was also suggested by the high gonadosomatic ratio at the end of the summer. Reduced recruitment could be due to poor spawning (as a result of resorption) but other factors that exert their influence after spawning (predation on larvae or postlarvae, lack of settlement substrate) or on the adult population (decrease in number of spawners) are also likely to be involved in the observed low recruitment during the last years.

In chapter 3, the timing of spawning and reproductive investment of the soft-shell clam *M. arenaria* and the cockle *C. edule* were followed. The aim was to analyse whether differences in timing of spawning and reproductive investment between species could be responsible for the observed differences in recruitment success in the field, which is in general higher in *C. edule* than in *M. arenaria*. Timing of spawning was similar in both species but individual reproductive investment was higher in *M. arenaria* than in *C. edule*. Moreover, due to the differences in body mass composition between species, absolute reproductive output of the *M. arenaria* population seems to be much higher than that of the *C. edule* population. Hence, differences in recruitment success between species are probably not due to differences in reproductive output. Differences in post-larval processes (such as differential predation pressure in early life stages) are more likely to be responsible for the observed recruitment patterns.

In chapter 4, growth and reproductive output of the Baltic clam *M. balthica* were determined in different habitats (intertidal and subtidal areas in the Wadden Sea, and a coastal North Sea area) to analyse which (sub)population contributes more to the persistence of the *M. balthica* population in the western Dutch Wadden Sea. Significant differences in growth and reproductive investment were found between locations. Young individuals were mostly found in the intertidal area, where growth in terms of somatic mass was good. In the subtidal, where the highest densities were found, somatic mass and gonadal mass indexes were low. Coastal areas had the lowest densities and showed high growth in terms of shell length and body mass. The subtidal habitat contributed the most to the reproductive output of the western Dutch Wadden Sea population (due to the high densities found) although the highest reproductive output per individual was in the coastal area.

The aim of chapter 5 was to test whether the latitudinal decrease of temperature towards the poles could potentially determine the ultimate northern limit of the geographical distribution of the Pacific oyster *C. gigas*. To analyse the physiological performance of this species, spatial and temporal variation in growth and reproductive output of *C. gigas* were studied in three populations, from France to the Netherlands. An increase in maximum shell length, somatic and gonadal mass was observed from France to the Netherlands. In addition, mean oocyte diameter decreased significantly from south to north. The combination of increasing gonadal mass and decreasing oocyte volume suggests an increasing reproductive output in terms of egg numbers from France to the Netherlands. Smaller eggs (oocytes) are thought to have a longer development time than larger eggs because small eggs need more time to reach a similar larval size (Kooijman 2000). This suggests that the environmental conditions along the Dutch coast may result in increased larval dispersal and possibly further population expansion. The fact that population expansion in the Wadden Sea appeared to be rather irregular might indicate that successful larval dispersal occurs only in years with a high reproduction, since with increasing development time also losses due to predation and other mortality factors increase.

The DEB model

Estimation of parameters

To be able to apply the DEB model to simulate growth in bivalves and to reconstruct food conditions in the field, several steps were taken. First, the assumptions of the DEB model regarding body-size scaling relationships for the comparison of species were tested. To this end, DEB model predictions for bivalves were validated with field and laboratory observations from literature (Chapter 6). Significant relationships were found between body size and, respectively, size at first reproduction and the Von Bertalanffy growth rate (coefficient); and between egg and larval size (volume) and egg and larval development time. The main discrepancy between field observations and DEB predictions was in the relationship between egg and larval volume and adult body volume. It was suggested that for successful settlement, the size or volume of the larvae must be in balance with the sediment composition, that is, the size or volume of the larvae must be similar to or larger than sediment grain size.

Next, DEB model parameter values were determined for each species, based on published experimental data (Chapter 7). Problems with parameter estimation were mainly due to the lack of reliable datasets. For none of the bivalve species, a complete set of DEB parameters could be estimated based on existing field data. Similarities among species were found in their volume-specific maintenance costs, the costs for growth and the maximum storage energy density. Differences between species were expressed in differences in some DEB parameters, especially the maximum surface area-specific assimilation rate and in the fraction of energy allocated to reproduction. These differences are reflected on the theoretical maximum total shell length of the species.

Model application

The estimated parameters from chapter 7 were used to run the model, with temperature and food conditions as input variables (Chapter 8). Field data (from Chapters 2-5) were combined with DEB model simulations and a reconstruction of the seasonal pattern in food conditions for the different species in the different habitats was made. Two types of simulations were done: [1] annual growth in shell length of the various species was predicted under prevailing temperature conditions and a range of food levels, expressed in the form of a functional response curve with values between 0 (starvation) and 1 (food *ad libitum*); and [2] seasonal variation in food level was reconstructed for each species in each habitat by simulating mass variation along the year under prevailing temperature conditions, and comparing the simulated mass with the observed mass in the field. This allowed a study of the interactions between bivalve species, and between different habitats of a species, in terms of food competition.

The reconstruction of the annual food conditions suggested that with respect to food consumption, *M. balthica* and *C. edule* are the most successful species, with an annual simulated food level between 0.7 and 1.0. The seasonal variation in food conditions showed high values of food level in spring and summer, corresponding to the main growing season. After the summer, the amount of food (phytoplankton and phytobenthos) in the water and sediment surface declines. As a result, the simulated food level for the various species decreased during summer and/or autumn, suggesting that strong food competition between species must have occurred during these periods. Overall, DEB model simulations, both on an annual and seasonal basis showed that, at the prevailing water temperatures, growth of all species studied was not maximal. This leads to the conclusion that for the various species in the different habitats, food conditions were different and insufficient for maximum growth.

Food limitation in suspension-feeding bivalves has been suggested by several authors (Beukema et al. 2002, Hummel 1985, Kamermans et al. 1992, Kamermans 1993, Cognie et al. 2001, Asmus et al. 1992, Jonsson et al. 2005). The results presented in this thesis suggest that food limitation also occurs in bivalve species in the Wadden Sea, pointing out to the importance of food as a limiting factor for benthic biomass and productivity. However, it is still questionable whether the apparent suboptimal food conditions (i.e. food conditions insufficient for maximum growth) for most species and habitats during most of the year are due to lack of food or/and to environmental conditions which limit feeding efficiency (such as silt content in the water).

Limitations and suggestions

Overall, the DEB model was successfully applied for the simulation of growth and reconstruction of food conditions in bivalves. To achieve this, several aspects are important. These are: field data on growth of bivalves, water temperature data and estimation of DEB parameters. With respect to field data, a correct age estimation of the different species is essential. For some species, such as *M. balthica* and *C. edule*, the analysis of external growth rings on the shell surface seems to give an accurate estimation of age. However, in other species, it is still unsure how to estimate age correctly. Therefore, validation of age determination in Wadden Sea bivalve species is essential in the near future. This could be done, for example, by marking experiments or by analysing carbon and oxygen isotopes. Since in many species age cannot be determined in an easy and straightforward way, a first impression of the pattern in seasonal food conditions can be given by reconstructing the seasonal functional response from variation in somatic mass index.

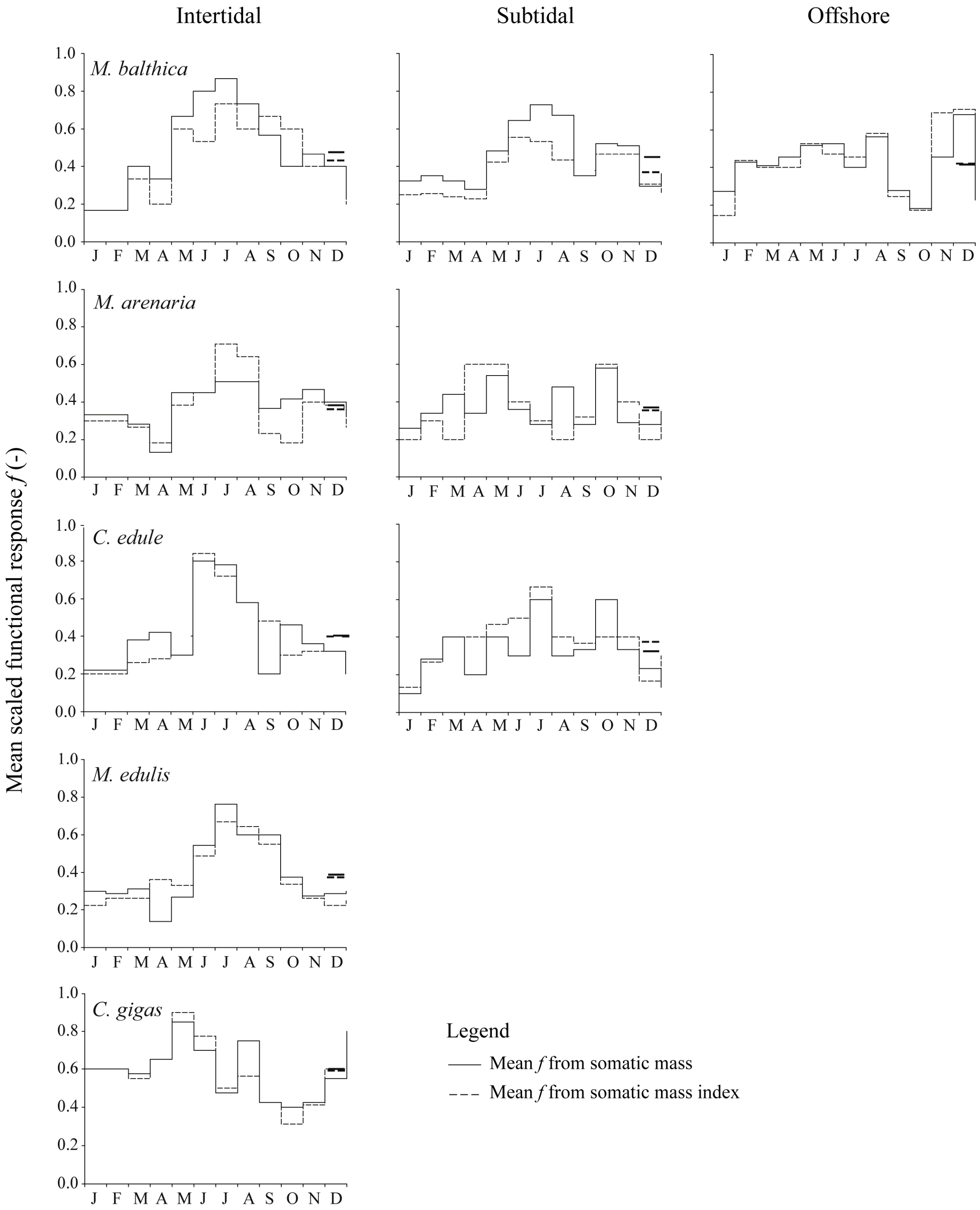

Fig. 9.1. Mean seasonal functional response (*f*, -) based on the reconstruction of food conditions for each age class (—; data from Chapter 8) and based on the reconstruction of food conditions using variation in somatic mass index (– –). Thicker marks indicate yearly mean. Simulations were done as described in Chapter 8.

However, it should be kept in mind that growth in terms of somatic mass index does not take into account the contribution of length growth over time and, therefore, during the growing season it will result in an underestimation of the reconstructed food patterns. Such underestimation will especially occur in fast growing, short living species and in younger age classes, since in older individuals there is hardly any change in somatic mass with age (Chapter 8). For the studied species, the seasonal reconstruction of food conditions by the two methods (somatic mass and somatic mass index) did not show large differences (Fig. 9.1).

Both the seasonal patterns as well as the yearly means are similar. However, the reconstructed functional response from somatic mass index was not always lower than the one from the instantaneous growth rate in somatic mass, as theoretically expected. This is a reflection of the large variability in individual growth. Also the lack of long-term data series on growth of bivalves may lead to some discrepancies between field data and model simulations since the variability in growth due to differences in environmental conditions from year to year was not considered.

With respect to temperature data, it is important to have accurate temperature measurements in the studied areas. Information on water temperature is necessary for model simulations. Continuous temperature measurements in the water column and the upper sediment layer by using temperature loggers would have been better. Also the availability of long-term data sets for the study areas is of interest since individuals are exposed to year-to-year variability in environmental conditions, which was not considered in this study. Furthermore, temperature was considered here the only environmental factor that controlled growth but differences in other factors such as salinity and water current speed, which have not been considered, could also influence growth.

The estimation of the DEB parameters for the different species was based on published literature data, mostly from laboratory experiments. However, the lack of good datasets led to difficulties in estimating some parameters and a protocol had to be adopted to fill in missing gaps and achieve parameter consistency (Chapter 7). For a reliable estimation of parameters, it is important to have well-designed experiments which allow a simultaneous determination of growth, reproduction, respiration, assimilation and body condition under varying feeding conditions and food intake. Furthermore, parameter values were considered species-specific, although some variability may be expected (Kooijman 2000). The fact that, in blue mussel *M. edulis*, resorption of gonads seems to occur due to unfavourable environmental conditions (too high temperature and food limitation during summer; Chapter 2), suggests that the parameter *k* may vary along the year. Also the shape coefficient of *M. balthica* was seen to differ between Wadden Sea areas (Luttikhuizen et al. 2003), and sediment load in the water seems to influence a bivalve's gill-palp ratio (Honkoop et al. 2003) and filtration efficiency (Kooijman 2006). Intra-specific variability in DEB parameters could be responsible for some patterns attributed to food conditions. A possibility for testing the intra-specific variability in DEB parameter is by applying the model to another area, using the same species-specific parameter values and changing only temperature conditions. A trial was done by applying the model to simulate growth in a northern European location (Valosen estuary, Bodø, Norway). Growth in the field was simulated for 4 different species and annual food conditions were reconstructed (Fig. 9.2), according to the methods described in chapter 8. For all species except *C. edule*, growth could be simulated without problems since the observed shell growth fell within the simulated curves. In *C. edule*, shell growth was faster than the maximum growth predicted by the DEB model, under maximal food conditions (*f*=1). The lack of accurate growth and temperature data could be responsible for this discrepancy (see above). However, variability in DEB parameter values can also not be ruled out. Applying the DEB model to simulate growth and to reconstruct food conditions in bivalves from other latitudes and habitats could be a first approach to evaluate with more detail the specificity of the parameters.

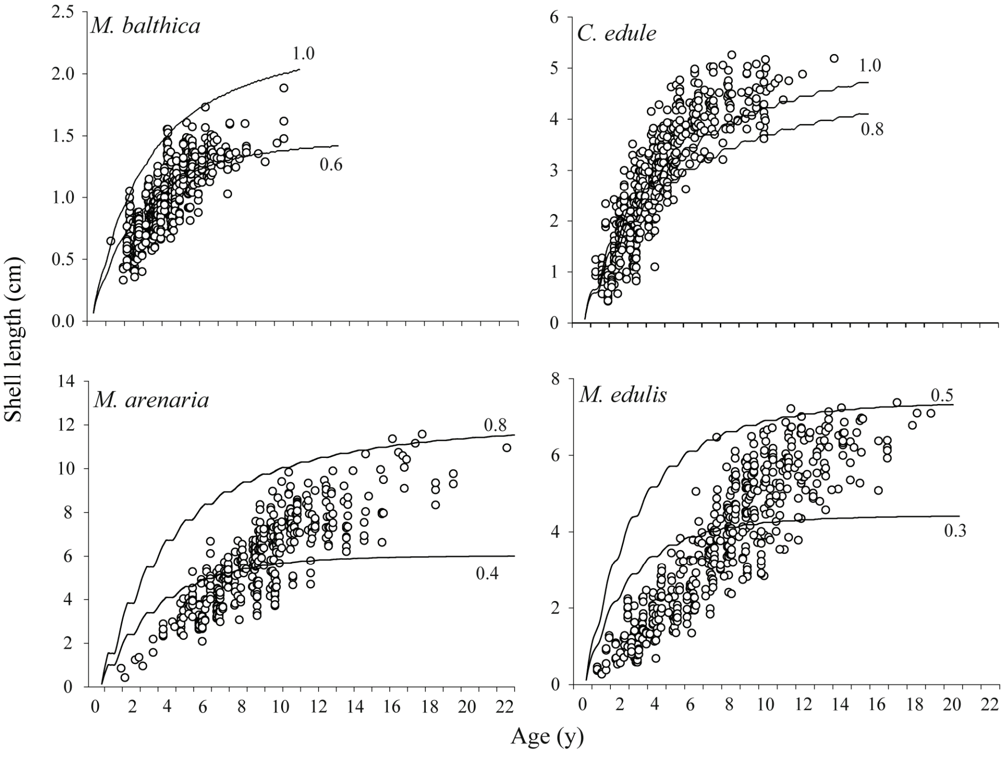

Fig. 9.2. Observed shell length (cm) with age (y) of *M. balthica*, *M. arenaria*, *C. edule* and *M. edulis* in Norway (Campos and Freitas, unpubl. data). Simulated growth curves were added for fast and slow growing individuals. Values indicate the average functional response used to simulate the curves under current water temperature conditions. Simulations were done as described in Chapter 8.

Concerning the recent attention and effort put into studying global climate change and its consequences in marine ecosystems, the DEB approach could to be an important tool to analyse the impact of climate change on bivalve productivity.

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Samenvatting

Tweekleppige schelpen zijn een belangrijke component in de bodemfauna in vele kustgebieden omdat zij in hoge dichtheden voorkomen en van belang zijn als voedselbron voor andere soorten, zoals kustvogels, vissen, schaaldieren en de mens. Ze zijn over de hele aarde te vinden in een scala aan leefgebieden, van de polen tot de tropen. Over zo'n groot verspreidingsgebied zijn er grote verschillen in omgevingsfactoren zoals watertemperatuur, zoutgehalte, voedselaanbod en stroming.. Deze verschillen beïnvloeden groei, overleving en voortplanting en uiteindelijk bepalen en beperken ze de distributie van de verschillende soorten.

In de ondiepe Waddenzee, het grootste estuariene gebied in Noord Europa, zijn enkele soorten tweekleppigen dominant aanwezig. 65% van het totale gewicht van alle grote bodemdieren bestaat uit schelpdieren, zowel op de wadplaten (gebieden drooggevallen tijdens laagwater) als in het sublitorale gebied (gebieden die altijd onder water staan). De belangrijkste soorten in deze habitats zijn het nonnetje *Macoma balthica,* de kokkel *Cerastoderma edule*, de mossel *Mytilus edulis*, de strandgaper *Mya arenaria* en de recent geïntroduceerde Amerikaanse zwaardschede *Ensis americanus* en de Japanse oester *Crassostrea gigas* (zie Fig. 1.1, Hoofdstuk 1).

Ondanks de overeenkomsten in de levenscyclus van de soorten zijn er ook vele verschillen. De jonge stadia van een aantal van deze soorten, onder andere *M. balthica* en *C. gigas*, vindt men bijna uitsluitend in hoge dichtheden op de wadplaten, dit in tegenstelling tot *C. edule*, *M. arenaria*, *E. americanus* en *M. edulis* waar de jonge dieren zich over een veel groter gebied op de wadplaten en in het sublitoraal vestigen. Volwassen exemplaren worden meestal verspreid over een groter gebied (wadplaten en sublitoraal gebied) gevonden. De verschillen in levenscyclus in de Waddenzee geven aan dat ruimtelijke verschillen in voedselverdeling en groeiomstandigheden in de Waddenzee tussen leefgebieden (getijgebieden, sublitoraal) bepalend kunnen zijn voor de groei, leeftijd van geslachtsrijpheid, en uiteindelijke grootte en leeftijd.

Doel van de studie

Dit proefschrift onderzoekt de groei en voortplanting van tweekleppigen in verschillende leefomstandigheden in de westelijke Waddenzee en het Noordzeekustgebied en hun relatie met de omgevingsfactoren vanuit een energetisch oogpunt. Deze aanpak zorgde ervoor dat interacties binnen- en tussen de soorten (o.a. competitie om voedsel) onderzocht konden worden. Hiertoe werd een wiskundig model toegepast, het 'Dynamic Energy Budget (DEB)' model dat de energiestromen in een dier beschrijft evenals de veranderingen van deze stromen in omgevingen waarin voedseldichtheden en temperaturen variëren (zie Fig. 1.4, Hoofdstuk 1).

Door het DEB model toe te passen is het mogelijk om de energiestromen in een enkel dier kwantitatief te beschrijven en om energetische investering in groei en voortplanting in relatie tot milieuomstandigheden (o.a. temperatuur) en voedselopname te analyseren. Alvorens het DEB model toegepast kan worden is basisinformatie over de diverse soorten vereist, zowel wat betreft groei en voortplanting in het veld, als wat betreft de DEB parameterwaarden om het model mee te voeden. In lijn hiermee bestaat het proefschrift uit de volgende drie hoofdgedeeltes:
[1] Veldstudies:
[2] Schatting van de parameters van het DEB model voor de verschillende soorten tweekleppigen;
[3] Toepassing van het DEB model voor de reconstructie van de voedselcondities in het veld om competitie tussen en binnen de soorten te analyseren.

Veldstudies

De bestudeerde soorten

Het onderzoek is uitgevoerd aan de vijf meest voorkomende soorten tweekleppigen: *M. balthica*, *C. edule*, *M. edulis*, *M. arenaria* en *C. gigas* in de Waddenzee. Deze soorten komen in verschillende gebieden voor. *M. balthica* heeft een ruime verspreiding en wordt aangetroffen zowel in de hoger gelegen gebieden van de wadplaten als in de subgetijdegebieden van de Waddenzee en in de buitendelta voor de kust.. Daarentegen komen *C. edule* and *M. edulis* alleen voor op de wadplaten tot in de ondiepe subgetijdegebieden van de Waddenzee. *M. arenaria* wordt meestal in dezelfde gebieden gevonden maar soms ook in dieper en verder van de kust gelegen gebieden. *C. gigas,* een van oorsprong Japanse soort, is tegenwoordig in groten getale aanwezig met een steeds verder uitbreidende populatie vooral op harde substraten in gebieden op de wadplaat en in het subgetijgebied. Tussen de soorten bestaan verschillen: de schelp van *M. balthica* heeft een maximale lengte van 2.5 cm, *M. arenaria* van 10-15 cm*, C. edule* van 5 cm, *M. edulis* van 15 cm en *C. gigas* tot een maximum van 20 cm. Alle soorten zijn in gedeeltelijk en soms in een volledig ingegraven toestand te vinden in modderige en zandige sedimenten. Daarentegen hechten mosselen en oesters zich vaak aan harde voorwerpen zoals rotsen, dode schelpen of aan stenen.

Deze vijf soorten werden tijdens het onderzoek verzameld op wadplaten en in subgetijdegebieden in de Waddenzee en langs de Noordzeekust (zie Fig. 1.2, Hoofdstuk 1). In de verschillende gebieden is de bemonsteringswijze aangepast aan het gebied en de verspreiding (talrijkheid, diepteverdeling) van de soort. Op de wadplaten zijn de schelpen met de handen en een riek verzameld. Voor het verzamelen van *M. baltica* schelpen is daarnaast gebruik gemaakt van een met de hand te bedienen monsterbuis. In subgetijdegebieden zijn *M. baltica* en *M. arenaria* vanaf een boot bemonsterd met behulp van een ‘Reineck’ box corer en is *C. edule* met een sleepnet opgevist. In kustgebieden is wederom gebruik gemaakt van een boot met in dit geval een ‘Van Veen’ grijper (zie Fig. 1.3, Hoofdstuk 1).

Groei en voortplanting

Voor alle soorten werden de groei en voortplanting in de verschillende gebieden een jaar lang gevolgd. Groei en voortplanting waren duidelijk seizoensgebonden. Voor alle soorten werd er een toename van de lichaamsconditie gevonden in de loop van het voorjaar, maar zowel tussen de soorten als tussen de leefgebieden waren er verschillen.. De toename van lichaamsgewicht begon het vroegst in *C. gigas*, al rond januari, voor *M. edulis* omstreeks februari en voor de ander soorten rond april. In *M. baltica* was er een duidelijk verschill tussen de gebieden: de groei in lichaamsgewicht was lager in het subgetijdegebied dan op de wadplaten of voor de kust (Hoofdstuk 4).

Naast groei, verschilt ook de voortplantingscyclus tussen de soorten. In *M. baltica* neemt het gewicht van de voorplantingsorganen vanaf de herfst toe met een maximum rond februari, waarna het paaien volgt (Hoofdstuk 4). Individuen in de kustzone stoppen duidelijk de meeste energie in voortplanting: de gewichten van hun voortplantingsorganen zijn het hoogste. Daarentegen is de totale voortplantingsinspanning per vierkante meter het hoogst in het subgetijgebied omdat hier de hoogste dichtheid van individuen wordt gevonden.

In *M. edulis* begint de toename van de voortplantingsorganen rond februari (Hoofdstuk 2). De belangrijkste voortplantingsperiode is rond april gezien het feit dat de hoogste aantallen larven in mei in het water worden gevonden. De laatste jaren is de aanwas van jonge mosselen verminderd. Onderzocht is of dit veroorzaakt kan zijn door de toename van de gemiddelde wintertemperatuur in de laatste decennia wat geleid heeft tot een hoger energiebehoefte en verbruik, waardoor lichaamsconditie en beschikbaarheid van energie voor voortplanting afgenomen zijn. Er werd echter geen relatie gevonden tussen lichaamsconditie en wintertemperatuur. Dit duidt op andere factoren die van belang zijn zoals overleving en vestigingssucces van de larven.

Opmerkelijke verschillen in voortplantingssucces werden er gevonden tussen *C. edule* en *M. arenaria* (Hoofdstuk 3) die beide voorkomen op zowel de wadplaat als in het subgetijgebied. Beide soorten hebben een identiek voortplantingsseizoen, maar *M. arenaria* investeert meer in voortplanting dan *C. edule.* Aangezien het gemiddeld lichaamsgewicht van *M. arenaria* veel hoger is dan dat van *C. edule*, zal de *M. arenaria* populatie in het westelijk deel van de Waddenzee een hogere voortplantingsinspanning vertonen dan die van *C. edule*. Desalniettemin is het voortplantingssucces van *C. edule* hoger dan dat van *M. arenaria*. Evenals bij *M. edulis* duidt dit op andere factoren die van belang zijn zoals overleving en vestigingssucces van de larven.

Voortplantingsproductiviteit kan op andere manieren worden gemeten dan door het meten van de hoeveelheid energie in de vorm van het gewicht van de voorplantingsorganen. Een alternatief is het meten van de aantallen en de grootte van de eicellen. Wat betreft eigrootte zijn er duidelijke verschillen tussen de soorten. *C. edule* heeft een gemiddelde eicelgrootte van 65 μm, daarentegen hebben de eicellen van *M. arenaria* een diameter van 57 μm en die van *C. gigas* van 50 μm. Binnen een soort kan de eigrootte ook verschillen tussen individuen in verschillende leefgebieden. In *C. gigas* verschilde de eiceldiameter tussen populaties in Frankrijk en Nederland (Hooftsuk 5), waarbij de gemiddelde eicel diameter -in tegenstelling tot de maximum schelplengte, het somatische gewicht en het gewicht van de voortplantingsorganen- af nam van zuid naar noord. De combinatie van toenemend gewicht van de voortplantingorganen en afnemende eiceldiameter impliceert een toename van het aantal geproduceerde eicellen van Frankrijk naar Nederland. Binnen een soort gezien hebben kleine eicellen meer tijd nodig om zich te kunnen ontwikkelen. Een langere ontwikkelingsduur betekent tegelijkertijd een langer verblijf in de watermassa en hierdoor meer tijd om zich te verspreiden. De recente verspreiding van de oester lijkt gerelateerd te zijn aan de toename van de watertemperatuur in de afgelopen jaren.

Het Dynamic Energy Budget (DEB) model

Het Dynamic Energy Budget is een model dat de energieverdeling in een organisme beschrijft in relatie tot voedselomstandigheden en temperatuur. Het DEB model is door S.A.L.M. Kooijman van de Vrije Universiteit ontwikkeld. De elegantie van het model is dat toepassen van het model slechts de schatting van een gering aantal parameters vereist en dat hetzelfde model kan worden toegepast voor verschillende soorten met alleen maar aanpassingen in de parameterwaarden. Wanneer twee van de drie vrijheidsgraden bekend zijn (temperatuur, voedselomstandigheden, waargenomen groei), kan de derde met behulp van het DEB model gereconstrueerd worden.

In deze studie is het DEB model gebruikt om, gegeven de waargenomen groei in het veld onder de gemeten temperatuurcondities, de voedselomstandigheden te reconstrueren voor de verschillende soorten in de diverse leefgebieden. Het doel was om verschillen tussen soorten (inter-species) en binnen een soort tussen leefgebieden (intra-species) te analyseren.

Aannames van het model en parameterschatting

Als eerste is, op grond van een literatuurstudie, nagegaan in hoeverre de onderliggende aannames van het DEB model aangaande relaties tussen, bijvoorbeeld, eigrootte, groeisnelheid, grootte bij innesteling en bij geslachtsrijpheid, en volwassen lichaamslengte geldig zijn voor schelpdieren (Hoofdstuk 6). Relaties zijn gevonden tussen lichaamsgrootte en, achtereenvolgens, de grootte bij de eerste voortplanting en de groeisnelheid; en tussen ei- en larvegrootte (volume) en ei- en larveontwikkelingstijd. Het voornaamste verschil tussen de veldwaarnemingen en de DEB voorspellingen was de relatie tussen ei- en larvevolume en het volwassen lichaamsvolume: kleine soorten bleken een groter volume te hebben dan voorspeld. Gesuggereerd is dat dit een weerspiegeling is van het feit dat een succesvolle settlement van de larven in en op het sediment vereist dat hun grootte zich verhoudt tot die van het sediment, waarbij de ondergrens gezet wordt door de korrelgrootte van slikkige sedimenten.

In Hoofdstuk 7 zijn de waarden van de DEB modelparameters geschat voor de verschillende soorten op basis van gepubliceerde experimentele gegevens. Voor geen enkele soort kon op basis van de beschikbare veldgegevens een complete set DEB parameters worden bepaald. De problemen rondom parameterschatting werden meestal veroorzaakt door een gebrek aan betrouwbare datasets. Een protocol is ontwikkeld hoe om te gaan met incomplete datasets om toch een consistente parameterset te verkrijgen. De taxonomische verwantschap tussen de soorten uitte zich in gelijke onderhoudskosten, kosten voor groei en kosten voor energieopslagdichtheid. Verschillen tussen soorten kwamen tot uitdrukking in met name de voedselopnamesnelheid en in de fractie van de energie bestemd voor voortplanting. Deze verschillen worden weerspiegeld in de verschillen in maximale totale schelplengte tussen de soorten.

Model simulaties

In Hoofdstuk 8 werden. de veldgegevens omtrent groei en watertemperatuur (van Hoofdstukken 2-5) gecombineerd met DEB modelsimulaties om een reconstructie van de voedselomstandigheden, geschaald tussen 0 (uithongering) en 1 (overmaat voedsel), voor de verschillende soorten en leefomgevingen te maken. Er werden 2 soorten simulaties gedaan:[1] reconstructie van de gemiddelde jaarlijkse voedselconditie op basis van lengte – leeftijd relaties en [2] seizoensvariatie in voedselniveau op grond van het seizoensverloop van groei en reproductie in het veld.

De voedselomstandigheden waren het best (vertoonden de hoogste waarden) in het voorjaar en de zomer, wat overeenkwam met het voornaamste groeiseizoen. Na de zomer vermindert de hoeveelheid voedsel voor tweekleppigen in het water en op het oppervlak van de bodem. Hierdoor neemt het gesimuleerde voedselniveau voor de verschillende soorten gedurende de zomer en/of de herfst af, hetgeen tevens aangeeft dat er tussen de soorten een sterke voedselcompetitie optreedt gedurende deze perioden. Bij voorbeeld, *M. balthica* en *C. edule* hebben een gesimuleerd jaarlijks voedselniveau tussen 0.7 en 1.0, dit in tegenstelling tot *M. edulis* met een gesimuleerd jaarlijks voedselniveau tussen 0.3 en 0.5. Het geeft aan dat de voedselomstandigheden in het veld beter zijn voor *M. balthica* en *C. edule* dan voor *M. edulis*

In het algemeen waren de voedselomstandigheden ontoereikend voor maximale groei wat een voedsellimitatie in de Waddenzee aangeeft. Hoewel, het is nog steeds onbekend of deze voedsellimitaties het gevolg zijn van voedseltekorten en/of van milieuomstandigheden die efficiënte voedselopname verhinderen (zoals het sedimentgehalte van het water).

Slotopmerkingen en suggesties voor verder onderzoek

De resultaten die in dit proefschrift gepresenteerd worden, laten zien dat het DEB model kan worden toegepast voor de simulatie van groei en de reconstructie van voedselcondities in tweekleppigen. Om optimale resultaten te verkrijgen is de kwaliteit van de gegevens van groot belang. Leeftijdsbepaling in de verschillende soorten is gebaseerd op uitwendige kenmerken zoals het aantal groeiringen. Hierin kunnen fouten opgetreden zijn die de discrepantie tussen veld en gesimuleerde gegevens kunnen verklaren. Daarom is het van groot belang om in de toekomst veel aandacht te schenken aan een nauwkeurige leeftijdsbepaling. Nauwkeurige leeftijdsbepaling kan onder andere met behulp van schelpkleuringen en/of zuurstof- en koolstofisotoopanalyse. Het gebrek van lange termijnseries van de groei van tweekleppigen kan ook hebben geleid tot verschillen tussen veldgegevens en modelsimulaties. Dit omdat de variatie in groei veroorzaakt door verschillen in omgevingsomstandigheden van jaar tot jaar niet in de simulaties zijn meegenomen.

Wat betreft temperatuursgegevens is het eveneens van belang om nauwkeurige en frequente metingen van de bestudeerde gebieden te hebben. Speciale temperatuurloggers lenen zich hier goed voor. In dynamische gebieden zoals de Waddenzee kan op deze manier meerdere keren per uur een nauwkeurige meting worden verkregen. Vooral de beschikbaarheid van lange meetseries (jaren) van uit de bestudeerde gebieden is van belang omdat individuen zijn blootgesteld aan jaar-tot-jaarvariatie in omgevingsomstandigheden, welke niet zijn meegenomen in deze studie.

De schatting van de DEB parameters voor de verschillende soorten was gebaseerd op gepubliceerde literatuurgegevens, meestal van laboratoriumexperimenten. Door het gebrek aan gegevensbestanden moest een protocol worden aangenomen om ontbrekende gaten te vullen en om een samenhang in de parameters te krijgen (Hoofdstuk 7). Optimaal zouden schattingen zijn gebaseerd op hiertoe opgezette laboratoriumexperimenten. die een gelijktijdige bepaling mogelijk maken van groei, voortplanting, zuurstofopname, energieopname en lichaamsconditie onder variërende voedselomstandigheden. Verder is het niet uit te sluiten dat er variatie bestaat in parameterwaarden binnen soorten. Dit aspect is niet meegenomen in deze studie. Het toepassen van het DEB model om groei te simuleren en om voedselomstandigheden te reconstrueren langs een breedtegraadgradiënt kan een eerste aanloop zijn om de specificiteit van de parameterwaarden binnen soorten in nader detail te bestuderen.

Dankwoord: Ik will graag Hans Witte, Ben Abbas, Henk van der Veer en Wim Wolff bedanken voor hun nuttige suggesties wat betreft Nederlands-Engels vertaling.

Resumo

Os bivalves são um componente principal de muitos estuários, quer devido à sua abundância quer ao seu valor como alimento para outras espécies tais como aves limícolas, peixes, crustáceos e o Homem. Por todo o globo terrestre, os bivalves ocupam uma variedade de habitats, estando expostos a diferentes condições ambientais de temperatura, salinidade, quantidade de alimento e correntes. Estas diferenças ambientais influenciam o seu crescimento, sobrevivência e reprodução, e limitam e determinam a distribuição das diferentes espécies.

No Mar de Wadden, a maior área estuarina do norte da Europa, algumas espécies de bivalves constituem mais de 65% da biomassa total do macrobentos, quer nas zonas intertidais (fora de água durante a maré baixa) quer nas zonas subtidais (sempre dentro de água). Estas espécies incluem a espécie de bivalve *Macoma balthica*, o berbigão *Cerastoderma edule*, o mexilhão *Mytilus edulis*, o clame-da-areia *Mya arenaria*, o lingueirão *Ensis americanus* e a recentemente introduzida ostra do Pacifico *Crassostrea gigas* (ver Fig. 1.1, Capítulo 1).

Embora estas espécies apresentem ciclos de vida semelhantes, dentro do estuário elas ocupam zonas diferentes. Em *M. balthica* e *C. gigas*, os juvenis são encontrados quase sempre nas zonas superiores do intertidal, enquanto os juvenis de *C. edule*, *M. arenaria*, *E. americanus* e *M. edulis* aparecem ao longo das zonas intertidais e subtidais. Os adultos das várias espécies tem uma distribuição mais alargada (intertidal, subtidal e zona costeira) que os juvenis. Estas diferenças na distribuição e uso dos habitats entre as várias espécies, implicam diferenças nas características do seu ciclo de vida. Isto é, diferenças no alimento e condições de crescimento entre as diferentes zonas tidais, provavelmente resultam em diferente idade máxima, tamanho máximo e idade de maturação atingidos.

Objectivo

O objectivo desta tese foi tentar perceber como é que diferentes espécies de bivalves conseguem sobreviver em diferentes condições ambientais, que são muitas das vezes desfavoráveis em termos de temperatura, alimento, competição (pelo alimento e espaço) e pressão de predação. Para começar, variações nas características de vida de diferentes espécies foram analisadas, em relação às condições ambientais. Para isso, diferenças em alimentação, crescimento, reprodução e altura da desova entre as espécies foram estudadas. De seguida, as diferenças dentro de cada espécie em diferentes habitats foram analisadas. Esta tese incide principalmente no crescimento e reprodução de bivalves em diferentes habitats da parte oeste do Mar de Wadden e da zona costeira do Mar do Norte. Esta abordagem possibilitou uma análise das interacções (tais como competição pelo alimento) entre diferentes espécies de bivalves (interacções inter-especificas) e dentro da mesma espécie em diferentes habitats (interacções intra-especificas). Para comparar as diferentes espécies em termos de competição pelo alimento, um modelo energético geral, o "Dynamic Energy Budget (DEB) model", foi usado. Este modelo descreve o fluxo energético através de um indivíduo e as variações deste fluxo em condições ambientais de quantidade de alimento e temperatura variáveis (ver Fig. 1.4, Capítulo 1). Ao usar o modelo DEB, é possível descrever quantitativamente o fluxo energético através de um indivíduo e analisar a repartição de energia entre o crescimento e a reprodução, em diferentes condições ambientais (temperatura) e de ingestão de alimento.

Esta tese está dividida em três partes:
[1] trabalho de campo,
[2] determinação dos parâmetros do modelo DEB para as várias espécies,
[3] aplicação do modelo DEB para reconstruir as condições de alimento no campo e analisar a competição intra e inter-especifica.

Trabalho de campo

Espécies estudadas

Este estudo incluiu cinco das espécies de bivalves mais comuns em águas Holandesas: *M. balthica*, *C. edule*, *M. edulis*, *M. arenaria* e *C. gigas*. No Mar de Wadden e zonas costeiras, estas espécies ocupam habitats diferentes. *M. balthica* é encontrada desde as zonas altas do intertidal ás zonas subtidais do estuário e também nas zonas costeiras do Mar do Norte. *C. edule* e *M. edulis* vivem nas zonas intertidais e subtidais do estuário. *M. arenaria* ocorre, no Mar de Wadden, nas zonas altas do intertidal e zonas subtidais pouco profundas mas no Mar do Norte pode ser encontrada a maiores profundidades. *C. gigas*, originária do Japão, é agora bastante abundante nas zonas intertidais do estuário embora também possa ser encontrada no subtidal. Estas espécies apresentam grandes diferenças em termos de tamanho máximo da concha: *M. balthica* pode atingir um tamanho máximo de 2.5 cm, *M. arenaria* pode atingir 10-15 cm, *C. edule* pode atingir 5 cm, *M. edulis* pode crescer até 15 cm e *C. gigas* pode atingir 20 cm. Todas as espécies vivem parcial ou completamente enterradas no sedimento, que varia entre lodoso e arenoso. No entanto, o mexilhão e a ostra necessitam de substrato mais duro como rochas ou conchas de bivalves mortos.

Estas cinco espécies foram amostradas em zonas intertidais e subtidais do Mar de Wadden e na zona costeira do Mar do Norte (ver Fig. 1.2, Capítulo 1). Durante as amostragens, diferentes tipos de equipamento foram usados. Nas zonas intertidais, *M. edulis*, *C. edule*, *M. arenaria* e *C. gigas* foram amostrados à mão, com a ajuda de uma pá, enquanto *M. balthica* foi amostrada com um cilindro de amostragem de mão. Nas zonas subtidais, a amostragem foi feita de barco. *M. balthica* e *M. arenaria* foram amostradas com uma caixa de amostragem "Reineck" e *C. edule* foi amostrado com uma rede de arrasto. Na zona costeira, *M. balthica* foi amostrada de barco com uma draga "Van Veen" (ver Fig. 1.3, Capítulo 1).

Crescimento e reprodução

Informação sobre o crescimento e reprodução das diferentes espécies em relação às condições ambientais foi recolhida no campo (Capítulos 2-5). Todas as espécies apresentaram um ciclo de crescimento anual, com um aumento da massa corporal durante a Primavera e Verão e uma diminuição no Inverno. No entanto, diferenças no periodo de crescimento foram observadas entre espécies. O crescimento em termos de massa corporal começou em Janeiro em *C. gigas*, em meados de Fevereiro em *M. edulis* e em Abril nas outras espécies. Diferenças de crescimento foram também encontradas dentro da mesma espécie em diferentes habitats. Por exemplo, em *M. balthica*, o crescimento da massa somática (massa do corpo excluindo a gónada) foi menor na zona subtidal do que nas zonas interidal e costeira (Capítulo 4).

O ciclo reprodutivo também foi diferente entre espécies. Em *M. balthica*, a gónada começou a crescer no final do Verão, e atingiu valores máximos em Fevereiro, mesmo antes da desova (Capítulo 4). Nos vários habitats, *M. balthica* também apresentou diferenças. O tamanho da gónada foi maior na zona costeira, onde as densidades eram menores. No entanto, analisando a quantidade de gonada por m^2 verificou-se que esta foi maior na zona subtidal devido à grande densidade de animais nesta zona. Assim sendo, é a zona subtidal que contribui mais em termos reprodutivos para a população de *M. balthica* no Mar de Wadden.

Em *M. edulis*, a massa da gónada começou a aumentar a partir de Janeiro (Capítulo 2). O pico de desova terá sido em Abril, pois as larvas são encontradas em maior número na água durante o mês de Maio. No entanto, o recrutamento (número de juvenis que entram na população) de *M. edulis* no Mar de Wadden é baixo. O aumento da temperatura durante o Inverno, que se tem feito sentir durante as últimas décadas, poderá causar uma diminuição geral da massa corporal e, logo, da massa da gónada. No entanto, isto não é o caso, uma vez que não foi encontrada nenhuma relação entre a temperatura e a condição do corpo. Provalvelmente, o baixo recrutamento observado, será devido a factores que actuam depois da desova (como predação das larvas) ou na população adulta (como diminuição do número de reprodutores).

Entre espécies que habitam o mesmo habitat, diferenças no recrutamento são também observadas, como é o caso de *C. edule* e *M. arenaria* (Capítulo 3). Ambas as espécies vivem em zonas intertidais e subtidais do Mar de Wadden mas o recrutamento de *C. edule* é geralmente maior que o de *M. arenaria*. Embora o período de desova tenha sido semelhante entre estas espécies, o investimento reprodutivo individual (em termos de tamanho da gónada) foi maior em *M. arenaria* do que em *C. edule*. Além disso, como a massa corporal de *M. arenaria* é muito maior que a de *C. edule*, o investimento reprodutivo da população de *M. arenaria* no oeste do Mar de Wadden tem que ser muito maior que o de *C. edule*. Logo, as diferenças em recrutamento entre as duas espécies não podem ser devidas a diferenças de sucesso reprodutivo entre populações. Diferenças na fase pós-larvar (como diferenças de predação nos estados juvenis) estarão provavelmente envolvidas nas diferenças de recrutamento.

O investimento de energia na reprodução pode ser avaliado não só em termos de massa da gónada mas também em termos de tamanho dos ovócitos. Diferenças no tamanho dos ovócitos são encontradas entre especies. Por exemplo, os ovócitos de *C. edule* têm em média um diâmetro de 65 µm, enquanto *M. arenaria* tem ovócitos de 57 µm e *C. gigas* de 50 µm. No entanto, dentro da mesma espécie, o tamanho dos ovócitos pode variar com as condições ambientais. Diferentes populações selvagens de *C. gigas* (da França à Holanda), apresentaram diferenças no tamanho dos ovócitos (Capítulo 5). O diâmetro médio dos ovócitos diminuiu da França para a Holanda. No entanto, o tamanho máximo da concha, a massa somática e a massa da gónada aumentaram da França para a Holanda. O aumento da massa da gónada em combinação com a diminuição do volume dos ovócitos sugere um aumento do sucesso reprodutivo em termos de número de ovos (ovócitos) produzidos da França à Holanda. Uma vez que, dentro da mesma espécie, ovócitos pequenos têm um desenvolvimento larvar mais longo que os ovócitos grandes, as condições ambientais ao longo da costa Holandesa poderão resultar num aumento da dispersão larvar e possível expansão da população. Uma vez que a expansão da ostra parece estar relacionada com o aumento da temperatura da água durante as última décadas, o limite norte da sua distribuição está dependente da diminuição latitudinal da temperatura em direcção aos pólos.

O modelo DEB

Para além dos dados de campo terem permitido estudar o crescimento e reprodução das várias espécies, permitiram também comparar as simulações do modelo DEB com as observações no campo.

Pressupostos do modelo DEB e determinação de parâmetros

O uso do modelo DEB na simulação do crescimento nas diferentes espécies e na reconstrução das condições de alimento no campo foi feito em diferentes fases. Em primeiro lugar, os pressupostos do modelo foram testados, nomeadamente a relação entre variáveis fisiológicas (tamanho de ovos, taxa de crescimento, tamanho de maturação) e o tamanho do corpo. Os pressupostos foram validados usando dados de campo e laboratório publicados na literatura (Capítulo 5). Relações significativas foram encontradas entre o tamanho do corpo e, respectivamente, o tamanho de maturação, e a taxa de crescimento; o mesmo se verificou entre o tamanho dos ovos e larvas e o tempo de desenvolvimento destes. A maior discrepância foi encontrada na relação ente o tamanho dos ovos e das larvas e o tamanho do adulto. Isto sugere que para as larvas poderem assentar no sedimento, o tamanho (ou volume) das larvas tem que ser semelhante ou maior que o tamanho dos grãos do sedimento.

Posteriormente, os parâmetros do modelo foram determinados para as diferentes espécies estudadas, com base em dados de laboratório publicados na literatura (Capítulo 6). Para nenhuma das espécies foi possível determinar todos os parâmetros. O principal problema na determinação dos parâmetros foi a falta de dados fidedignos. Por isso, um plano foi usado para ajudar a lidar com falhas na determinação de parâmetros (devido à falta de dados) e conseguir consistência entre os parâmetros determinados. Semelhanças entre espécies foram encontradas nos custos com manutenção dos tecidos, nos custos de crescimento e na quantidade máxima de energia acumulada. Diferenças entre espécies são expressas em diferenças em certos parâmetros, nomeadamente na taxa máxima de assimilação e na quantidade de energia usada na reprodução. Estas diferenças reflectem-se no tamanho máximo teórico (em termos de comprimento da concha) das diferentes espécies.

Simulações com o modelo DEB

Finalmente, os parâmetros determinados no capítulo7 foram usados para correr o modelo, com a temperatura e alimento como variáveis (Capítulo 6). Os dados de crescimento recolhidos no campo (Capítulos 2-5) foram combinados com as simulações do modelo e a reconstrução das condições de alimento para as diferentes espécies foi feita. Dois tipos de simulações foram feitas: [1] o crescimento anual das espécies foi simulado em condições de temperatura dentro do intervalo de valores normalmente verificado e com diferentes níveis de alimento (entre 0 – sem alimento, e 1 – alimento *ad libitum*); [2] a variação sazonal no nível de alimento foi reconstruída para cada espécie em cada habitat, através da simulação do crescimento ao longo do ano em condições de temperatura dentro do intervalo de valores normalmente verificado, e da comparação destas simulações com o crescimento observado no campo. Isto permitiu um estudo das interacções (em termos de competição pelo alimento) entre as diferentes espécies e dentro da mesma espécie em diferentes habitats.

A reconstrução sazonal do nível de alimento apresentou valores elevados na Primavera e Verão, o que correspondeu com a altura de crescimento no campo. Depois do verão, a quantidade de alimento na água e na superfície do sedimento geralmente diminui. Como resultado, o nível de alimento simulado diminuíu durante o Verão e Outono, sugerindo que competição pelo alimento entre espécies deve ter ocorrido nesta altura. Diferenças no nível de alimento foram encontradas entre espécies. Por exemplo, em *M. balthica* e *C. edule* o nível de alimento simulado variou entre 0.7 e 1, enquanto que em *M. edulis*, o nível de alimento simulado variou entre 0.3 e 0.5. Isto significa que as condições alimentares no Mar de Wadden são melhores para o crescimento de *M. balthica* e *C. edule* do que de *M. edulis*.

Em geral, as condições de alimento para as várias espécies foram insuficientes para atingir o crescimento máximo previsto, sugerindo que as espécies de bivalves do Mar de Wadden sofrem de limitação de alimento. No entanto, ainda não se sabe se as condições de alimento aparentemente sub-óptimas (isto é, insuficientes para o crescimento máximo), durante grande parte do ano, são devidas à falta de alimento e/ou a condições ambientais que limitam a eficiência de alimentação (como por exemplo a quantidade de silte dissolvida na água).

Conclusões e sugestões para o futuro

Os resultados apresentados nesta tese demonstram que o modelo DEB pode ser usado para a simulação do crescimento e reconstrução das condições de alimento em bivalves. No entanto, vários aspectos importantes ainda requerem um estudo mais detalhado. As discrepâncias entre as simulações e os dados de campo podem ser causadas por erros na determinação da idade dos indivíduos das diferentes espécies. Sendo assim, é importante, no futuro, fazer uma validação da determinação da idade. Isto pode ser feito, por exemplo, através de experiências de marcação ou da análise de isótopos na concha. A falta de séries temporais de dados de crescimento também pode causar diferenças entre simulações e observações, uma vez que a variabilidade no crescimento devido a diferenças nas condições ambientais de ano para ano não foram consideradas.

Relativamente à temperatura, é importante ter dados correctos de medições de temperatura nas diferentes áreas, uma vez que estes dados são necessários para as simulações. Ter acesso a este tipo de dados é essencial pois as condições variam de ano para ano. Esta variabilidade não foi considerada neste estudo.

A determinação dos parâmetros do modelo DEB para as diferentes espécies foi feita com base em dados publicados, principalmente dados resultantes de experiências no laboratório. No entanto, a falta de dados causou dificuldades na determinação dos parâmetros. Para uma correcta estimação dos parâmetros, é importante fazer experiências bem controladas, que permitam a determinação simultânea do crescimento, reprodução, respiração, assimilação e condição corporal sob vários níveis de alimento. Além destes aspectos, parece haver também alguma variabilidade em parâmetros dentro da mesma espécie, o que não foi abordado neste estudo. A utilização do modelo para simular o crescimento e reconstruir condições alimentares na mesma espécie ao longo de um gradiente latitudinal e em diferentes habitats poderá ser o primeiro passo para avaliar com mais cuidado a especificidade dos parâmetros.

Agradecimentos: Gostaria de agradecer ao Prof. Paulo Santos e à Vânia Freitas pelos comentários dados ao resumo.

Acknowledgements

Finally, the anticipated, yet most anxious, moment of my PhD has arrived. On the one hand, the relief of finishing the thesis, on the other hand the question of what will happen next. But I would certainly not reach this point without the help, support and friendship of a group of people, whom I would like to acknowledge.

First of all, I would like to thank my supervisor Henk van der Veer, for giving me the possibility of staying at the NIOZ for my PhD, and for all the help and support, especially during the final writing phase (which lasted a few years!).

Next, I would like to thank Hans Witte, without whom half of the work in this thesis wouldn't have been possible. Thank you Hans for all your help with the heavy jobs of digging, pulling and lifting; and for keeping me going with your good mood and singing abilities.

A large part of my samples was collected by boat. Some moments will always stay in my memory. One of them is the delicious soup of Ewout Adriaans from RV 'Stern', which we had every time we were back on the boat after working hours on the tidal flat. Also the cosy atmosphere RV 'Navicula': the working schedule of Cees van der Star (starting at 5 a.m.!); Tony van der Vis and the 6 o'clock session of 'Klokhuis', and of course the 'grandma duck suit' (a warm suit that I used to wear to survive the cold temperatures outside and made me look like a duck!); and the nice food of Hein de Vries. Thank you all for all the help.

I would also like to thank my promoters Prof. dr. Wim Wolff and Prof. dr. Paulo Santos for all the help with the paperwork necessary for defending a thesis and for always being willing to read and comment on the chapters.

Thank you to all my colleagues at the NIOZ and MEE department for the cosy and friendly working environment. Special thanks go to Jaap van der Meer and Tineke Troost for statistical help, to Tanya Compton for help with the English language, to Anneke Bol for working on the oyster genetics and to Rob Witbaard for helping with shell analysis.

Sampling outside Texel wouldn't have been as easy without the help of Karin Troost and Pauline Kamermans in Yerseke and Marianne Alunno-Bruscia in La Rochelle. Also working at the University of Paris was facilitated by the help and support of Denis Langlet, Marc Rafelis and Franck Lartaud. In addition, I would like to thank everyone who helped me sampling when needed. Thanks also go to my students Ana Martins and José Loff for all the help.

During the last couple of years, I've also been working in the molecular lab. I would like to thank everyone for their help, especially to Judith van Bleijswijk and Harry Witte. I really enjoyed the work and the friendly atmosphere of the lab.

Life on Texel wouldn't be the same without a group of people: thank you to all of those who shared with me the crazy and unforgettable times in the Potvis and later the more quite times in Den Burg. Special thanks go my housemates Tanya, Micha and Anne-Claire; but also to Alina and Leon, Marie-Aude, Claire, Thomas and Barbara, Astrid, Seb and Nicola, Denis, Marta, Eva, Joaquín, Wolfgang, Thomas Richter, Marco and Cornelia, Neven, Hélène, Judith and François + children, Ismael and Khalid for the nice times we've spent together. Thank you Claire for offering me a place to stay in Paris and for the company then. Thank you Alina for sharing with me the good and bad moments, and for your wonderful teas. Thank you Tanya and Micha for all the good moments we've spent together and for your friendship.

The small "Portuguese community" on Texel brought some Portuguese warmth to this northern island. Thank you Luisa, Teresa, Sara, Isabel and Pedro for all the conversations and good food.

I would also like to thank my Portuguese colleagues Joana Campos and Vânia Freitas, who visit the NIOZ a few times per year, for the nice company, active conversations and gossips, the Portuguese newspaper and the bottles of wine.

Although the ferry to Texel only takes 20 min. it's still quite a barrier to pass since unfortunately not many of my Portuguese friends have visited me. Nevertheless, they were always there for me when I went back to Porto and my visits were so often that I managed to see them almost as often as they saw each other. Than you Sofia, Rogério, Ana, Pedro M., Mafalda and Pedro F. for your long-term friendship.

A big thank you goes to Ben. Thank you Ben for all your love and patience. Thank you also to Marika and Albert Abbas for always welcoming in their house.

At last, but not least, I would like to thank my family for all the love and support. Without you I would not be here now. Thank you for all the survival packages with smelly codfish, tasty sausages, strong cheese, Portuguese magazines and (even) clothes.

Curriculum Vitae

Joana Cardoso was born on the 6th November 1975 in Porto, Portugal. In 1993 she began studying Biology at the Faculty of Sciences from Porto University. In July 1998 she obtained her Licentiate degree in Biology, with specialisation in Applied Animal Biology. Soon after, in October 1998, she started a practical period of 9 months at the Royal Netherlands Institute for Sea Research (NIOZ). She worked on the food conversion efficiency in shrimps and on the resistance to low temperatures of various benthic invertebrate species of the North Sea. In July 1999 she started another practical period of 1 year at the NIOZ, working on the distribution and abundance of benthic and epibenthic organisms in the Frisian Front, North Sea. In September 2000, she was granted a 4-year scholarship from the Portuguese Science Foundation to do her PhD at the NIOZ, where she has been until now. The results from these years are documented in this thesis. In addition, from March 2005 until August 2006 she worked as a technician at the NIOZ, first on the molecular identification of bivalve larvae, then on the molecular identification of bacteria and archaea associated to sponges and later on the identification of benthos from sediment samples. Recently she has been awarded a 3-year post-doctoral scholarship from the Portuguese Science Foundation to work on the biochemistry of shells for age determination in different bivalve species along the European coast. This project is a collaboration between CIIMAR in Porto and the NIOZ and will start in October this year.

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