Patterns of distribution, dynamics and genetic variation in the peppery furrow shell Scrobicularia plana

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Patterns of distribution, dynamics and genetic variation in the peppery furrow shell *Scrobicularia plana*

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te Ribeirão, Portugal

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“… Ah, que ninguém me dê piedosas intenções!
Ninguém me peça definições!
Ninguém me diga: “vem por aqui”!
A minha vida é um vendaval que se soltou.
É uma onda que se alevantou.
É um átomo a mais que se animou...
Não sei por onde vou,
Não sei para onde vou
- Sei que não vou por aí!”

José Régio

Para a minha família.
To my family.

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Chapter 1

Introduction

General Introduction

Many marine bivalve species are characterized by wide distribution areas, usually indicative of a high physiological tolerance, as individuals are repeatedly subjected to different environmental regimes. Moreover, they commonly live in large populations and display external fertilization followed by a long, planktonic, larval phase which allows (in theory) wide dispersal. The high potential for dispersal, in combination with large population sizes and the absence of obvious barriers to gene flow in marine environments, are expected to enhance physiological flexibility, which leads to a better genetic potential for adaptation when compared to isolated populations. At the same time, however - at a more local scale - many species show an aggregated (patchy, clumped) distribution (e.g. Meire et al., 1989; Strasser et al., 1999; Bocher et al., 2007), which is normally associated with proximal factors (Ysebaert and Herman, 2002; Bocher et al., 2007), suggesting the existence of specific environmental requirements and high (biological) sensitivity. If the degree of aggregation also results in reproductive isolation over time, populations will become demographically isolated and potentially more vulnerable to environmental changes as reduced genetic diversity and reduced gene flow will generally result in lower fitness. This suggests that locally adapted populations may go extinct if local conditions shift too rapidly.

Scrobicularia plana da Costa 1778 (Bivalvia, Semelidae) exemplifies many of the above attributes. On the one hand, it has a wide geographical range and shows a high tolerance to physical and chemical variation, and a rapid demographic adaptation to environmental changes (Hughes 1970). Along its distributional range, *S. plana* needs to be able to cope with a large spatial and temporal variation in temperature and salinity regimes. The species has been recorded in areas with mean water temperatures ranging from 6-15.5°C in North Wales (Hughes, 1971), 13-28°C in Morocco (Bazaïri et al., 2003), and 10-27°C in the Mediterranean (Casagranda and Boudouresque, 2005); and salinities ranging from 11 psu (Green, 1957) to 34.5 psu (Freeman and Rigler, 1957), suggesting a higher tolerance to low salinities than most common estuarine bivalves (Spooner and Moore, 1940). On the other hand, at a local scale, *S. plana* is patchy in its spatial distribution (Hughes, 1970; Langston, 1986; pers. obs.). Although patchiness is commonly observed for other bivalve species along the European Atlantic coast - such as *Mya arenaria* (Strasser et al., 1999; Bocher et al., 2007), *Macoma balthica* and *Cerastoderma edule* (Meire et al., 1989) - *S. plana* is seemingly even more aggregated than these co-distributed soft sediment bivalves (pers. obs.). This suggests that despite the species apparent high tolerance to environmental variation, a higher sensitivity to local factors, e.g., salinity, sediment type and immersion periods play a significant role (Hughes, 1970; Compton et al., 2009).

The different scales of spatial variation must be taken into account when trying to understand abiotic and biotic interactions such as small-scale behavioural responses to habitat, competition and predation; and to larger-scale patterns of dispersal and mortality/recruitment, which operate simultaneously. Understanding spatial distributions and their demographic dynamics should then help us to disentangle the relative importance of particular factors.

The main aim of this thesis was to study the temporal and spatial patterns of population structure and population dynamics in *Scrobicularia plana*. Knowledge about large-scale, latitudinal patterns provide insights into historical effects of climate and temperature on overall distribution, whereas small-scale patterns, characteristic of specific estuaries or coastlines, provide insights about dispersal capacity, local population turnover and the maintenance of other demographic parameters such as growth and age structure. Together these patterns provide fundamental information about adaptation potential, dispersal, effects of habitat fragmentation/isolation and the trajectory that management must adopt in order to preserve population sustainability (areas that are fished) and ecosystem integrity (in both fished and unfished areas).

The peppery furrow shell *Scrobicularia plana*

The peppery furrow shell *Scrobicularia plana* (Fig. 1.1) is one of the most common and abundant bivalve species in soft-sediment intertidal areas along the NE Atlantic coast (Bocher et al., 2007). The species is distributed along the Atlantic coast, from the Norwegian Sea to Senegal, and also in the Mediterranean Sea (Fig. 1.2, Tebble, 1976). It inhabits intertidal areas of soft bottoms, with a clear preference for muddy sediments (Freeman and Rigler, 1957; Bazaïri et al., 2003; Carvalho et al., 2005; Bocher et al., 2007) . *S. plana* is a member of the Class Bivalvia, Family Semelidae and constitutes with *Scrobicularia cottardi* the genus *Scrobicularia* (ERMS, 2011; Le Renard, 2011). It has a thin, equivalve, rounded and nearly perfectly flattened shell, a morphological feature that distinguishes it from other bivalves (Pizzolla, 2002).

Fig. 1.1. The peppery furrow shell *Scrobicularia plana*.

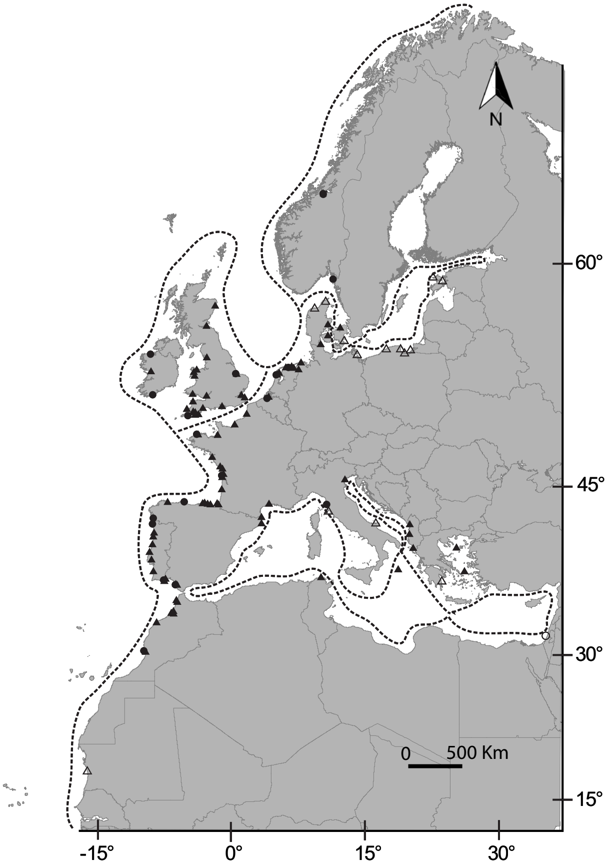

Fig. 1.2. Geographical distribution of *Scrobicularia plana*. Dotted line is assumed distribution based on reference from Tebble 1976. Symbols represent confirmed distribution locations of *S. plana* based on: literature records of live specimens (full triangles), references to the presence of dead shells in the sediment (open triangles) and pers. obs./comm. (full circles).

Scrobicularia plana is a key species of intertidal communities (Tebble, 1976; Keegan, 1986), and is an important food source for avifauna and ichtyofauna (Hughes, 1970; Moreira, 1997; Wanink and Zwarts, 2001). Due to higher densities in southern latitudes (Bachelet, 1979; Sola, 1997; Bazaïri et al., 2003; Verdelhos et al., 2005; Bocher et al., 2007), *S. plana* is very often the most abundant species in Southern Europe (e.g. Rodrigues et al., 2006; Salgado et al., 2007) and the main prey species of numerous bird communities (Moreira, 1997). Although the species is not as abundant in more northern latitudes, it is still an important food source for wading birds (Hughes, 1970; Boates and Goss-Custard, 1989; Wanink and Zwarts, 2001).

Scrobicularia plana is also of direct importance to humans, being commercially exploited in southern European countries (Langston et al., 2007). The decline (or even disappearance) of populations may, therefore, have serious consequences for both the ecosystem and socio-economic well-being. A recent study in the Mondego Estuary (Portugal) revealed that the decline of *S. plana* populations, due to extreme flooding and drought, led to lower quality and stability of the macrobenthic ecosystem and simultaneously, negatively affected local fisheries (Rito, 2009). In turn, harvesting pressure is also expected to affect population dynamics. For example, intense commercial exploitation of *S. plana* in the Tagus Estuary (Portugal) led to changes in population structure and turnover with smaller sized individuals dominating (Salgado et al., 2007). This emphasizes the need for a more quantitative understanding of natural spatial distributions and population dynamics of *S. plana* in order to determine how the species may respond to environmental and community changes and how those responses will affect ecosystem functioning.

Population dynamics

Age structure of a population provides a key to understanding how spatial processes - dispersal, connectivity - related to growth, maturation, mortality, recruitment, turnover (Pulliam, 1988; Mouquet and Loreau, 2003). As an indicator of population health, it is an essential parameter in the development of successful management programs. Age determination and its validation is critical as incorrect age determination can easily lead to an over- or underestimate of population turnover which effects exploitation. Moreover, as legislation on the commercial size of mollusk species varies depending on location, knowledge about the lifespan of local populations is essential.

In bivalves, age determination commonly relies on the interpretation of external lines or annual growth rings. For temperately distributed bivalves, the annual temperature cycle involves alternating periods of high temperature that favors growth (the growing season) with low temperature that inhibits growth as energy reserves are utilized to fuel metabolism (the wintering period). It is during this period that a winter line is usually formed on the surface of the shell (e.g. Richardson et al., 1980; Brousseau, 1984; Richardson and Walker, 1991; Richardson, 2001; Moura et al., 2009). The number of lines provides an estimate of age. In warmer latitudes, however, metabolic rates may not decrease enough during the winter to form clearly visible winter rings; while in normally colder areas that have experienced temperature fluctuations, individuals may form a “disturbance” ring, which can be indistinguishable from the true annual rings (e.g. Hughes, 1970; Haag and Commens-Carson, 2008).

A number of researchers have reported difficulties in application of the line method (Hughes, 1970; Bachelet, 1981; Neves and Moyer, 1988; Richardson and Walker, 1991; Moura et al., 2009). Thus, an alternative approach to molluscan sclerochronology needed to be found. One approach is to analyze the variation of a stable isotope across the shell in order to identify major shell-growth increments and their periodicity. The approach relies on the assumption that the precipitation of carbonate in marine mollusks occurs in isotopic equilibrium with the ambient water, a direct relationship between the oxygen isotope composition ($\delta^{18}O$) of molluscan shells and temperature (Epstein et al., 1953; Dettman et al., 1999). The seasonality in water temperature is then expected to result in annual periodicity in the $\delta^{18}O$ composition of shells, which can be used to validate the annual formation of growth bands and estimate age.

Spatial scale

Spatial patterns of macrobenthic invertebrates are shaped by environmental conditions. Since environmental conditions are rarely homogeneous and the relative influence of pattern-forming processes changes across scales, ecological variability tends to be scale-dependent (Legendre and Fortin, 1989; Englund, 1997). Such processes can operate on scales from thousands of kilometres, e.g., with large-scale abiotic factors determining the geographical distribution of a species (Barry and Dayton, 1991), to centimetre-scales governed by behavioural responses of the individuals to microhabitat variation (de Wolf, 1989; Underwood and Chapman, 1996). As organisms respond to more than one of these levels (Hewitt et al., 1996), analyzing distribution patterns at different scales is necessary.

Large-scale patterns

Analysis of distribution patterns at large biogeographic scales provides information about the species history over thousands to millions of years, mainly in response to paleoclimates and, in particular, the most recent glacial and inter-glacial periods. Information about dispersal, location of refugia and historical demographic estimates of effective population size can be inferred using a phylogeographic approach. Present-day distribution patterns in the North Atlantic have mainly been shaped by the last two glacial periods, the last one ending some 20,000 years ago. Such information is useful in forward projections related to climate change scenarios. Nested within the evolutionary context is the physiological. Here, the distribution patterns of marine communities at this latitudinal scale are mainly determined by temperature (Barry and Dayton, 1991), a factor that also influences physiological rates and timing of reproductive cycles (Hayward and Ryland, 1990).

Temperature generally decreases with increased latitude which is then reflected in the physiological performance of bivalves. Several studies have documented physiological differences in temperature tolerances among latitudinally separated populations of marine organisms (e.g. Dehnel, 1955; Clarke, 1987; Present and Conover, 1992; Jonassen et al., 2000; Terossi et al., 2010; Wehrtmann et al., 2011). Temperature tolerance and relative acclimation capacity are the main factors determining southern and northern boundaries and changes in sea surface temperatures are already producing shifts in the distributional ranges for many species (e.g., Walther et al., 2002; Beaugrand and Reid, 2003; Brander et al., 2003; Philippart, 2007) as well as phenological shifts in life history traits (Parmesan and

Yohe, 2003; Philippart et al., 2003). For example, rising sea temperatures are affecting the recruitment in bivalves, such as *Macoma balthica*, by decreasing their reproductive output and advancing the timing of their spawning period and thus creating a mismatch with the phytoplankton bloom and the settlement of juvenile shrimps on the tidal flats (Philippart et al., 2003). It is then expected that shifting temperatures will also affect recruitment success in *S. plana.*

Small-scale patterns

At smaller spatial and temporal scales, biotic and/or abiotic processes will further modify distributions and abundances nested within the larger, biogeographic-scale patterns (Thrush, 1991; Warwick et al., 1991; Sousa et al., 2008; Westerbom et al., 2008; Kraufvelin et al., 2011). Understanding small-scale patterns (e.g., patchiness, aggregations) provides information on the ecological component related to density-dependent processes since, at local scales, behavioural interactions among organisms or between them and small-scale environmental variables are likely to exert a stronger, more immediate influence on spatial patterns (Underwood and Chapman, 1996).

Where the need for contact among sessile individuals is necessary for copulation, spatial pattern is governed by a balance between the cost of increased starvation with proximity to one another (Heip, 1975). However, because bivalves do not copulate, suggesting that physical closeness is not required, a random or uniform distribution would be predicted based on intraspecific competition for space and surrounding resources (Gaines and Roughgarden, 1985; Meire et al., 1989; Thrush, 1991). In reality, the situation is more complex as aggregation is the most commonly observed condition (Hughes, 1970; Meire et al., 1989; Strasser et al., 1999; Bocher et al., 2007).

Causes of aggregation are several. It could be that external fertilization does require some degree of aggregation (Levitan and Young, 1995; Luttikhuizen et al., 2011) after all. In addition, post-settlement dispersal of juveniles and adults (Beukema and De Vlas, 1989; Commito et al., 1995; Cummings et al., 1995; Hewitt et al., 1997) and predation (Jensen and Jensen, 1985; Reise, 1985; Günther, 1992) are also important. Post-settlement migration confers small-scale dispersal abilities and can occur as a response to unfavourable conditions as juveniles search for an optimal site for development (Hughes, 1970; Bouchet et al., 2005). This active habitat selection helps determine which patches are best suited for occupation. As for predation, it may affect the species spatial distribution as it inhibits recruitment (Hughes, 1970; Richards et al., 1999; Hunt and Mullineaux, 2002), while the distribution and intake rate of the predators (Sutherland, 1996) as well as predator's mobility (Schneider, 1992), can also influence the degree of aggregation of the prey. Only by determining the relative importance of different factors can we disentangle the relative strength of a particular factor alone and in combination.

Approach

This study used a comparative, analytical and inferential approach to investigate: 1) life history, growth dynamics and phenological characteristics of *Scrobicularia plana* over its latitudinal range; 2) large-scale biogeographic distribution patterns including population history using a phylogeographic approach; and 3) small-scale patterns related to both abiotic and biotic factors using spatial autocorrelation. The approach involved an extensive review and analysis of the literature in order to identify gaps in our knowledge of the natural history of *S. plana*, a comparative survey of growth and reproductive cycles at three locations along a latitudinal gradient, a large-scale phylogeographic survey based on mitochondrial DNA and an assessment of medium and local scale patchiness in relation to abiotic and biotic factors.

Thesis outline

In **Chapter 2** we reviewed what is known about the general biology and ecology of the peppery furrow shell, *Scrobicularia plana*, in order to identify which factors may determine the species' spatial pattern and at which stage(s) of its life cycle. Temperature, salinity, sediment type, hydrographic conditions and the role of predation were evaluated. In **Chapter 3**, seasonal growth and reproduction patterns of *S. plana* were evaluated with respect to temperature and food availability at three locations along a latitudinal gradient in order to assess physiological performance and tolerances in relation to possible range shifts under climate change scenarios. In commercially exploited species, such as *S. plana*, it is very important to have information on stock health for which accurate age estimation is vital. Given the uncertainties associated with the methodologies commonly used, such as counting external surface lines or the analysis of internal lines shell cross-sections, in **Chapter 4** we used sclerochronology to validate age estimates. In **Chapter 5**, the phylogeography and historical demography of *S. plana* were determined using mtDNA-COI. The goal was to infer the species history in an evolutionary context as a baseline for future projections given the relative patchiness of its latitudinal distribution and potential isolation due to poor connectivity. The main objective in **Chapter 6** was to analyze spatial distribution patterns of *S. plana* at smaller regional (Wadden Sea) and local (<2 km^2) scales in order to shed some light on the degree to which patterns are species or site-specific in nature. Spatial correlograms were constructed for four intertidal areas. In **Chapter 7** the overall findings are synthesized.

Chapter 2

Spatial distribution patterns of the peppery furrow shell *Scrobicularia plana* (da Costa, 1778) along the European coast: A review

Sílvia Santos, Pieternella C. Luttikhuizen, Joana Campos, Carlo H.R. Heip, Henk W. van der Veer

Abstract

The bivalve *Scrobicularia plana* is an important species of shallow water benthic communities with a wide geographic distribution but also with a general patchy pattern, i.e. irregular in occurrence and in density. This review aims to determine the processes responsible for the species' spatial distribution pattern based on the available information on *S. plana*. Although several pre- and post-settlement processes are believed to influence spatial patterns of marine invertebrates, the general patchy distribution of *S. plana* seems to be determined by the existence of specific environmental conditions during settlement. Factors such as temperature, salinity, sediment type, hydrographic conditions and predation affect settlement and spat survival and not one but a combination of factors seems to explain the species distribution pattern. Future work should focus on determining the scale of patchiness, using hierarchical sampling, as well as the connectivity between populations by analysing the population genetic structure.

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Introduction

Intertidal mudflats are valuable ecological entities with productive flora and fauna, supporting large populations of birds (e.g. Beukema et al., 1993), and acting as nursery and feeding areas for fish and crustaceans (e.g. Beukema, 1992; Campos et al., 2010; van der Veer et al., 2001; van der Veer and Bergman, 1986). Bivalves are an essential component of these intertidal soft-sediment communities (Beukema, 1976; Piersma, 1987; Piersma and Beukema, 1993; Raffaelli and Hawkins, 1996; Reise, 2001). Worldwide, bivalves are found in a variety of environments from the poles to the tropics (Dance, 2002; Hayward and Ryland, 1990; Tebble, 1976). Along the European coast a few bivalve species account for most of the biomass (Bocher et al., 2007). These species 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*, the white furrow shell *Abra alba*, an invasive species the Pacific oyster *Crassostrea gigas* and the peppery furrow shell *Scrobicularia plana* (Beukema, 1976; Bocher et al., 2007; Cardoso et al., 2007a; Dankers et al., 2006; Dekker and Waasdorp, 2005; Wolff, 1983).

S. plana is a member of the Class Bivalvia, Family Semelidae and constitutes with *S. cottardi* (Payraudeau, 1826) the genus *Scrobicularia*, according to the European Register of Marine Species (ERMS, 2011) and the Check List of European Marine Mollusca (CLEMAN; Le Renard, 2011). It has a thin, equivalve, rounded and nearly perfect flattened shell, a morphological feature that distinguishes it fromother bivalves (Pizzolla, 2002). *S. plana* has been considered a "key species" because of its ubiquity, local abundance and importance in the estuarine trophic chain (Keegan, 1986). It is a natural resource of high importance not only as a food resource for the avifauna and the ichtyofauna, but also due to its economic value, especially in southern Portugal and Spain (Langston et al., 2007). *S. plana* is a temperate to warm water species that is often dominant in shallow water communities (Tebble, 1976). The species is characterized by a general patchy structure, in the sense that seemingly suitable locations either house high densities of the species or are vacant (Bocher et al., 2007; Hughes, 1970). Some southern populations in high muddy substrates suggest however a more continuous distribution (Bocher et al., 2007) which could be an artefact of the higher densities in southern areas (Bachelet, 1979; Bazaïri et al., 2003; Bocher et al., 2007; Sola, 1997; Verdelhos et al., 2005), although, in a study in SW Netherlands, *S. plana* was the only mollusc species with a random (not aggregated) pattern and the one with lowest mean density (Meire et al., 1989).

Spatial structure is an important characteristic of species' ecology, reflecting the influence of several factors such as life-history, competition, predation, motility and feeding modes, with organisms that are closer to one another in space or in time being more likely influenced by the same factors (Legendre and Fortin, 1989). Analysis of these spatial patterns and the underlying relationships may help identify the scales over which particular processes become important (Andrew and Mapstone, 1987; Thrush, 1991), essential for data interpretation since scale determines the types of statistical analysis that can be performed and the inferences that can be made (Thrush, 1991). However, basic information about *S. plana* is still missing. The aim of this review is to identify which factors may determine the species spatial pattern and at which stage(s) of its life cycle is it occurring, based on available information about the biology and ecology. In addition, gaps in basic knowledge will be identified and an agenda for future research will be formulated. Such information is essential for a better understanding on population dynamics and spatial aspects of the distribution of individuals in relation to the species ecology and habitat characteristics.

Distribution

The geographic distribution of *S. plana* (Fig. 2.1) ranges from the Norwegian and Baltic Seas, along the Atlantic coast to Senegal, and into the Mediterranean Sea (Tebble, 1976). The species inhabits intertidal soft bottoms, rich in organic matter, with a preference for muddy sediments (Bazaïri et al., 2003; Bocher et al., 2007; Carvalho et al., 2005; Casagranda and Boudouresque, 2005; Freeman and Rigler, 1957; Guerreiro, 1998). *S. plana* is normally absent from coarser and more mobile sediments that prevent the maintenance of the long, narrow burrows for the siphons (Hughes, 1970). Animals live burrowed at a depth of 5 to 20 cm (Akberali and Davenport, 1981), laying vertically (Hughes, 1969), although it has been shown that they can rest, horizontally, on either valve (Rasmussen, 1973). They are found, regularly, with the shells almost touching and the inhalant siphons overlapping while deposit-feeding, without any effect whatsoever on their activities (Hughes, 1970). Their presence can be identified by the star-shaped markings made on the surface of the sediment by their inhalant siphons (Pizzolla, 2002).

S. plana is usually more abundant in southern areas: 2.1 ind m^{-2} in the Dutch Wadden Sea, 19.9 ind m^{-2} in The Wash (England) and 2.7 ind m^{-2} in Mont Saint-Michel Bay, 126 ind m^{-2} in Aiguillon Bay and 41.5-83.4 ind m^{-2} in Marennes-Oléron Bay, in France (Bocher et al., 2007). Maximum densities of 1025 ind m^{-2} were observed in South Wales (Green, 1957) while considerably higher values were calculated for the Atlantic coast of France (3055 ind m^{-2}; Bocher et al., 2007) and Spain (5892ind m^{-2}; Sola, 1997), and the Mediterranean (4901 ind m^{-2}; Guelorget and Mazoyer-Mayère, 1983). Despite the suggested overall trend of increased density with decreasing latitude, year-to-year variability can be high (Essink et al., 1991), with mean densities in Southern Netherlands varying between 0 and 394 ind m^{-2} in a seven years period (Ysebaert and Herman, 2002).

Highly patchy spatial distributions are typical for many hard substrate invertebrates while soft bottom infauna is generally more evenly distributed, possibly as a result of intraspecific food competition (Meire et al., 1989). The increased risk of starvation due to an uneven partitioning of space is usually outweighed by the need for contact between individuals, particularly in species where copulation occurs. Though external fertilization may require aggregation, too (Levitan and Young, 1995; Luttikhuizen et al., 2011), copulation is not part of the reproductive scheme in bivalves, which may explain why they are usually not aggregated (Heip, 1975). Nevertheless, a patchy distribution has been recorded not only for populations of *S. plana* (Bocher et al., 2007; Hughes, 1970) but also for other soft bottom bivalves such as, e.g., *M. arenaria* (Bocher et al., 2007; Strasser et al., 1999), *M. balthica* and *C. edule* (Meire et al., 1989), which co-occur with *S. plana* in many areas. However, *S. plana* seems to be more aggregated than other co-distributed soft sediment bivalves of the intertidal.

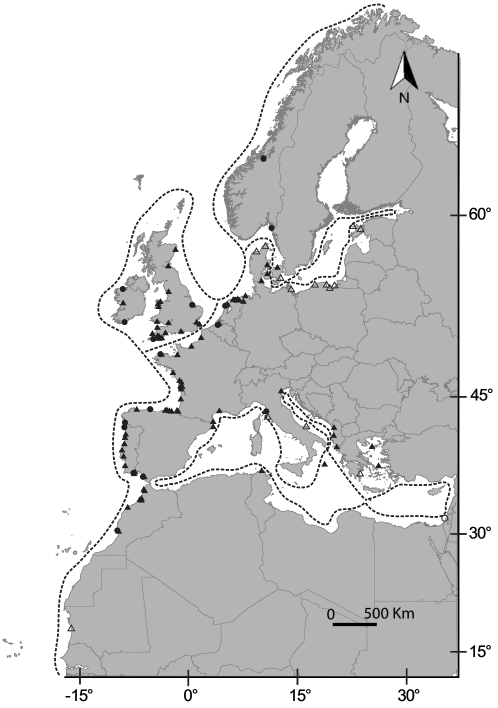

Fig. 2.1. Geographical distribution of *Scrobicularia plana*. Dotted line is assumed distribution based on reference from Tebble 1976. Symbols represent confirmed distribution locations of *S. plana* based on: literature records of live specimens (full triangles), references to the presence of dead shells in the sediment (open triangles) and pers. obs./comm. (full circles). List, coordinates and references of locations where the species was recorded can be found as online as electronic supplementary material.

Life cycle

Bivalves are usually dioecious species (separate sexes), with gametes being released into the water, where fertilization, embryonic and larval development occur. After fertilization, eggs develop into free-swimming trochophores, a non-feeding stage, followed by veliger larvae, a swimming planktotrophic phase that can last from a few days to several months. At metamorphosis, larvae will settle on the substrate, beginning their benthic existence, and further growth (Dame, 1996; Thorson, 1950). This general pattern also applies to *S. plana*.

Reproduction

S. plana is considered to be a gonochoristic species (Hughes, 1971; Rodriguez-Rua et al., 2003), although a few cases of hermaphroditism have been reported (Paes-da Franca, 1956; Raleigh and Keegan, 2006). The disturbance of the 'normal' gonadal development of male *S. plana* by endocrine-disrupting chemicals has been recently observed at sites in the SW of the United Kingdom, with varying degrees of incidence and severity of intersex. It was suggested that the few cases of "hermaphroditism" detected previously could be examples of intersex in other populations (Langston et al., 2007).

Sexual maturity is obtained between age classes 2 and 3 (Guerreiro, 1998; Paes-da Franca, 1956; Sola, 1997), with only individuals with a shell length greater than 20 mm undergoing sexual development (Raleigh and Keegan, 2006).

Gonadal development

Gonadal development in bivalves follows one of two models (Lammens, 1967): [1] repose period during the winter months, with empty gonads; gametogenesis initiated at the end of winter/beginning of spring; spawning in the warmer months in response to increases in temperature and food supply; or [2] the sexual reserves are stored during summer and autumn, and maintained in winter, with spawning also taking place between spring and summer. Development of *S. plana* follows the first model (Hughes, 1971; Paes-da Franca, 1956).

Gonadal development of *S. plana* has been studied at several locations (Table 2.1). In SW England, gametogenesis begun between March and May (Worrall et al., 1983); while in northern Wales, it started around the same period, in April and lasted until September (Hughes, 1971). Gonadal development started earlier (January/February) in a population from the west coast of Ireland (Raleigh and Keegan, 2006) similarly to more southern populations. In SW Spain, it occurred between February and October (Rodriguez-Rua et al., 2003), while in a Portuguese population differentiated gametes were present in all months of the year, except for December (Paes-da Franca, 1956). It seems that gonadal development starts earlier in the year and lasts longer in more southern areas, suggesting the existence of a latitudinal gradient which in turn would play a major role on the species biogeography.

Environmental factors such as increased temperature and food availability are thought to trigger gametogenic development (Raleigh and Keegan, 2006; Rodriguez-Rua et al., 2003; Worrall et al., 1983; Worrall and Widdows, 1983). Gametogenesis started in Ireland when water and substrate temperatures were on average 9°C (Raleigh and Keegan, 2006). In turn, the period of sexual repose usually coincides with the lowest temperatures, occurring at temperatures of 12°C in SW Spain (Rodriguez-Rua et al., 2003) and 14°C in Portugal (Paes-da-Franca, 1956). With increasing latitude and consequently decreasing temperature and food availability, the time available for gonadal development is expected to decrease until just beyond the geographical limit of the species, as time for complete maturation becomes insufficient (Hughes, 1971). The observation that the period of gonadal development is shorter in northern areas supports this hypothesis.

Table 2.1. Timing of gametogenesis periods of several populations of *Scrobicularia plana*, ordered along a latitudinal gradient from north to south (†† end of the period; * timing of the peak).

	Winter			Spring			Summer			Autumn			
Location	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Reference**
Conway Bay, Wales				▨	▨	▨	▨	▨	▨				Hughes 1971
Mweeloon Bay, Ireland	▨	▨	▨	▨	▨	▨	▨	▨	▨				Raleigh & Keegan 2006
Torridge estuary, England								▨ ††					Ruiz 1995a
Plym estuary, England						▨	▨	▨ *					Ruiz 1995a
Avon estuary, England						▨	▨ *	▨ *	▨	▨			Langston et al. 2007
Cornwall, England			▨	▨	▨								Worrall et al. 1983
Seine, Loire and Bourgeuf, France	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨			Mouneyrac et al. 2008
Cádiz, Spain		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		Rodríguez-Rua et al. 2003

Spawning

Being a broadcast spawner (Langston et al., 2007), *S. plana* releases its gametes into the water, where fertilization takes place. Eggs are released repeatedly, with mature oocytes present in the ovary throughout the reproductive season (Raleigh and Keegan, 2006).

Timing of spawning differs between locations (Table 2.2). More northern populations are usually characterized by a single spawning period in summer, as observed in the Dutch Wadden Sea (Zwarts, 1991) and SW England (Worrall et al., 1983). The population from Ireland was once again closer to more southern populations with spawning occurring from May to September (Raleigh and Keegan, 2006). Two spawning periods are often observed in southern populations, namely in winter and in late spring in SW France (Bachelet, 1981) and Portugal (Paes-da Franca, 1956), while in Moroccan estuaries, the second period occurred in late summer (Cheggour et al., 2005). Nevertheless, in some southern locations, only one protracted spawning event is recorded such as in northern Spain, between June and mid September (Sola, 1997), and in SW Spain and in two Portuguese estuaries, although starting 1 and 2 months earlier, respectively (Guerreiro, 1991). Further south, on the Atlantic coast of Morocco, the single spawning event observed occurred from August to November (Elkaïm, 1976). In general, as latitude decreases, the occurrence of two spawning periods is common with spawning starting usually earlier in the year. However, despite a general trend with latitude, the exceptions observed suggest that local site-specific characteristics also play a role (Sola, 1997).

Spawning, like gonadal development, can be triggered by environmental factors (Raleigh and Keegan, 2006; Worrall et al., 1983). In SW England, spawning occurred when sediment temperatures were at 15-17°C, but was also influenced by the algal spring bloom in the water column (Worrall et al., 1983).

Egg and larval stage

Mean diameter of mature oocytes ranges from 42 μm in Ireland (Raleigh and Keegan, 2006), 57 μm in Portugal (Paes-da Franca, 1956), 75-80 μm (Frenkiel and Mouëza, 1979) and 91.0±6.9 μm (Hughes, 1971) in Wales. The number of oocytes produced by females is unknown. The sperm cells are characterized by long (30 μm) sickle-shaped heads, and slow, pendular movements (Frenkiel and Mouëza, 1979). When the gametes are mature they are released into the water, where fertilization takes place. Fertilized eggs have a thick chorion inside which the development of the early larval stages occurs (Frenkiel and Mouëza, 1979).

Larval development of *S. plana* consists of three well-defined stages: (1) a non-feeding stage between fertilization and hatching, lasting from 60 h to 4 days (Frenkiel and Mouëza, 1979); (2) from hatching to settling pediveliger larvae, with veligers dwelling in an entirely pelagic environment; and (3) from settlement to the completion of metamorphosis, with individuals mostly burrowed into the top few mm layer of the substratum but probably frequently experiencing resuspension (Ruiz et al., 1995). At 18°C, recently hatched veliger larvae take around 30 days to attain the settling pediveliger stage, and several more weeks to complete metamorphosis and become siphoned juveniles. Development of veligers is three times faster at 23°C, suggesting that is temperature dependent (Frenkiel and Mouëza, 1979).

A relationship between egg/larval volume and egg/larval development time in bivalves was proposed by Cardoso et al. (2006). Assuming an oocyte diameter of 75 μm and egg size at hatching of 106 μm (Frenkiel and Mouëza, 1979), oocyte and egg volumes are, respectively, 220781.3 and 623298.4 μm^3. The model suggests that for such volumes, egg development time (from fertilization to hatching) at a mean temperature of 16°C would be around 90 hours and larval development at a temperature of 18°C would take about 23 days. *S. plana* seems to fit the general relationship observed for bivalves, although the larval development time calculated from the model is a week shorter than what has been observed by Frenkiel and Mouëza (1979).

Settlement

When reaching substrate with appropriate characteristics, the pediveliger larvae of *S. plana* settle and start growing. Settlement size is in the range of 250-300 μm (Frenkiel and Mouëza, 1979). No quantitative information is available on the supply of settling larvae to local adult populations preventing an analysis of pelagic-benthic coupling. Information is only present from small just-settled juveniles onwards giving insight into the period of settlement of *S. plana*. Timing of settlement is a reflection of the spawning period with a time lag representing egg and larval development and therefore also shows a latitudinal trend (Table 2.3).

Settlement in a northern location (England) occurred between June and July (Stopford, 1951). In more southern locations, two settlement periods are usually recorded, starting earlier in the year. Settlement was almost uninterrupted between April and September in the Gironde estuary and Arcachon Basin in France (Bachelet, 1981),while it occurred in October at Prévost (Guelorget and Mazoyer-Mayère, 1983). In northern Spain, an uninterrupted settlement period occurred from June-July to October-December (Sola, 1997). In Portugal as well as in several estuaries in Morocco and in the Mediterranean Sea, a first settlement period in spring and a second one in late summer/autumn were recorded (Casagranda and Boudouresque, 2005; Cheggour et al., 2005; Guerreiro, 1998). An exception to this general trend is the single settlement period observed in Portugal from early spring to summer (Guerreiro, 1991; Verdelhos et al., 2005). Settlement can be prevented by environmental conditions such as low salinity, coarser sediment, high temperature and low rate of dissolved oxygen. Increased post-settlement mortality is also commonly observed as spat usually lives within 5 cm of the mud surface due to their reduced siphon size, which makes them more vulnerable to extreme temperatures, wave action and predation (Casagranda and Boudouresque, 2005; Hughes, 1970; Ruiz et al., 1994). Moreover, consumption of settling larvae and spat by adults can also occur (Green, 1957; Hughes, 1970). The very long inhalant siphon of up to 28 cm in *S. plana* (Green, 1967), and the consequent large feeding area, could potentially lead to greater than normal recruitment impediment by resident adults.

Table 2.2. Timing of spawning periods of several populations of *Scrobicularia plana*, ordered along a latitudinal gradient from north to south († beginning of the period; * timing of the peak).

	Winter			Spring			Summer			Autumn			
Location	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Reference**
Wadden Sea, The Netherlands								▨ †					Zwarts 1991
Conway Bay, Wales							▨	▨					Hughes 1971
Menai Strait, Wales						▨		▨					Frenkiel & Mouëza 1979
Mweeloon Bay, Ireland					▨	▨	▨	▨	▨				Raleigh & Keegan 2006
Avon estuary, England						▨ †							Langston et al. 2007
Cornwall, England							▨	▨	▨				Worrall et al. 1983
Seine, Loire and Bourgeuf, France					▨	▨	▨						Mouneyrac et al. 2008
Gironde estuary, France		▨	▨	▨		▨	▨	▨	▨				Bachelet 1981
Arcachon Basin, France	▨	▨			▨	▨							Bachelet 1981
Bidasoa estuary, Spain						▨	▨ *	▨ *	▨				Sola 1997
Tagus estuary, Portugal			▨	▨		▨	▨	▨	▨				Paes-da-Franca 1956
				▨	▨	▨	▨	▨	▨				Guerreiro 1991
Mira estuary, Portugal				▨	▨	▨	▨	▨	▨				Guerreiro 1991
Cádiz, Spain			▨	▨	▨ *	▨	▨ *	▨	▨				Rodríguez-Rua et al. 2003
Bou Regreg estuary, Morocco								▨	▨	▨	▨		Elkaïm 1976
Four Atlantic estuaries, Morocco			▨					▨	▨				Cheggour et al. 2005

Table 2.3. Timing of settlement periods of several populations of *Scrobicularia plana*, ordered along a latitudinal gradient from north to south.

	Winter			Spring			Summer			Autumn			
Location	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Reference**
Liverpool, England						▨	▨						Stopford 1951
Lynher estuary, England				▨									Warwick & Price 1975
Gironde estuary, France				▨	▨	▨	▨	▨	▨				Bachelet 1981
Arcachon Basin, France				▨	▨	▨	▨	▨	▨				Bachelet 1981
Prévost, France										▨			Guelorget & Mazoyer-Mayére 1983
Bidasoa estuary, Spain						▨	▨			▨	▨	▨	Sola 1997
Mondego estuary, Portugal	▨	▨	▨	▨	▨								Verdelhos 2005
Tagus estuary, Portugal								▨	▨	▨	▨	▨	Silva et al. 2006
				▨	▨			▨	▨				Guerreiro 1998
Mira estuary, Portugal					▨				▨	▨			Guerreiro 1998
Lake Ichkeul,Tunisia				▨	▨			▨	▨				Casagranda & Boudouresque 2005
Bou Regreg estuary, Morocco							▨	▨	▨	▨	▨	▨	Cheggour 1989
Four Atlantic estuaries, Morocco				▨	▨	▨				▨	▨	▨	Cheggour et al. 2005
l’Oued Souss, Morocco			▨	▨				▨	▨				Bergayou et al. 2009

Recruitment

Recruitment is expected to reflect settlement but only when post-settlement mortality is density-independent (Connell, 1985) which may not be the case for *S. plana* as inhibition of recruitment by adults is a density-dependent process. Intraspecific competition for food and space results in unsuccessful recruitment with cohorts from the years of successful recruitment dominating the population, i.e. the “dominant-age-class” phenomenon (Guerreiro, 1998). Successful recruitment is an irregular event and increased densities do not last longer than 2-3 years (Essink et al., 1991). Recruitment failures are common after severe winters and recruitment may even be absent for several years, particularly in northern areas (Essink et al., 1991; Zwarts and Wanink, 1993). Generally, for winter sensitive species, subtidal populations may assure repopulation of the intertidal habitat, however *S. plana* is not very common in subtidal areas (Dekker, 1989; Essink et al., 1991).

In species with long longevity, such as *S. plana* which is able to reach 18 years (Green, 1957), regular yearly recruitment may not be essential for the population’s maintenance. In addition, the protracted spawning of southern populations (Elkaïm, 1976; Guerreiro, 1991; Rodriguez-Rua et al., 2003; Sola, 1997), may contribute tomore stable adult populations since the effects of unsuccessful settlement events are likely reduced (Seed and Brown, 1977).

Post-settlement migration in *S. plana* can occur but is uncommon (Hughes, 1970). A massive arrival of juveniles (size > 10 mm) in search of an optimal site for development was observed in France (Bouchet et al., 2005). Also, migration of large numbers of one-year-old *S. plana* individuals has been observed in the northern coast of Tunisia, as a response to unfavorable conditions (Casagranda and Boudouresque, 2005).

Juvenile and adult stages

Feeding and growth

Once settled, *S. plana* can act both as deposit feeders, collecting food by siphoning sediment surrounding their burrow during emersion periods, and as suspension feeders filtering suspended matter from the water column during immersion periods. However, they primarily behave as deposit feeders due to the long emersion periods, as they are confined to upper shore levels of intertidal mudflats (Bocher et al., 2007; Freeman and Rigler, 1957; Hughes, 1970; Orvain, 2005; Orvain et al., 2007). When filter-feeding, *S. plana* ingests a mix of sedimenting phytoplankton and particulates, as well as resuspended sedimentary material (Hughes, 1969), while bacterial populations constitute the basis of the diet when deposit-feeding (Lopez and Levinton, 1987). Due to the extension of its large siphons when feeding resulting in large grazing areas, interspecific competition may be particularly important.

A latitudinal trend of higher growth with decreasing latitude has been suggested (Bachelet, 1981; Sola, 1997). The analysis of growth rates for several populations shows indeed a general inverse trend with latitude (Table 2.4). Some exceptions occur, namely the low growth rates in some Portuguese estuaries, close to values for northern Europe populations. In southern latitudes not only a faster growth but also higher density of individuals of smaller size and a shorter life span are usually observed (Coelho et al., 2006; Sola, 1997; Verdelhos et al., 2005). However, Bocher et al. (2007) detected an absence of a clear latitudinal pattern in average sizes and age-composition of *S. plana* populations, suggesting that growth rates and lifespan mainly differ according to local characteristics. In addition, the absence of a latitudinal gradient in winter weight was also observed (Zwarts, 1991) possibly due to higher food availability/consumption during winter in southern areas which can counterbalance the higher costs of living. Therefore, *S. plana* might show maximum growth rates in intermediate latitudes as has been suggested for *M. balthica* along European coasts (Beukema and Meehan, 1985).

Growth rates also differ within locations and are usually higher during spring and summer (Sola, 1997; Verdelhos et al., 2005; Wanink and Zwarts, 2001). When growth slows down during winter, *S. plana* produces quite well defined rings. These rings in principle allow the study of age structure and population dynamics but, as individuals grow older, the earlier rings become less visible and in some winters hardly any ring is laid down at all. Also, as with most bivalves, if *S. plana* is disturbed during the growth season, a “disturbance” ring is formed that is indistinguishable from the true winter rings. The problem of age determination is more complicated in southern regions, where winter conditions are not as marked and do not cause a sufficient decrease in metabolism to form clearly visible winter rings (Hughes, 1970; Sola, 1997).

Table 2.4. Growth rate constants (K value) for several populations of *Scrobicularia plana* along a latitudinal gradient.

Location	**K value**	**Author**
Conway Bay, Wales	0.194	Hughes, 1970
Gwendraeth estuary, Wales	0.139	Green, 1957
Lynher estuary, England	0.07	Warwick and Price, 1975
Le Guillec estuary , France	0.347	Chassé, 1972 (in Bachelet 1981)
Croisic Bay, France	0.32	Chassé, 1972 (in Bachelet 1981)
Bourgneuf Bay, France	0.38	Chassé, 1972 (in Bachelet 1981)
Gironde estuary, France	0.625	Bachelet, 1981
Arcachon Bay, France	0.322	Bachelet, 1981
Bidasoa estuary, Spain	0.815	Sola, 1997
Mira estuary, Portugal	0.07-0.54	Guerreiro, 1998
Tagus estuary, Portugal	0.15	Guerreiro, 1998

Ecophysiology and ecological constraints

The species has been found in locations with variable temperature regimes, with mean water temperatures ranging from 6°C in north Wales (Hughes, 1971) to 27-28°C in more southern areas (Bazaïri et al., 2003; Casagranda and Boudouresque, 2005; Rodriguez-Rua et al., 2003; Sola, 1997; Sprung, 1993). Moreover, during periods of emersion *S. plana* needs to cope with an even larger range of temperatures. Salinity can also vary, with salinities ranging from 11 (Green, 1957) to 34.5 PPM (Freeman and Rigler, 1957). Being more tolerant to low salinities than most common estuarine bivalves, *S. plana* can be found further upstream than species like *M. balthica*, *M. edulis* and *C. edule* (Bryan and Hummerstone, 1977; Spooner and Moore, 1940). Over the last years, an unexplained decline or even disappearance of populations of *S. plana* has been reported (Essink et al., 1991; Langston et al., 1987). Factors such as low winter temperatures, sediment instability, hydrological conditions, predation, pollution and parasites have been suggested as causes (Essink et al., 1991; Ruiz et al., 1994; Thiel and Zubillaga, 1997). The main predator of adult *S. plana* is the oystercatcher Haematopus ostralegus, although several other bird species also feed on it (Hughes, 1970; Moreira, 1997). Oystercatchers can be an important source of mortality, consuming about 10% of resident populations (Hughes, 1970). Parasites may also be a reason for the observed decline in densities. Several meiogymnophallid trematodes are known to use *S. plana* as first intermediate host (Bowers et al., 1990; Russell-Pinto et al., 1996; Thieltges et al., 2006). These parasites can infect the gonad tissues of *S. plana* causing lysis of the tissues and inducing castration (Russell-Pinto et al., 1996), ultimately removing the infected individuals from the reproductive population. Pollution is a factor that can cause recruitment failures. Moderate TBT levels, commonly found in waters of a number of European estuaries and coastal areas during the 1980s, prevented the successful and timely progress of the veliger larvae of *S. plana* (Ruiz et al., 1995). Since *S. plana* can live so long, yearly successful recruitment may be unnecessary. However, production of large amounts of larvae or larvae with high survival rates would be required (Essink et al., 1991).

Discussion

Several theories have been proposed about population dynamics and spatial aspects of the distribution of individuals in relation to its ecological and habitat characteristics. The member/vagrant hypothesis (Sinclair, 1988) is built on the premise that "populations can only exist in those geographic locations within which there can be continuity in the life cycle". Populations are discrete units and at any stage of the life cycle an individual can become separated from its population (vagrants) either by spatial (losses from distributional areas, inability to find a genetically sufficiently similar mate) or by energetic (predation, starvation, disease) processes. In contrast, the metapopulation theory recognizes the importance of vagrants, or more precisely migrants, for the survival, persistence and expansion of the metapopulation (McQuinn, 1997). Local populations are largely independent but interconnected by migration, a concept particularly important for species living in patchy environments (Hanski, 1999). Despite the differences both models agree that spatial distribution patterns reflect the relation between the environment and the species' ecological requirements.

Since the majority of natural environments are patchy and environmental heterogeneity tends to cause aggregated distributions, most populations are to some extent aggregated in their spatial pattern (Heip, 1975; Levinton, 1972). Patchiness can occur on different temporal and spatial scales (De Wolf, 1989; Thrush et al., 1989; Ysebaert and Herman, 2002) and often varies during different stages of an organism's life cycle (e.g. Armonies, 1996; Flach and Beukema, 1994; Haubois et al., 2004). Consequently, at different stages it may be determined by different biological and physical factors (e.g. Foldvik et al., 2010; Maynou et al., 2006) that can affect both pre- and post-settlement processes (Ólafsson et al., 1994; Strasser et al., 1999).

One of these processes is protracted spawning. The degree to which spawning is synchronised and the time window over which spawning is stretched (trickle spawning vs. batch spawning) influences the number of and extent to which populations are supplied with larvae yearly and, hence, spatial recruitment patterns (McQuaid and Phillips, 2006). A long spawning period suggests a bet hedging strategy, which means that a large degree of randomness is associated with the chances of successful settlement of a batch of larvae. Trickle spawning might lead to small batches of larvae and to small patches of recruits and, thus, to patchiness. Spawning periods of *S. plana* can be in fact extremely long but patchiness is more marked in more northern locations where spawning periods actually tend to be shorter.

Pelagic larval duration is also a very important process in shaping species distribution patterns (e.g. Johnson et al., 2001; Norkko et al., 2001; Palmer et al., 1996). A short or absent planktonic larval stage tends to lead to aggregated distributions as a result of low mobility (Johannesson, 1988) and usually results in a smaller geographic range (Lester et al., 2007; Mileikovsky, 1971). In turn a long pelagic stage allows dispersal over broad geographic areas since larvae can be transported for the majority of their development (Scheltema, 1986; Scheltema and Williams, 1983; Strathmann, 1985). The long pelagic stage of *S. plana* implies high mobility which suggests that patchiness in this species is not related with limited larval dispersal.

After attaining the settling stage, settlement of juvenile *S. plana* occurs in areas with appropriate characteristics (Frenkiel and Mouëza, 1979). If a given area was uniformly suitable for settlement, and even if adults influence recruitment success and the distribution of settlers, a random distribution would be expected over time due to adults themselves being randomly distributed (Hughes, 1970). The aggregated distributions observed for *S. plana* imply that settlement areas may not be homogeneous. Furthermore, if annual spat settlement is a random process and successful every year, patches composed of single age classes would be expected due to dispersal of larvae and random settlement. Spatial autocorrelation in age structure, meaning that spatially close sites tend to exhibit more similar proportions of different cohorts, has in fact been observed for several species (David et al., 1997; Lindegarth et al., 1995). However, for *S. plana*, such age composition is generally not observed, with several age classes being found in the patches (Green, 1957; Hughes, 1970; Guerreiro, 1998; Verdelhos et al., 2005; pers. obs.). This suggests that successful settlement is a spatially non-random process but determined by specific environmental or biological factors which may result in an aggregated distribution (e.g. Butman, 1987; Zimmer et al., 2008).

For organisms with planktonic larvae, an important mechanism determining spatial patterns is habitat selection which, in sessile or sedentary organisms, occurs during larval settlement. In a study with marine gastropods, larvae settled gregariously as a result of selective settlement, with juveniles that colonized suboptimal habitats showing enhanced mortality, a risk associated to random settlement (McGee and Targett, 1989). Environmental factors are then expected to determine settlement success which in turn affects at least to some extent, the spatial structure of *S. plana*. While conditions such as insufficient immersion time, coarse sediment and low salinity are believed to prevent successful settlement and growth of this species, environmental factors such as higher water flow and turbidity tend to result in high densities (Hughes, 1970). Sediment composition has also been suggested to be involved as a direct or indirect determinant of variation in patchiness between areas (Gage and Geekie, 1973). Although Hughes (1970) found no correlation between the overall distribution and substrate type, a more recent study showed that sediment was a significant term predicting occurrence of *S. plana* in several systems (Compton et al., 2009).

Given the importance of physical and biological parameters as determinants of settlement success, the high tolerance of *S. plana* to environmental changes could favour the species' distribution. This species is more tolerant to environmental conditions than most bivalves occupying a similar niche which may allow it to occupy areas that are uninhabitable for other species while being driven out of less extreme environments through interspecific competition. Such effect would result in an aggregated distribution. However, co-occurrence of *S. plana* and other bivalves such as *C. edule*, *M. balthica* and *M. arenaria* is commonly observed (e.g. Bocher et al., 2007; Compton et al., 2009; Zwarts, 1991) suggesting that it may not be the case. A better understanding of species associations of *S. plana*, not only with bivalves but other groups such as crustaceans and polychaetes is required since these may also affect recruitment and consequently species spatial distribution (Thrush, 1991; van der Veer et al., 1998).

Regarding post-settlement processes, one aspect that influences spatial structure is feeding mode. Suspension feeders may exhibit either aggregated or random distribution patterns, while deposit feeders tend to be more uniformly distributed due to competition and territoriality (Hewitt et al., 1996; Levinton, 1972). The fact that *S. plana* acts mainly as a deposit-feeder, implies that feeding mode does not seem to be responsible for the species patchy distribution. Nevertheless, while deposit-feeding, adults will inevitably consume some larvae which will reduce the probability of successful recruitment within range of the inhalant siphon (André and Rosenberg, 1991; Green, 1957; Ólafsson et al., 1994). The long inhalant siphon of *S. plana* will likely result in a large feeding area and, consequently, it may lead to a strong recruitment impediment by resident adults. However, this hypothesis is not supported by a study in several European tidal flats (Compton et al., 2009) that observed matching distributions of juvenile and adult *S. plana*. This suggests that either juveniles actively settle in the proximity of the adults or that settlement success is lower away from the adults. Other factors, however, like low temperatures, wave action or predation can also result in increased spat mortality (Hughes, 1970), affecting recruitment success and consequently the species spatial distribution.

Finally, dispersal of juveniles and adults (e.g. Beukema and De Vlas, 1989; Commito et al., 1995; Cummings et al., 1995; Hewitt et al., 1997; Poulton et al., 2004) and predation (e.g. Günther, 1992; Jensen and Jensen, 1985; Reise, 1985) have also been suggested to be involved in determining spatial patterns of populations. Since the optimal areas for settlement are not always the most adequate for subsequent survival (Bertness and Grosholz, 1985; Holme, 1949), redistribution of juveniles and/or adults is sometimes necessary. Horizontal migration does occur in *S. plana* (Bouchet et al., 2005; Casagranda and Boudouresque, 2005) but only rarely, so post-settlement migration does not seem to be an important factor defining the species spatial structure. Spatial variation in predation can also affect prey distribution (Sutherland, 1996). Several bird species feed on *S. plana* (Hughes, 1970; Moreira, 1997) and the general patchy distribution of shorebirds, concentrating their feeding efforts on particularly food-rich areas, could explain the patchy structure of *S. plana* (Jensen and Mouritsen, 1992). However, according to Schneider (1992), predators with high mobility, such as birds, actually tend to decrease patchiness. Therefore, it is not likely that predation of adult *S. plana* is responsible for the patchy patterns observed.

Conclusion

Analysis of spatial patterns is essential to understand the structure and dynamics of populations. Changes in spatial distributions, e.g. along environmental gradients, can provide insight in a species' ecological processes as well as its responses to environmental stress (Thrush et al., 1989). Several pre- and post-settlement processes are believed to influence spatial patterns such as method of spawning, pelagic larval stage duration, settlement success, feeding mode, dispersal of juveniles/adults and predation. For *S. plana*, most of these factors were not able to explain the species' patchiness which seems to be determined mainly by the existence of specific environmental conditions at the time of settlement. Since no environmental factor seems to fully explain the species distribution, a combination of multiple factors is likely needed with factors such as temperature, salinity, sediment type, hydrographic conditions and predation, playing an important role in settlement success and spat survival. Nevertheless, the relationship between the spatial pattern of *S. plana* and the processes that determine it are still not fully understood and further research is necessary.

Future research

Several gaps in basic knowledge can be identified from this review. First and foremost, the present general notion that *S. plana* has a patchy distribution needs to be further quantified; at what spatial scale, at which life stages does patchiness occur and to what extent? In addition, the question whether a relationship between latitude and patchiness exists should be addressed. This would require (1) hierarchical samples of recruits and adults for autocorrelation analysis at various latitudes and (2) long term monitoring of populations and the space around those populations. Long term data have been collected before (Bocher et al., 2007; Essink et al., 1991; Sola, 1997; Wanink and Zwarts, 2001; Zwarts, 1991; Zwarts and Wanink, 1993) but have not been analysed in terms of patchiness; in addition, hierarchical (re)sampling of a number of populations in different geographical areas will be essential. Moreover, spatial sampling to determine species associations as well as differences in microhabitat between occupied and empty locations will give valuable information about the biotic and/or abiotic factors that may be determining the species distribution.

A methodological point of consideration is the need for improved ageing methods, since the study of cohorts and failed recruitment requires accurate age estimation. This can be done by studying the relationship between the shell surface rings, the stable isotope composition of the aragonite in the shell and the seasonal cycles of seawater temperature (Cardoso et al., 2007b; Lartaud et al., 2010).

Finally, the spatial structure of a species also determines connectivity, defining the number of different populations able to persist in a given geographic area (Sinclair, 1988). Connectivity between different *S. plana* populations can be assessed by analysing the population genetic structure, which in turn gives an indication of the level of population dynamical buffering through interchange of recruitment between populations. No such studies have been done and the only study available on intrapopulation variation in *S. plana* (Skibinski et al., 1978), which described variability in seventeen allozyme loci, showed high variation. The results were congruent with the high potential for gene flow during the larval phase, large sensus population sizes and obligate sexuality of the species. In addition, *S. plana* is an interesting case for comparative phylogeography in light of its patchy distribution, which makes it a likely candidate for self recruitment and low levels of inferred migration rates (Cruzeiro, 2009).

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Chapter 3

Seasonal variability in somatic and reproductive investment of the bivalve *Scrobicularia plana* (da Costa, 1778) along a latitudinal gradient

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Abstract

Monthly investment in soma and gonads in the bivalve *Scrobicularia plana* is described for three populations along its distributional range: Minho estuary, Portugal; Westerschelde estuary, The Netherlands and Buvika estuary, Norway. Seasonal cycles in body mass (BMI), somatic mass (SMI) and gonadal mass (GMI) indices were observed for all populations. In Portugal, BMI and SMI peaked in mid-autumn, while in The Netherlands both indices were at their highest in mid-spring. Norway showed a different pattern with two distinct peaks: one in mid-autumn and a second peak in spring. GMI reached maximum values in July in Portugal and Netherlands and in June in Norway. Overall, mean BMI and SMI were lower in Portugal while mean GMI was lower in Norway. The spawning period lasted the whole summer in Portugal, but was shorter (only two months) in The Netherlands and Norway. The reproductive investment in The Netherlands was significantly higher than in Portugal and Norway, with the lowest values being observed in Norway. Differences in annual cycles between populations were attributed to environmental factors, namely temperature and food availability. Temperature seems important in shaping the reproductive pattern with more northern populations showing shorter reproductive periods starting later in the year, and a lower reproductive output. In addition, winter water temperatures can explain the lower mean body and somatic mass values observed in Portugal. Food availability influenced the physiological performance of the species with peaks in somatic mass coinciding with phytoplankton blooms. This relation between physiological performance and environmental factors influences *S. plana* distribution, densities and even survival, with natural consequences on its commercial importance.

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Introduction

Bivalves are a main component of the intertidal benthic faunal communities, with top-down effects on sediment characteristics and primary producers, and bottom-up effects on a variety of invertebrate, fish and shorebird predators (Piersma and Beukema, 1993; Raffaelli and Hawkins, 1996; Seitz and Lipcius, 2001; Tulp et al., 2010). They can be found in a variety of habitats where they are exposed to temporal and spatial differences in the environmental conditions, influencing physiological processes in these organisms. Two of the main abiotic factors are temperature and food availability, affecting physiological rates (Clarke, 1987; Sprung, 1991; Masilamoni et al., 2002) and reproduction (MacDonald and Thompson, 1986; De Goeij and Honkoop, 2003; Burke et al., 2008). Other factors such as salinity, water flow, immersion time and sediment type seem to be more important at a local scale (Warwick et al.,1991; Dame, 1996; Bocher et al., 2007). Therefore, it is expected that the general inverse trend of temperature and food availability with latitude (Thorson, 1950; Barry and Carleton, 2001; Pidwirny, 2006; Jansen et al., 2007), will be reflected in the physiological performance of bivalves.

Latitudinal trends in the physiological performance of marine invertebrates are commonly observed. Studies showed that egg and larval size, initial energy reserves of eggs and larvae and reproductive effort tend to increase with increasing latitude, and fecundity, age at first maturity and reproductive output (Clarke, 1987; Thatje et al., 2004; Ward and Hirst, 2007) as well as growth and mortality tend to decrease (Contreras and Jaramillo, 2003; Petracco et al., 2010). Some hypotheses are still under debate (e.g. Mileikovsky, 1971; Pearse et al., 1991; Arntz and Gili, 2001; Gallardo and Penchaszadeh, 2001; Laptikhovsky, 2005), such as "Thorson's rule", which suggests that the occurrence of species with pelagic planktotrophic larvae decreases with latitude due to increased food seasonality and lower temperatures in colder areas (Thorson, 1950). Therefore, knowledge of large-scale patterns of growth and reproduction in marine invertebrates and the factors determining them is essential for understanding spatial patterns of population dynamics and for management of commercially exploited species. This requires species with a large distributional range such as for instance the peppery furrow shell, *Scrobicularia plana*.

Scrobicularia plana is an important species in shallow water benthic communities (Keegan,1986) and commercially exploited in several European countries (Langston et al., 2007). It ranges from the Norwegian Sea in the north, along the Atlantic coast into the Mediterranean until Senegal (Tebble, 1976). *S. plana* usually remains confined to the upper intertidal (Hughes, 1970; Orvain et al., 2007), not being very common in subtidal areas (Dekker, 1989) and preferably inhabiting muddy sediments (Bocher et al., 2007). It burrows to a depth of 5-20 cm (Akberali and Davenport, 1981) and can act both as a deposit and as a suspension feeder, although it behaves mainly as a deposit-feeder when emersion periods are long (Hughes, 1970). The species has been found in salinities ranging from 11 (Green, 1957) to 34.5 PSU (Freeman and Rigler, 1957), suggesting a higher tolerance to low salinities than most common estuarine bivalves (Spooner and Moore, 1940). *S. plana* occurs in areas with water temperature regimes ranging from 6-15.5°C in North Wales (Hughes, 1971) to 13-28°C in Morocco (Bazaïri et al., 2003), and 10-27°C in the Mediterranean (Casagranda and Boudouresque, 2005). In the intertidal, organisms may need to cope with even a larger spatial and temporal variation in temperature (Harrison and Phizacklea, 1987).

Although studies on growth and reproduction are available for *Scrobicularia plana* (Paes-da-Franca, 1956; Hughes, 1971; Bachelet, 1981; Worrall et al., 1983; Zwarts, 1991; Sola, 1997; Guerreiro, 1998), none of these assessed the reproductive output in a quantitative way and in relation to somatic mass, which is essential to determine the allocation strategies of a population to growth and reproduction. Therefore, we studied growth and reproduction patterns of three populations along a latitudinal gradient, from Norway (Trondheim) to the north of Portugal (Caminha). The aim was to interrogate spatial and temporal variation in growth and reproduction by analyzing: (1) body and somatic mass cycles; (2) reproductive strategies (by assessing the seasonal patterns in gonadal mass) and (3) mass allocation to growth and reproduction (by following changes in somatic and gonadal cycles throughout the year).

Material & Methods

Field sampling

Scrobicularia plana was collected at three different locations along the European coast (Fig. 3.1): Minho estuary, Portugal (N41°52’46’’ , E08°50’14’’);Westerschelde estuary, The Netherlands (N51°21’01’’, E03°44’01’’); and Buvika estuary, Norway (N63°18’42’’, E10°10’12’’). Sites were exposed for 3-4 hours per day in Portugal and Norway and 7-8 hours per day in The Netherlands. Sampling took place once a month over a total period of 2 years: between April 2007 and May 2008 in Portugal, June 2008 and September 2009 in The Netherlands, and from April 2008 to April 2009 in Norway. At each sampling, around 50 individuals were randomly collected by hand at each location, in an area of 1-2 km^2. They were then transported to the laboratory the same day, stored overnight in seawater at 5°C and processed within the next 24 h.

At each location, sediment surface temperature was recorded every 15 min. during the sampling period with loggers (Stow-Away® TidbiT®). Since there was an overestimation of low water temperatures due to the intertidal location and structure of the logger, mean monthly temperatures were calculated using only high water data. In addition, long-term data series of sea surface water temperatures were obtained from subtidal areas in the vicinity of the sampling stations. Data were obtained for Portugal by the “CISL Research Data Archive” (via http://dss.ucar.edu/), for the Netherlands by “Rijkswaterstaat” (via http://www.waterbase.nl), and for Norway by J.-A. Sneli (pers. comm.).

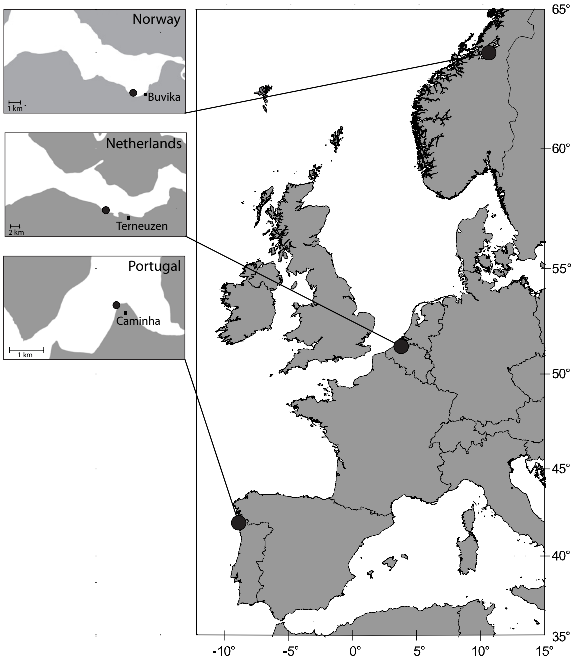

Fig. 3.1. Sampling locations of *Scrobicularia plana* along the European coast: Minho estuary, Portugal; Westerschelde estuary, The Netherlands; Buvika estuary, Norway.

Data analysis

For each specimen, shell length was measured to the nearest 0.01 mm with electronic callipers, after which bivalves were opened and reproductive tissue was separated from the somatic mass under a binocular microscope (10x). For both gonadal and somatic tissues, ash-free dry mass (AFDM) was determined to the nearest 0.01 mg, as the difference in dry and ash mass by first drying for 48 h at 60°C followed by incineration for 2 h at 580°C. In order to determine body condition, the body mass index (BMI) was calculated as the total body AFDM (soma + gonads) divided by cubic shell length. To follow the investment in somatic and gonadal mass, the somatic mass index (SMI) and the gonadal mass index (GMI) were estimated as the AFDM of the soma, or gonads respectively, divided by cubic shell length. Mass was divided by cubic shell length to allow comparison in terms of condition between animals of different sizes. If more than 50 individuals were collected, the extra animals were burnt as a whole and only information regarding BMI, and not SMI or GMI, would be available. The relative investment in reproduction compared to body mass was calculated as the gonadosomatic ratio (GSR), i.e. the gonadal AFDM divided by the total body AFDM.

ANOVA was performed to test for differences in seasonality of BMI, SMI and GMI between locations. Due to the lack of independence between samples from each month within location, monthly averages were used for all indices. Moreover, since no significant differences in the monthly averages of BMI, SMI and GMI were found between males and females during the year or between locations (ANOVA, all interaction terms Sex*Location, Sex*Month and Sex*Season for all indices with $p > 0.05$, not shown), data from both sexes were analyzed together. Due to an unbalanced design (sampling periods differed between locations) the effect of time could not be described as differences among all sampling months (i.e. by using sampling month as a categorical variable) since there would be loss of degrees of freedom. Therefore, a non-linear model was developed to fit the effect of time. For BMI and SMI, time was then expressed as a linear trend and combined with a sinusoidal seasonal effect: β_1*Month + β_2*Season, where season was described as $\sin(2\pi\ ((\text{Month} - \beta_3)/12))$. The parameters β_1, β_2 and β_3 refer to the direction and steepness of the seasonal trend, the amplitude of the seasonality, and the timing of the peak, respectively. Month was a continuous variable that ran from the first until the last sampling month. To correct for differences in sampling dates, the variable Month was calculated as a fraction, i.e. each month (1 unit) was divided by the number of days, that fraction was then multiplied by the sampling day and added to the value of the month. Since this model is only linear when β_3 is known beforehand, the model was run for all possible values of β_3 (i.e. the values 1-12), for each location and each index separately, and the models with the lowest residual mean squares (RSS) were selected. Given that different values of β_3 for each location adds to the complexity of the model, these models were then tested (F-test) for significance against a model that used the same value of β_3 for all locations. Since no significant differences were observed between models, the simpler model (only one value for β_3) was selected. For GMI, a similar analysis was performed although Season was not described as a sinusoidal function but as a block function which divided the year into two periods (that could differ between sites): months with low GMI (0) and with high GMI (1). Different combinations of months were tested (GLM), for each location, and the one with the lowest RSS was selected. Due to the fact that individuals with no gonadal development were present the whole year and normality could not be obtained even after transformation, this analysis of variance (GLM) was done using only individuals with gonadal development (zeroes excluded) after square root transformation. A second analysis was then necessary to test if animals without gonads showed similar trends and for that a binomial analysis of the presence and absence of gonadal development was performed using logistic regression.

All statistical analyses were performed with the software package SYSTAT 12 (Wilkinson, 1996).

Results

Temperature conditions

Seawater temperature patterns over the last 15 years showed some interannual variability (only statistically significant in The Netherlands) but with clear differences between locations (Fig. 3.2a). Winter temperatures were always higher in Portugal and similar at the other two locations, and summer temperatures were lower in Norway and similar in Portugal and The Netherlands. During the sampling period, the logger data followed the same trend (Fig. 3.2b). Portugal had a much higher winter temperature whereas the other two locations showed similar values. Norway had the lowest summer temperatures. In Minho estuary (Portugal), mean monthly temperature varied between 18.2°C in July and 12.5°C in January. In the Westerschelde estuary (The Netherlands), mean values varied between 2.4°C in February and 19.3°C in August, while in the Buvika estuary (Norway), mean temperatures were between 17.2°C in July and 3.5°C in December.

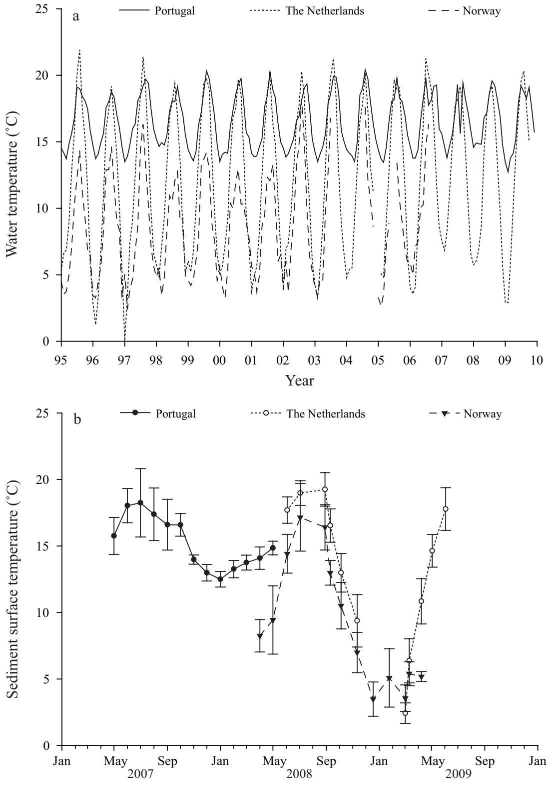

Fig. 3.2. Water temperatures at the three sampling locations. (a) Mean monthly temperature (°C) profiles from the last 15 years, obtained from online databases (http://dss.ucar.edu, http://www.waterbase.nl) and personal communication (by J.-A. Sneli). (b) Monthly sediment surface temperature (C, mean SD) during sampling period. Mean values were calculated based on daily temperatures at high tide.

Seasonal cycles

Seasonal patterns of BMI and SMI were observed (Fig. 3.3), but only SMI showed significant differences in amplitude of the seasonal pattern between locations (Table 3.1). For both indices, no significant differences were found in timing of seasonality between locations. Parameters of the seasonal effect (β_1*Month + β_2*Season) estimated for the different populations are given in Table 3.2. In Portugal, both BMI and SMI reached maximum values in November and minimum values in April 2008 (Fig. 3.3a and b). For The Netherlands, the BMI and SMI cycles showed a clearer seasonality in 2009 with indices increasing for BMI from March to May (Fig. 3.3d), and for SMI to the end of April (Fig. 3.3e). In Norway, BMI was lowest between May and August 2008 and highest in November, although it decreased again to a minimum in January 2009 (Fig. 3.3g). SMI showed lowest values in June 2008, followed by an increase that peaked in November 2008, decreasing again till January 2009 (Fig. 3.3h). On average, BMI and SMI were lower in Portugal than at the two other locations.

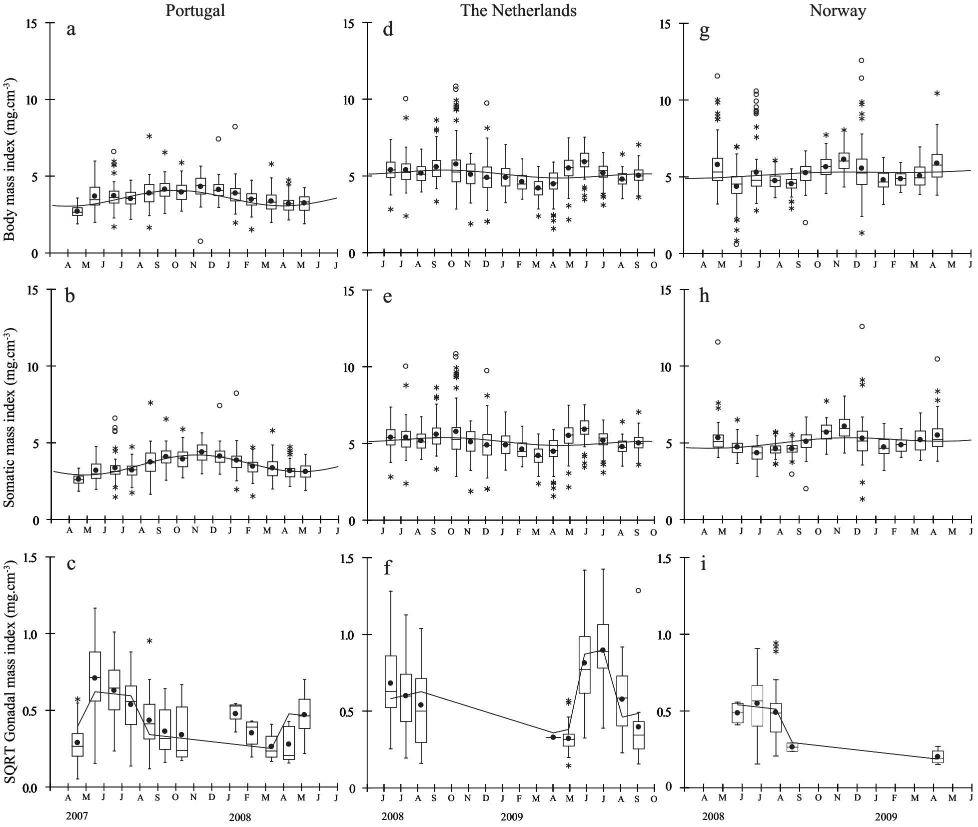

Fig. 3.3. Body mass index (mg.cm^{-3}), somatic mass index (mg.cm^{-3}) and gonadal mass index (mg.cm^{-3}) of *S. plana* along the year, for the three sampling locations. Gonadal mass index values are square root transformed. Curves and lines are model (β_1*Month + β_2*Season) predictions. Full dots and horizontal bars indicate mean and median values, respectively; boxes represent the range within which the central 50% of the values fall; bars correspond to the data range excluding outliers; outliers and extreme values are, respectively, observations more than 1.5 and 3 times the box range and are represent by asterisks and open circles.

Seasonal cycles of GMI were also observed for all populations (Fig. 3.3c, f, and i). In Portugal and the Netherlands, about 100% of the individuals developed gonads, while in Norway the highest percentage was only about 50%. Significant differences were detected between locations, for animals that developed gonads, for both amplitude and timing of seasonality (Table 3.1). In Portugal, GMI was highest in May (Fig. 3.3c), while in The Netherlands and in Norway the peak was in June (Fig. 3.3f and i). On average, GMI was highest in The Netherlands and lowest in Norway. A similar trend was observed in animals without gonads. Sexual development was observed in individuals >14.8 mm in the Netherlands, > 17.4 mm in Norway and > 25.0 mm in Portugal. For all locations, the percentage of animals that developed gonads was considerably higher in spring/summer, being zero or close to zero for most of the autumn/winter period, in The Netherlands and Norway (Fig. 3.4).

Table 3.1. Analysis of variance for comparison of body, somatic and gonadal mass indices of *S. plana* between locations. Month was a continuous variable that runs from the first until the last sampling month; Season was described as the function sin $(2\pi((\text{Month} - \beta_3)/12))$. Probabilities are expressed as: ***p< 0.001; **p<0.01; *p<0.05; ns - not significant. To obtain a final model, non-significant factors for BMI and SMI were dropped one at a time until all factors were significant. The final model had the same significant factors as the original model for both BMI (Location: SS = 20.466, *F*-ratio = 10.233, *p* = 0.000; Season: SS = 1.423, *F*-ratio = 6.922, *p* = 0.012) and SMI (Location: SS = 17.535, *F*-ratio = 65.892, *p* = 0.000; Season: SS = 1.817, *F*-ratio = 13.653, *p* = 0.001; Location*Season: SS = 1.193, *F*-ratio = 4.482, *p* = 0.018).

Effect	SS	df	*F*-ratio
Body Mass Index			
Location	2.082	2	5.081*
Month	0.074	1	0.363^{ns}
Season	1.397	1	6.819*
Location*Month	0.371	2	0.905^{ns}
Location*Season	0.655	2	1.598^{ns}
Error	6.966	34	
Somatic Mass Index			
Location	1.841	2	6.976**
Month	0.086	1	0.65ns
Season	1.513	1	11.468**
Location*Month	0.42	2	1.592^{ns}
Location*Season	1.302	2	4.934*
Error	4.486	34	
Gonadal Mass Index (SQRT-transformation)			
Location	0.286	2	11.951**
Month	0	1	0.002^{ns}
Season	0.733	1	61.294***
Location*Month	0.475	2	19.836***
Location*Season	0.093	2	3.883*
Error	0.203	17	

Table 3.2. Estimated parameters for the model β_1*Month + β_2*Season, where Season = sin (2π((Month - β_3)/12)), for body mass index, somatic mass index and gonadal mass index (square root transformed) for three populations of *S. plana*.

	Constant	**β_1**	**β_2**	**β_3**
Portugal				
BMI	3.548	0.020	0.509	4
SMI	3.464	0.018	5.960	5
GMI (SQRT)	0.375	0.035	0.302	(-)
The Netherlands				
BMI	5.563	-0.020	0.190	4
SMI	5.360	-0.021	0.001	5
GMI (SQRT)	-0.655	0.031	0.554	(-)
Norway				
BMI	4.568	0.033	0.087	4
SMI	4.344	0.038	0.217	5
GMI (SQRT)	0.038	-0.002	0.362	(-)

Timing of spawning and reproductive investment

Spawning was considered to start when a decrease in GMI was observed. In Portugal, two spawning periods occurred. A first stronger peak lasted from May until October while a later and weaker peak occurred in January. In The Netherlands, spawning started in July and by September only a very small percentage of animals with gonads was observed. In Norway, spawning occurred during a short period between June and August (Fig. 3.3c, f and i). The overall reproductive investment (Fig. 3.5), just before the spawning period (when GMI was at its highest), was significantly higher in The Netherlands than in Portugal or Norway, with no significant differences being found between these two locations (not shown).

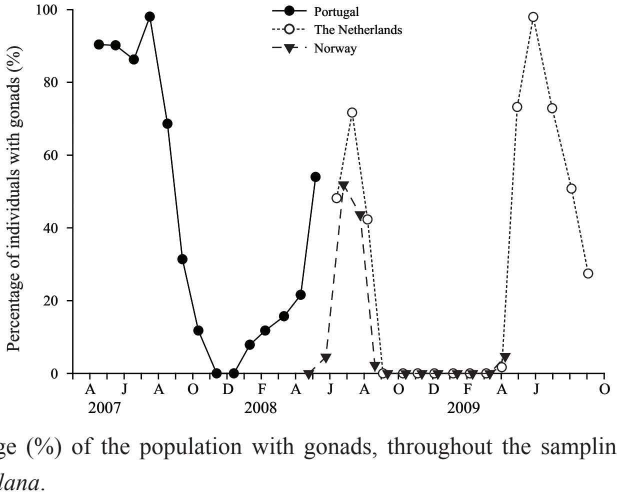

Fig. 3.4. Percentage (%) of the population with gonads, throughout the sampling period, for three populations of *S. plana*.

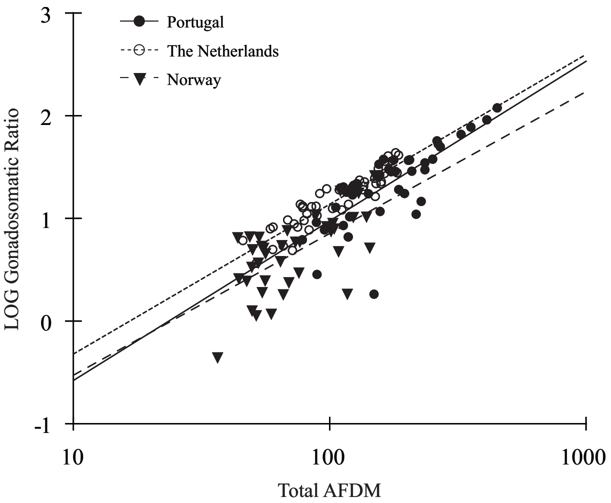

Fig. 3.5. Gonadosomatic ratio (-) plotted against total mass (mg AFDM), for the month when GMI was at its highest, with fitted regression lines for three populations of *S. plana*. Gonadosomatic ratio and total mass data were log transformed. Values shown in the x-axis were back transformed.

Discussion

Body and somatic mass cycles

Seasonal variations in bivalve body mass are usually observed in temperate waters, with a general pattern of increases in late spring and early summer followed by losses in autumn/ winter (e.g. Zwarts, 1991; Honkoop and Beukema, 1997; Cardoso et al., 2007a,b, 2009). For *Scrobicularia plana*, such a pattern has been described in populations from Spain, The Netherlands and Portugal (Sola, 1997; Wanink and Zwarts, 2001; Verdelhos et al., 2005) and a similar pattern has been found in the Dutch *S. plana* population studied here. In most animal populations, the annual energy intake appears to be limited primarily by food availability (Clarke,1987) suggesting that food is the most important factor controlling growth in marine invertebrates. As a consequence, the observed peak in body mass is likely associated with the phytoplankton spring bloom occurring around April/May (Forster et al., 2006). Although *S. plana* is mainly a deposit-feeder, data on phytoplankton blooms are a good indication of food availability due to the sedimentation of organic matter from the pelagic to the benthic system during the blooms (Graf et al., 1982; Smetacek, 1985). For the Portuguese population, however, body and somatic mass peaked in mid-autumn, with minimum values in spring which can be explained by the presence of food during most of the growing season (Schmidt et al., 2010). Food seasonality will increase with latitude, resulting in a restricted period of growth in colder areas (Thorson, 1950; Clarke, 1987). This explains the pattern observed for the Norwegian population with two peaks in body mass, in spring and autumn, simultaneously with the phytoplankton blooms in the area (Sakshaug and Mykiestad, 1973; Tangen and Arff, 2003).

Body mass, temperature and metabolism are closely coupled in marine invertebrates, as demonstrated, for example, in crustaceans (Ikeda et al., 2001) and echinoderms (Brockington and Clarke, 2001). As a response to temperature conditions, different compensatory responses may occur at the metabolic level (Newell, 1969). As a result of higher metabolic rates with increasing water temperatures an increase in the rate of mass loss (Zwarts,1991) and in the period of loss (Honkoop and Beukema, 1997) can occur. The overall higher water temperatures observed in Portugal, especially during the winter months, may therefore explain the lower mean body and somatic mass values observed in the beginning of the growing season at this location supporting the idea that species living in colder areas start growing at a better body condition than conspecifics from warmer areas (Honkoop and Beukema, 1997). Due to a lower basal metabolic rate in cold water, less maintenance energy will be required, so a relatively greater proportion of energy can be directed to growth resulting in higher remaining body mass (Clarke, 1987; Honkoop and Beukema, 1997).

Reproductive strategies

Temperature is known to be one of the most important physical factors mediating the life history strategies of aquatic organisms with effects not only on growth but also on reproductive cycles (e.g. Ram et al., 1996; De Goeij and Honkoop, 2003; Lawrence and Soame, 2004). Latitudinal differences in temperature have been suggested as the cause of the differences in the reproductive cycle of *Scrobicularia plana* along its distributional range (Hughes, 1970; Bachelet, 1981; Guerreiro, 1998) and a latitudinal trend was also observed in our study. A minimum threshold temperature is usually necessary to trigger the different reproductive stages of marine invertebrates, e.g. polychaetes (Watson et al., 2000), echinoderms (Nunes and Jangoux, 2004) and bivalves (Ram et al., 1996; Verween et al., 2009). Previous studies with *S. plana* suggested indeed that temperature is an important environmental factor in determining timing and duration of reproductive stages in this species (Worrall et al., 1983; Worrall and Widdows, 1983; Rodrígúez-Rua et al., 2003; Raleigh and Keegan, 2006). Therefore, earlier gametogenesis/spawning are expected in warmer areas. In Portugal, gametogenesis in January and spawning in May is expected to be related to the relatively high temperatures at those times of the year, respectively 12°C and 15-16°C. Similar temperatures (Paes-da-Franca, 1956; Worrall et al., 1983; Rodrígúez-Rua et al., 2003) and reproductive patterns (Paes-da-Franca, 1956; Raleigh and Keegan, 2006; Mouneyrac et al., 2008) have been observed in other populations. However, the importance of food availability in the reproductive cycle of *S. plana* cannot be overlooked (Worrall et al., 1983). Although temperature increase may initiate gametogenesis, it occurs only if sufficient energy reserves have been built up or if an adequate food supply is present as shown for bivalves (MacDonald and Thompson, 1986; Darriba et al., 2005) and echinoderms (Garrido and Barber, 2001). Therefore, the increased seasonality of food availability with latitude tends to limit growth and reproduction of marine invertebrates to summer, when food is available (Thorson, 1950; Clarke, 1987). As a result, in more northern populations, spawning periods of *S. plana* tend to be shorter and occur in mid-summer (Hughes, 1971; Worrall et al., 1983; Zwarts, 1991). In our study we did observe such a pattern with longer gametogenesis and spawning periods in Portugal, while for the two more northern locations, animals were in repose all autumn/winter. Furthermore, a second but smaller spawning period seems to have occurred at the beginning of the year in Portugal, when temperatures were around 12°C, although in a very small number of individuals only. This second spawning period of the population is commonly detected in southern populations of *S. plana* (Paes-da-Franca,1956; Bachelet,1981; Cheggour et al., 2005), and is likely linked to the extended reproductive period in the area as a result of higher water temperatures (Hughes, 1971; Bachelet, 1981).

In The Netherlands and Norway, gametogenesis and spawning started later in the year and in general at lower temperatures than what was observed for Portugal. In The Netherlands, gametogenesis and spawning started when temperatures were around 11°C and 20-21°C (Ysebaert, pers. comm.) and in Norway at 9°C and 14.4°C, respectively. A temperature of 9°C at the beginning of gametogenesis was also observed in a population from Ireland (Raleigh and Keegan, 2006). These differences in temperatures suggest that temperature is not a fixed parameter for *Scrobicularia plana* which is in disagreement with Orton's (1920) suggestion that the temperature threshold for spawning is a physiological constant of marine species. Other studies with bivalves (Korringa, 1957; Sastry, 1970) also observed that latitudinally separated populations reproduced at different temperatures. Size at first reproduction also varied between populations suggesting that sexual maturity in this species is a function of age and not size since reproduction was reported to occur in individuals above 2 years old (Paes-da-Franca, 1956; Sola, 1997; Guerreiro, 1998). Finally, the lowest gonadosomatic ratio (GSR) and percentage of individuals with gonads (at the peak in gonadal mass) in Norway supports the existence of an inverse trend of reproductive output with latitude (Clarke, 1987; Philippart et al., 2003).

Latitudinal patterns in reproductive traits are commonly detected in marine invertebrates (e.g. Lewis, 1986; Brante et al., 2003; Béguer et al., 2010) affecting population dynamics, particularly recruitment (Lewis,1986). However, reproductive strategies of a species also play a major role on survival and hence the biogeography of the species. Aspects such as developmental mode affect the distribution of marine invertebrate species (Thorson, 1950). In species with pelagic larvae, such as *Scrobicularia plana*, the increased food seasonality with latitude tends to result in shorter and earlier spawning periods so that the larvae can benefit from the phytoplankton bloom. Moreover, the time available for gonadal development is expected to decrease with increasing latitude until there is no more time to complete gonadal maturation, just beyond the geographical limit of the species (Hughes, 1971).

Allocation strategies

Intraspecific differences in relative gonadal biomass may be the result of differences in energy allocation strategies as a response to environmental variability (Jokela and Mutikainen, 1995). In all populations, the start of gametogenesis coincided with the spring blooms suggesting that energy from ingested food was used to build up gonadal tissue. However, the Dutch and the Portuguese populations also used stored energy during part of the year to build up gonads at the expense of somatic mass. After spawning, Portuguese and Norwegian populations still had energy to grow, suggesting priority of investment on somatic tissues rather than on reproduction. It is possible that exposure to high winter temperatures in Portugal leads to higher tissue losses and higher maintenance costs, and energy obtained from food must be sufficient to guarantee survival over the next winter; while in Norway, growth is only possible during a short period in spring/summer. In the Netherlands, somatic mass started to increase just before an increase in gonadal mass was observed and spawning occurred when somatic mass was decreasing, suggesting priority of investment in reproduction rather than in somatic growth. The same was observed in a Dutch population of *Mya arenaria* (Cardoso et al., 2009), suggesting that this species invested more energy in reproduction than in somatic growth under unfavorable conditions (Roseberry et al., 1991). Therefore, *Scrobicularia plana* seems to be able to change its allocation pattern in response to the environmental conditions. This phenotypic flexibility, although energetically costly, constitutes a selective advantage for the species (Jokela and Mutikainen, 1995; Kingsolver and Huey, 1998).

These intraspecific physiological differences can be caused not only by phenotypic adaptation but also by genetic divergence or even a combination of both. While limited population structure may be expected in the geographic range studied here (because of recent recolonization and high potential for gene flow), this is observed for some (e.g. Luttikhuizen et al., 2008) but not all marine invertebrates of the region (e.g. Luttikhuizen et al., 2003; Maggs et al., 2008). Therefore, also in *Scrobicularia plana* some of the observed growth and reproductive patterns might be due to genetic differences.

Conclusion

In the present study, the three *Scrobicularia plana* populations showed distinct somatic and reproductive patterns that appeared to be related to differences in temperature and food availability. The seasonal cycles in somatic mass reflected the increased seasonality in food availability at higher latitudes. Regarding the reproductive cycles, the two more northern populations were characterized by shorter reproductive periods that started later in the year, as well as lower reproductive output attributed to the lower temperatures and increased food seasonality at higher latitudes. The existence of latitudinal, environmentally controlled gradients suggests that changes in the environmental conditions will affect population dynamics and the biogeography of the species.

Acknowledgements: This study was co-financed by the Portuguese Foundation for Science and Technology (FCT) and Fundo Social Europeu (FSE) through grants awarded to Sílvia Santos (SFRH/BD/28370/2006) and Joana Cardoso (SFRH/BPD/34773/2007). Thanks are due to everyone that helped during sampling: Alfredo Santos, Catarina Cruzeiro, Filipe Amador, Jannes Heusinkveld, Jeremy Smith and Vânia Freitas; and also to all personnel from CIIMAR, in particular Joana Campos, and from Trondheim Biological Station (TBS - NTNU), specially Jarle Mork, Jon-Arne Sneli, Jussi Evertsen and

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Chapter 4

Isotopic fractionation between seawater and the shell of *Scrobicularia plana* (Bivalvia) and its application for age validation

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Abstract

This study analyzed the isotopic profiles of four aragonitic shells of *Scrobicularia plana* in conjunction with measured seawater temperatures and salinities. Comparison of $\delta^{18}O_{SHELL}$ with expected values revealed fractionation of $\delta^{18}O$ in near equilibrium with the ambient environment. Growth cessation occurred between November and March. Carbonate deposition stopped when temperatures were <12°C. Analysis of $\delta^{13}C_{SHELL}$ values suggested that carbon in the shell does not reflect the DIC in ambient water, likely due to the incorporation of metabolic carbon. An ontogenetic trend of increasing $\delta^{13}C$ values over time was observed, likely related to changes in metabolic activity. Annual growth patterns were inferred from $\delta^{18}O_{SHELL}$ profiles and compared with internal and external growth lines. Estimations of age based on external lines were unreliable, resulting in overestimation of age and underestimation of growth rates, likely due to the disturbance lines being wrongly identified as annual. Analysis of internal lines may lead to over- or underestimation of age and was more reliable in recent portions of the shell.

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Introduction

The bivalve *Scrobicularia plana*, peppery furrow shell, is a key species in shallow water benthic communities (Keegan 1986) and commercially exploited in several European countries (Langston et al. 2007). Its geographic distribution ranges from the Norwegian Sea in the north, along the Atlantic coast to Senegal, and in the Mediterranean Sea (Tebble 1976). The species, which is mainly a deposit feeder, is commonly found in the upper intertidal with preference for muddy areas (Hughes 1970; Bocher et al. 2007). It lives to about 18 years and has a maximum shell length (SL) of 54 mm (Green 1957). Although its reproductive cycle differs between locations (Santos et al. 2011b), in the Netherlands, gametogenesis starts around April, with spawning occurring from July to September (Santos et al. 2011a). Only individuals with a SL >15 mm undergo sexual development (Santos et al. 2011a). In commercial species, such as *S. plana*, information on the growth and the age structure of populations is necessary for understanding population dynamics, which in turn is crucial for the development of successful management and conservation programs. Studies on growth in several temperate estuaries and bays are available for this species (Green 1957; Hughes 1970; Bachelet 1981; Sola 1997; Guerreiro 1998). However, all studies revealed difficulties in estimating ages based exclusively on annual growth lines. When considering all external surface lines as annual, an overestimation of age by a factor of three and underestimation of growth rates were observed (Bachelet 1981). If only the more distinct lines were considered, growth rates were considerably increased and age estimations more accurate (Hughes 1970; Bachelet 1981).

Age determination commonly relies on the interpretation of external lines as representing years of growth. Mollusk shells are mainly composed of calcium carbonate and usually formed by three major layers: a thin outer periostracum composed of horny conchiolin; a middle prismatic layer of aragonite or calcite; and an inner calcareous (nacreous) layer (Gosling 2003). Shell growth occurs through the deposition of successive layers of carbonate material. Although several environmental factors can affect growth, temperature is one of the most important factors directly or indirectly (by influencing food availability) determining growth rates (e.g., Gosling 2003). During the warmer months of the year, when growth rates are usually higher (Bachelet 1980; Moura et al. 2009), the distance between layers is also higher. As growth slows during winter and early spring, the deposition of carbonate material occurs in thinner layers, forming a winter line on the shell surface. However, this aging method has clear limitations. In areas where winter conditions are not as marked, metabolic rates may not decrease enough to form clearly visible winter lines. Also, as individuals grow older, the earlier lines become less visible and in some winters hardly any line at all is formed. If disturbed during growth season, bivalves can form a ‘‘disturbance’’ line, which can be indistinguishable from the annual lines (Hughes 1970; Haag and Commens-Carson 2008). As disturbance lines can also be formed internally (Haag and Commens-Carson 2008), the uncertainties associated with the use of external and internal lines as an indication of annual growth lead to the development of a new method: the analysis of stable isotope variations across shell increments.

Bivalve mollusks can record environmental variation in their shells which, associated with their wide geographic distribution and ability to occupy a variety of habitats, makes these organisms very attractive environmental proxies. High-resolution records of those environmental changes can be provided by analyzing stable isotopes in shell carbonates. The use of isotope ratios as biologic recorders often relies on the assumption that bivalves fractionate isotopes in equilibrium with ambient water (e.g., Schöne et al. 2007; Bucci et al. 2009; Goodwin et al. 2009). Assuming equilibrium conditions, variations in the oxygen isotope composition ($\delta^{18}O$) of bivalve shells are a direct function of temperature and water $\delta^{18}O$ (Epstein et al. 1953; Grossman and Ku 1986; Dettman et al. 1999), the latter varying with salinity (Ingram et al. 1996; Gillikin et al. 2005). The seasonality in water temperature is expected to result in an annual periodicity in the $\delta^{18}O$ composition of molluskan shells that can be used to validate the annual formation of growth lines and estimate age. However, departure from equilibrium has been observed in several studies (Gillikin et al. 2005; Hallmann et al. 2008), making verification necessary for any considered species. Analysis of the carbon isotope ratio ($\delta^{13}C$) of carbonate shells is a bit more complex. $\delta^{13}C$ in carbonate shells can be obtained not only from dissolved inorganic carbon (DIC) in the water but also from respiratory CO_2 originating from food metabolism (Geist et al. 2005; McConnaughey and Gillikin 2008; Lartaud et al. 2010; Poulain et al. 2010). This is in turn influenced by kinetic effects (McConnaughey 1989), which results in a complex relationship between environmental factors and $\delta^{13}C$.

This study aimed to (1) determine whether shells of *S. plana* preserve seasonal environmental records as variation in $\delta^{18}O$ and $\delta^{13}C$; (2) assess whether isotope analysis can be used to estimate the age of S. plana; and (3) investigate the reliability of external and internal lines as age estimators. $\delta^{18}O_{SHELL}$ profiles were compared with a prediction of $\delta^{18}O_{SHELL}$ values to test the hypothesis that S. plana precipitates its shell in oxygen isotope equilibrium with ambient water. The relation between carbon in the shell and in the ambient water was also assessed. Seasonality of isotopic profiles was used to infer growth history. Age was determined using external and internal growth lines and compared to results from isotopic records in order to determine the reliability of different aging methods. Since the calculation of growth rates, vital for studies of population dynamics, depends on the reliability of growth lines as age estimators, validation of this methodology is of extreme importance.

Material & Methods

Experimental setup

To validate age estimation in *S. plana*, growth increments in 55 individuals were measured from July 2008 to December 2009. Experimental *S. plana* were collected in the Westerschelde estuary in the south of the Netherlands (N51°21′01″, E03°44′01″) and transported to the

NIOZ lab on Texel (N53°00′18″, E04°47′45″). For each individual, shell height (SH, defined as the distance from the umbo to the opposite shell margin) was measured to the nearest 0.01 mm with electronic calipers, and a numeric tag (http://www.hallprint.com) was glued to the valve. The experimental bivalves were then divided into two groups, and each group was placed in a floating platform in the NIOZ harbor, in a container with sediment from the original location, at a depth of ~50 cm. The first platform, carrying 22 *S. plana*, was left undisturbed during the experiment, while the 33 individuals in the second platform were measured monthly. At each sampling date, water samples were collected for the analysis of oxygen and carbon isotopic composition. Samples for the analysis of carbon profiles were poisoned with 0.1 ml of saturated $HgCl_2$ solution to prevent any further biologic activity. Temperature and salinity data were also collected, close to the experimental site, at 30-min intervals (van Aken 2001). At the end of the experiment, all individuals were collected and killed, and shells were measured and stored for the analysis of isotopic profiles.

Growth lines

Four shells, one from the platform left undisturbed (shell 1243) and three that were measured monthly (shells 1291, 1317, and 1338), were selected from the experiment based on the highest growth increments. For each individual, age was estimated by counting the external surface growth lines, defined as the dark lines on the shell surface extending circumferentially from the umbo and occurring on both valves (Fig. 4.1). Internal lines were also counted in the selected shells. For that purpose, left valves were embedded in epoxy resin (Poly Service, THV-500 epoxy and hardener 155), following Ropes (1985). Once hardened, a 5- to 6-mm crosssection was obtained by sectioning the blocks longitudinally through the hinge. The surface of each cross-section was then ground flat under successively finer grit (600, 800, 1,200, and 4,000 lm) and wet polished. To prepare acetate peels, the polished cross-sections were submerged in 1% HCl for about 20 s, rinsed with water, and covered with drops of acetone followed by an acetate sheet to obtain an imprint of the cross-section surface. Acetate peels were analyzed under a Zeiss Axiostar Plus microscope, and pictures were taken by using an AxioCam ICc3 digital camera and the Axio-Vision 4.7.1 software (both by Zeiss). The number of internal lines, defined as the dark lines that extended from the umbo to a discontinuity in the prismatic layer (Fig. 4.1), was determined.

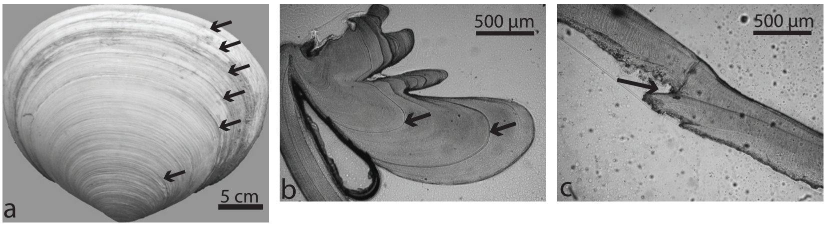

Fig. 4.1. Photographs of valve and cross-sections of *S. plana* shells: **a** lines identified as external growth lines, in shell 1243; **b** indication of internal growth lines in umbo of shell 1291; **c** discontinuity in prismatic layer indicative of an internal line, in shell 1338

Isotopic profiles

Using the Feigl test in a shell cross-section (Feigl 1937), we determined that shells of *S. plana* are mainly composed of aragonite (Appendix 4.1). For the determination of carbon and oxygen isotopic composition in the shell, the right valve was filled with epoxy resin to reinforce it. Using a micro-sampler attached to a binocular microscope (Micromill, New Wave Research) and equipped with an 800-µm drill bit, calcium carbonate powder was sampled in equally spaced (0.25- to 0.5-mm) intervals along the outer surface of the valve, following the growth lines. Twenty to 80 µg was required for mass spectrometry.

Oxygen and carbon stable isotopes ratios in the shell were measured using a Thermo Finnigan MAT 253 mass spectrometer coupled to a Kiel IV carbonate preparation line. Reproducibility of the external standard NBS 19 amounted to ≤0.1 and ≤0.05% (1 SD) for $\delta^{18}O$ and $\delta^{13}C$, respectively. Water samples collected monthly during the field experiment were also analyzed for oxygen and carbon isotope ratios. δ^{13}CDIC and $\delta^{18}O_{WATER}$ values were determined by headspace analysis using a Thermo Finnigan Delta+ mass spectrometer equipped with a GasBench-II preparation device. $\delta^{13}C_{DIC}$ ratios were determined relative to laboratory standards calibrated against NBS 19 and Na_2CO_3, with a reproducibility of ≤0.1 and ≤0.2%(1 SD), respectively. The long-term standard deviation of routinely analyzed in-house water standards is <0.1% (1 SD) for $\delta^{18}O_{WATER}$ values.

To establish a relationship between environmental factors and the isotopic composition of the water, the correlation of $\delta^{18}O_{WATER}$ and $\delta^{13}C_{DIC}$ values with daily mean temperature and salinity was estimated. The correlation between $\delta^{13}C_{SHELL}$ and $\delta^{18}O_{SHELL}$ values was estimated to determine the role of kinetic effects in the observed isotopic patterns (McConnaughey 1989).

Agreement with equilibrium fractionation was verified by comparing measured $\delta^{18}O_{SHELL}$values to predicted values, and measured $\delta^{13}C_{SHELL}$ to predicted values for inorganic aragonite. Given the high variability of salinity in intertidal areas, measured $\delta^{18}O_{WATER}$ and $\delta^{13}C_{DIC}$ values were first corrected for changes in salinity using leastsquares regression equations. Predicted $\delta^{18}O_{SHELL}$ values were then calculated from measured water temperature and $\delta^{18}O$ corrected values following the equation suggested by Dettman et al. (1999):

(1) $1000 \ln(\alpha) = 2.559\ (10^6 T^{-2}) + 0.715$

where *T* is temperature in degrees Kelvin and a is the fractionation factor between water and aragonite described by the equation:

(2) $\alpha = (1000 + \delta^{18}O_{ARAGONITE(VSMOW)}) / (1000 + \delta^{18}O_{WATER(VSMOW)})$

where $\delta^{18}O_{ARAGONITE}$ is the $\delta^{18}O$ of the shell. Shell $\delta^{18}O$ values calculated relatively to Vienna Standard Mean Ocean Water (VSMOW) were converted to Vienna Pee Dee Belemnite (VPDB), using the following equation (Gonfiantini et al. 1995):

(3) $\delta^{18}O_{ARAGONITE(VPDB)} = (\delta^{18}O_{ARAGONITE(VSMOW)} - 30.91) / 1.03091$

Predicted $\delta^{13}C_{SHELL}$ values were calculated as equilibrium $\delta^{13}C$ values for inorganic aragonite using the inorganic aragonite-HCO3$^-$ carbon fractionation of Romanek et al. (1992). This fractionation is +2.7±0.6% and is independent of temperature. To calculate equilibrium values, the fractionation value was simply added to $\delta^{13}C_{DIC}$ values, corrected for salinity. Predicted values were calculated for the period of the experiment since $\delta^{18}O_{WATER}$ and $\delta^{13}C_{DIC}$ data were only available for that period. To align measured shell $\delta^{18}O$ and $\delta^{13}C$ with predicted values, a time scale was assigned to the individual data points of the shell isotopic record. Anchoring growth increments to the time of harvest, and accounting for differential growth rates and periods of no growth, calendar dates were assigned to measured shell $\delta^{18}O$ and $\delta^{13}C$ records. Dates were estimated by extrapolating the distance from the umbo of each sample based on a plot of width against the date of the monthly measurements during the experiment. Given that estimated dates differed from dates of measurements of isotopes in the water, a sinusoidal model (adapted from Santos et al. 2011a) was fitted to the predicted $\delta^{18}O_{SHELL}$ and $\delta^{13}C_{SHELL}$ data, and values were extrapolated for estimated dates. The goodness of fit between measured and predicted values was then determined using a linear regression. Based on estimated dates, growth period was determined. Knowledge on water temperatures at the last date recorded in the shell allows the determination of temperature of growth cessation. In addition, predicted seawater temperatures ($T_{\delta^{18}O}$) were calculated. $T_{\delta^{18}O}$ were derived from measured $\delta^{18}O_{SHELL}$ and reconstructed $\delta^{18}O_{WATER}$ from the relationship between salinity and $\delta^{18}O_{WATER}$ described in this study, using the temperature equation from Grossman and Ku (1986):

(4) $T_{\delta^{18}O} = 20.6 - 4.34\ (\delta^{18}O_{SHELL(VPDB)} - \delta^{18}O_{WATER(VPDB)})$

where $\delta^{18}O_{WATER}$ is subtracted by 0.27% (Gonfiantini et al. 1995) in order to relate to the VPDB standard.

Winter lines were identified as a peak in $\delta^{18}O$ profiles that followed a period of considerably lower values (summer). Age, length-at-age, and growth rates were estimated based on the position of the winter lines. The number and position of the peaks in the isotope records were then compared to external and internal growth lines to determine which methods provide an accurate estimation of age.

Results

Water temperature, salinity and isotopic composition

Water temperature varied sinusoidally (Appendix 4.2) with daily means ranging from 2.9°C in January to 20.7°C in July 2008 and from 0.9°C in February to 20.6°C in July 2009. A seasonal pattern could also be observed for salinity (Appendix 4.2), with a minimum daily value of 23.0 in March 2009 and a maximum of 33.2 in September 2009. Estimated seawater temperatures closely resembled the observed field temperatures during spring/summer, while the fall/winter signal was completely missed (Appendix 4.3). Temperature estimates ranged from 13.0°C to 22.4°C in shell 1291 and 14.1°C to 18.7°C in shell 1317, in 2008 and 2009 respectively, and from 20.9°C in 2008 to 12.9°C in 2009, for shell 1338.

Oxygen isotope composition of water measured monthly varied seasonally with salinity (Appendix 4.2). $\delta^{18}O_{WATER}$ values had a stronger correlation with salinity than with temperature ($r^2_S = 0.77$, $r^2_{T°C} = 0.47$, $n = 28$). The relationship between salinity and $\delta^{18}O_{WATER}$ values was represented by the following least-squares regression equation ($p < 0.001$):

(5) $\delta^{18}O_{WATER} = 0.2333(\pm 0.02) * S - 7.9456\ (\pm 0.72)$

where S is salinity.

$\delta^{13}C_{DIC}$ values were more strongly correlated with temperature than with salinity ($r^2_S = 0.31$, $r^2_{T°C} = 0.56$, $n = 28$). Nevertheless, a significant correlation was found between carbon isotope composition of water and salinity ($p = 0.002$), described by the following least-squares regression equation:

(6) $\delta^{13}C_{DIC} = 0.1654(\pm 0.05) * S - 5.8933(\pm 1.40)$

where S is salinity.

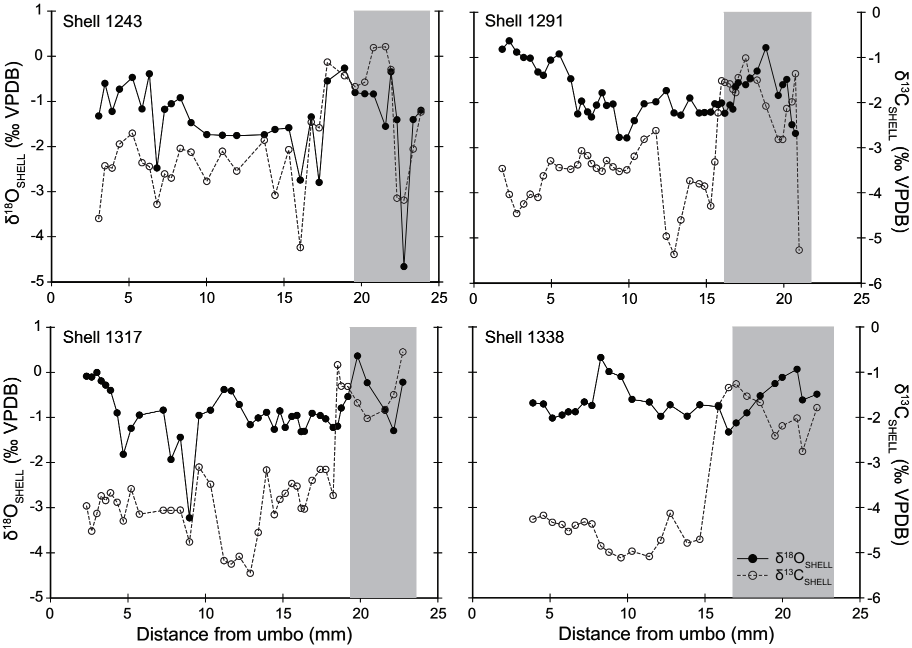

Fig. 4.2. $\delta^{18}O_{SHELL}$ and $\delta^{13}C_{SHELL}$ values of individual *S. plana* shells plotted against distance from umbo. Gray area corresponds to experimental period.

Measured and expected shell $\delta^{18}O$ and $\delta^{13}C$

Measured δ^{18}O and δ^{13}C values in the four shells are represented in Fig. 4.2. A significant correlation between $\delta^{18}O_{SHELL}$ and $\delta^{13}C_{SHELL}$ values was observed for shell 1243 ($r^2 = 0.24$, $p = 0.004$), while in the remaining three shells, these were not significantly correlated (Appendix 4.4). After correction of observed $\delta^{18}O_{WATER}$ and $\delta^{13}C_{DIC}$ values for changes in salinity, using Eqs. 5 and 6, respectively, predicted values of $\delta^{18}O_{SHELL}$ and $\delta^{13}C_{SHELL}$ were calculated. Predicted $\delta^{18}O_{SHELL}$ values, calculated based on Eqs. 1–3, followed a sinusoidal trend (Fig. 4.3) and showed an overall higher range of variation than observed values, with exception of shell 1243. Maximum predicted $\delta^{18}O_{SHELL}$ value was more than 2 units higher than the maximum observed value (shell 1291), while minimum values were always lower for the measured than the predicted $\delta^{18}O_{SHELL}$ values. Predicted $\delta^{18}O_{SHELL}$ values showed a stronger correlation with seasonal temperature than with salinity ($r^2_S = 0.06$, $r^2_{T°C} = 0.86$). Comparison of predicted with observed $\delta^{18}O_{SHELL}$ records of the shell portion that grew during the experiment (4-7 mm) showed a good correspondence between profiles (linear regression: $r^2 = 0.37$, $F_{1,25} = 14.63$, $p < 0.001$). The most positive values of $\delta^{18}O_{SHELL}$, from October/November 2008 until April 2009, were, however, not represented in the observed shells.

Predicted $\delta^{13}C_{SHELL}$ values showed a weak correlation with temperature ($r^2_{T°C} = 0.34$). No correspondence between predicted and measured $\delta^{13}C_{SHELL}$ values was observed (linear regression: $r^2 = 0.08$, $F_{1,25} = 2.23$, $p = 0.15$).

Growth and age estimation

Growth, as determined from the repeated size measurements, stopped around October 2008, when a monthly mean temperature of 12.8°C was recorded, and resumed in April 2009, at a mean temperature of 10.1°C (Appendix 4.5). From November 2008 to March 2009, there was virtually no growth (mean growth rates <0.001 mm d^{-1}). Based on $\delta^{18}O_{SHELL}$ records, growth cessation also occurred between November and March.

A peak in $\delta^{18}O_{SHELL}$ profiles of the four shells analyzed was identified during this period, corresponding to the winter growth cessation (Fig. 4.4). Although in shell 1243 the peak appears slightly before the first measurement, it was assumed as corresponding to the 2008-09 winter and the mismatch attributed to an inaccuracy in SH measurement. Seasonality of $\delta^{18}O_{SHELL}$ profiles was not always clear for the preceding period, and as a result, the identification of winter lines was not straightforward. Peaks and/or troughs in δ^{18}O profiles, i.e., values that considerably differed (>0.5%) from the mean value, were identified and information was combined to determine winter lines. Apart from the line formed during the experimental period, one more line was detected in shells 1243 and 1338, assigning them to the 2007 cohort. In shells 1291 and 1317, two more lines were identified in the δ^{18}O profiles, indicating they belonged to the 2006 cohort.

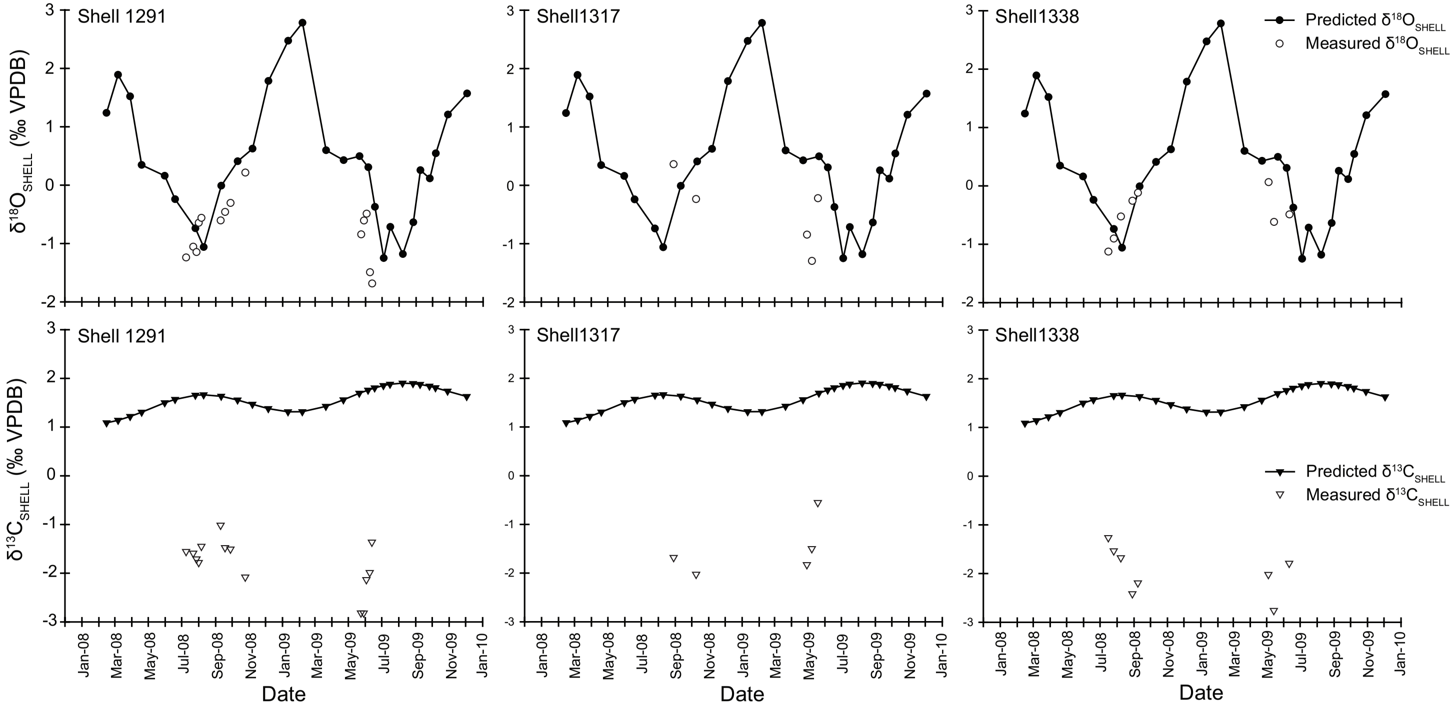

Fig. 4.3. Predicted $\delta^{18}O_{SHELL}$ and $\delta^{13}C_{SHELL}$ values of three *S. plana* shells plotted with individual shell $\delta^{18}O$ and $\delta^{13}C$ values, respectively

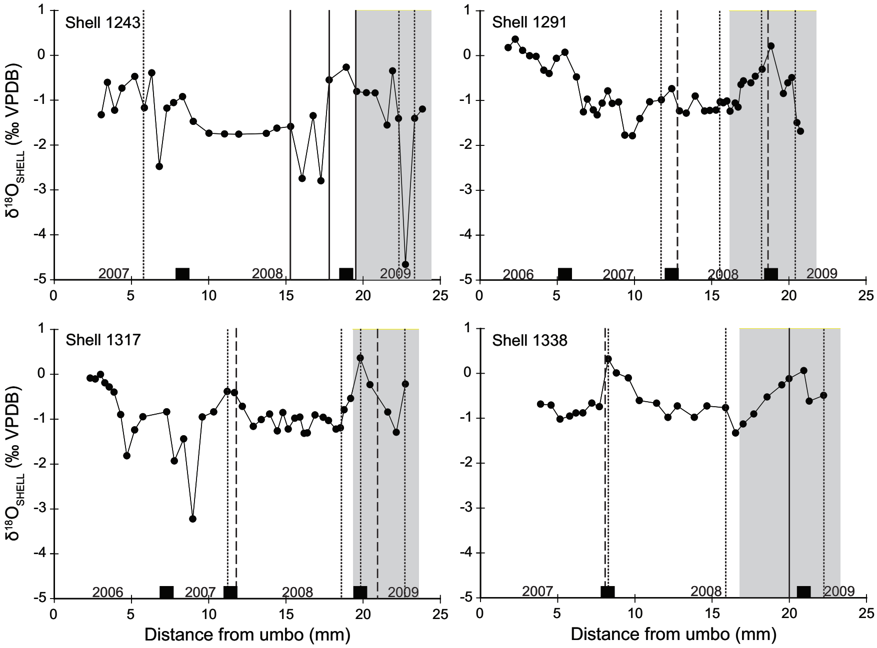

Fig. 4.4. Variation in $\delta^{18}O_{SHELL}$ values of four *S. plana* shells versus distance from umbo. Gray area corresponds to experimental period. Black bars at bottom of each plot indicate location of growth lines identified from $\delta^{18}O$ profiles. Growth years were defined based on these bars. External (dotted) and internal (dashed) lines are also indicated in the plot, with solid lines corresponding to a position where both an external and an internal line were identified during the sampling period. Mean values were calculated based on daily temperatures at high tide.

Table 4.1. Length-at-age (mm) of four *S. plana* shells determined as distance between shell umbo and each annual line identified in the $\delta^{18}O$ profiles.

Age	**Length-at-age (mm)**				**Mean length-at-age**	**Mean yearly growth**
	Shell 1243	*Shell 1291*	*Shell 1317*	*Shell 1338*	**(±SD)**	**(±SD)**
1	8.31	5.50	7.28	8.27	7.34 (±1.32)	7.34 (±1.32)
2	18.91	12.42	11.20	20.94	15.87 (±3.88)	8.53 (±4.78)
3	24.41	18.85	19.82	23.25	21.58 (±2.62)	5.71 (±2.67)
4		21.80	23.62		22.71 (±0.60)	3.38 (±1.29)

Length-at-age and growth rate values varied among shells (Table 4.1). Growth rates for the first 2 years were higher in shells 1243 and 1338, both from the 2007 cohort, with the highest growth being observed in shell 1243. During the experimental period, growth was considerably lower than in previous years. If the information for 2009 is then removed, a growth rate of 7.52±1.54 mm year^{-1} is obtained for the third year, similar to the rates observed in the first 2 years, with a mean length-at-age of 19.33±0.68 mm. Mortality rates were higher in the undisturbed platform.

External and internal lines identified in the four shells analyzed are represented in Fig. 4.4. The number of external lines varied between 6 in shell 1243 and 4 in the remaining shells, while 3 internal lines were observed in shell 1243 and 2 lines in the remaining three shells (Table 4.2). The number of external lines was always higher than that of internal lines (±2-3) and peaks in the $\delta^{18}O_{SHELL}$ profiles (1-4 years). The difference between estimations by counting internal lines or peaks in the $\delta^{18}O_{SHELL}$ profiles was never more than ±1 year. Internal lines seemed to have an overall good agreement with the isotopic profiles, especially in the younger portions of the shells. Only in shell 1243, two internal lines did not have a correspondence with the $\delta^{18}O$ profiles. In the older portion of some of the shells, however, winter growth lines, as determined by $\delta^{18}O_{SHELL}$ profiles, were not represented as internal dark lines.

Table 4.2. Age estimations of four *S. plana* shells using three distinct methods.

Method	**Age (years)**			
	Shell 1243	*Shell 1291*	*Shell 1317*	*Shell 1338*
External lines	6	4	4	4
Internal lines	3	2	2	2
Stable isotopes	2	3	3	2

Discussion

Comparison of measured and predicted $\delta^{18}O_{SHELL}$

We examined whether *S. plana* precipitated its shell in near isotopic equilibrium with the ambient water by comparing measured and predicted $\delta^{18}O_{SHELL}$ values. The correction of predicted values using the $\delta^{18}O$-salinity relation determined for our area allowed us to account for salinity variation. The comparison revealed that the most positive shell values were not represented in the isotopic profiles. This observation suggests the growth cessation of *S. plana* during winter months, which is in agreement with previous studies along the Atlantic Coast and in the Mediterranean Sea (Hughes 1970; Casagranda and Boudouresque 2005). Although winter values were not represented due to growth cessation, a good correspondence between predicted and measured $\delta^{18}O_{SHELL}$ values was observed for the growing period, suggesting that oxygen is incorporated in the shell at or near isotopic equilibrium with the water.

Reconstruction of seawater temperatures based on shell isotopic data revealed that the annual cycle in seawater temperature is recorded in the shell, although the fall/winter signal is missed due to the growth cessation. An overall good correspondence between measured and reconstructed spring/summer temperatures is observed, although there is a slight overestimation and shift to the left of reconstructed temperatures. Overestimation of predicted temperatures may result from *S. plana* being exposed to locally higher seawater temperatures, as water currents within the experimental setup were low and pots were placed rather close to the surface which could lead to warming of the water over the pots, while the horizontal shift could be due to a small imprecision in the assignment of calendar dates to isotopic profiles. Nevertheless, we can conclude that $\delta^{18}O_{SHELL}$ values are representative of the environment in which the shells grew.

Measured $\delta^{13}C_{SHELL}$

The $\delta^{13}C_{SHELL}$ profiles followed a fairly sinusoidal trend suggesting that variation in $\delta^{13}C$ is influenced by seasonal factors. However, the non-overlapping of $\delta^{13}C_{DIC}$ with predicted values (corrected $\delta^{13}C_{DIC}$ values) suggests that carbon in the shell does not reflect the DIC in the ambient water. This is likely due to the incorporation in the shell of metabolic carbon from respiratory CO_2, which can result in measurable deviation from an equilibrium model (Lorrain et al. 2004; Gillikin et al. 2006; Goewert et al. 2007). As we found no relation between $\delta^{13}C_{DIC}$ and $\delta^{13}C_{SHELL}$ values (not shown), it was not possible to determine the offset from equilibrium. Moreover, the contribution of metabolic carbon can result in an ontogenetic trend of decreasing $\delta^{13}C$ values over time. The onset of sexual maturity and the physiologic changes associated with gametogenesis and slower growth rates can lead to elevated incorporation of metabolically derived CO_2 and consequent decline in $\delta^{13}C_{SHELL}$ values (Krantz et al. 1987; Lorrain et al. 2004). In our study, we observed an opposite trend with values shifting toward heavier $\delta^{13}C$, similarly to what Brey and Mackensen (1997) described for the Antarctic bivalve *Laternula elliptica*, suggesting that less metabolic carbon is incorporated into larger shells. It is possible that the higher metabolic rates of juveniles would lead to a stronger depletion of metabolic carbon (Rohling and Cooke 1999). Departure from equilibrium can also occur due to the kinetic effects that refer to the simultaneous depletion of ^{18}O and ^{13}C during CO_2 hydration and hydroxylation (McConnaughey 1989). Their role can be tested by analyzing the correlation between shell $\delta^{13}C$ and $\delta^{18}O$ values. As a significant correlation was only found for one shell, kinetic effects seem to contribute little to the overall pattern of $\delta^{13}C_{SHELL}$.

Growth

Variation in measured $\delta^{18}O_{SHELL}$ values followed a truncated sinusoidal pattern suggesting a seasonal growth in *S. plana* (Dettman and Lohmann 1993). Further supporting this observation is the absence of the most positive predicted values of $\delta^{18}O_{SHELL}$ in the analyzed shells. Growth cessation started in November, similar to what has been observed in a population from a Mediterranean brackish lagoon (Casagranda and Boudouresque 2005), lasting until

March. As temperature becomes too low, the mantle draws away from the edges of the shell and deposition of carbonate is interrupted (Richardson 2001), explaining why no oxygen isotope record was identified in this portion of the year. When shell growth is resumed, as environmental conditions become more suitable, a new layer that extends past the older regions of the shell is formed, resulting in an obvious growth line (Richardson 2001).

As *S. plana*'s shell is precipitated in near equilibrium with ambient water, the shutdown temperature below which growth stops can be estimated. In our study, there was virtually no growth when temperatures were below 12°C. The estimated shutdown temperature is relatively high when compared with other North Atlantic species such as *Arctica islandica* (Witbaard et al. 1994). However, similar shutdown temperatures (12-13°C) were observed in a *S. plana* estuarine population from northern Spain (Sola 1997). In a Mediterranean brackish lagoon, weak growth lines were produced during winter, when a minimum mean temperature of 10.6°C was registered (Casagranda and Boudouresque 2005). The high shutdown temperatures observed may suggest that S. plana has a different thermal tolerance range from other North Atlantic species such as *M. balthica*, *Mya arenaria*, and *Mytilus edulis* (Freitas et al. 2007), possibly related to its geographic distribution since it inhabits more southern areas than the other species. In the Mediterranean population, stronger growth lines were formed between July and August, when water temperature and salinity were highest (Casagranda and Boudouresque 2005). Growth cessation in summer can occur due to thermal stress as the physiologic limits of thermal tolerance of the species are exceeded (Kirby et al. 1998), or to the stress associated with spawning (Jones 1980; Richardson 2001). The good correspondence between predicted and observed values in the summer suggests that, unlike the Mediterranean population, growth does not stop during warmer months. Nevertheless, growth slows down during summer which may also be related to spawning, shown to begin in July/August in two intertidal mudflats in the Netherlands (Zwarts 1991; Santos et al. 2011a). The investment in spawning involves the channeling of energy toward egg and sperm production, which would result in less energy being available for growth, explaining the lower growth rates.

The growth pattern observed for *S. plana* may, however, not be directly related to temperature but rather to food availability, which in turn is closely regulated by the seasonal cycle. The onset of the spring phytoplankton bloom in the area (Philippart et al. 2010) would explain the initiation of the high growth rate observed in spring. Then, the increase in temperature would be predicted to result in increasing growth rates. However, a continued decrease in growth rates was detected which may be attributed to the post-bloom decrease in food availability. In a previous study of three populations of *S. plana* along the species distributional range (Santos et al. 2011a), body and somatic mass cycles were observed to be related to food availability, particularly the phytoplankton blooms. It is likely that food availability, and the temperature at which food becomes available, is the main determinant of growth in *S. plana*. If so, variation in temperature at which phytoplankton blooms occur could result in different temperatures for growth cessation.

Growth increments between consecutive winter lines can be used to calculate the individual growth rates. Growth rates calculated in this study, for the period of 2009, are most likely an inaccurate representation of growth rates in natural populations. Stress caused by handling likely resulted in increased energy expenditure and slower growth. Comparison of growth increments between the two experimental platforms suggests that the individual left undisturbed grew faster, although the high mortality in the undisturbed individuals did not allow further analysis. When data from 2009 are excluded, annual growth rates were higher than those of Green (1957) in the Gwendraeth estuary (South Wales), where *S. plana* reached a length of 5 mm by the first winter, with another 5 mm being added during each of the next 2 years and 6-7 mm added between the third and fourth winters. Our rates are, however, lower than what Verdelhos et al. (2005) calculated for a population of the Mondego estuary in Portugal (~10 mm year^{-1}), while in the Bidasoa estuary (Spain) bivalves reached a mean length of 21.8 mm at the age of ~16 mo, growing up to 30 mm in the following year (Sola 1997). The observed differences are in agreement with the general trend of increasing growth rates with decreasing latitude suggested for *S. plana* (Bachelet 1981; Sola 1997).

Age validation

Oxygen isotopic profiles should provide an accurate estimate of age in *S. plana* given the seasonality observed in the deposition of $\delta^{18}O$ during the experiment. However, when analyzing the shell, the seasonality of the oxygen isotopic profiles is not always very clear. This could be due to limitations associated with the sampling methodology. Given the overlap of growth increments, sampling too deeply into the outer layer can result in contamination with shell material from inner layers that were deposited more recently. In our study, since shells were drilled to a depth of only 20 μm, it is extremely unlikely that more than one layer was sampled at a time. Different parts of the shell can also vary in their chemical composition and analysis of one part of a shell is not necessarily a good representation of the whole shell (Rosenberg 1980; Carriker et al. 1991), although, regarding $\delta^{18}O_{SHELL}$ composition, different portions of the shell were not found to be isotopically different from each other in bivalve species such as the American oyster *Crassostrea virginica* (Surge et al. 2001) and the marine mussel *Mytilus trossulus* (Klein et al. 1996). Finally, given the smaller surface area that could be sampled as we got closer to the umbo, some samples were pooled to obtain enough material for analysis. This will lead to less distinct (seasonal) profiles in the older portion of the shell, as the temperature signal is averaged over longer time intervals, hampering the correct identification of the first winter growth check. In our study, we believe that the first winter was correctly identified based on the values of mean yearly growth. These values are congruent with previous studies on the growth of *S. plana* in two European temperate estuaries (Green 1957; Verdelhos et al. 2005). Nevertheless, age estimations should be viewed as minimum values.

Although it can now be concluded that isotopic analysis provides an accurate indication of age in *S. plana*, it is an expensive and time-consuming method. To process large samples, a preferred method would be the analysis of shell surface lines since it costs little money and time. Unfortunately, the number of peaks identified in the $\delta^{18}O$ profiles of our shells did not correspond well with the number of external lines. More lines were counted on the shell surface than in shell cross-sections or isotopic profiles which can result from disturbance lines being wrongly identified on the shell surface as annual. Difficulties in using this method to accurately estimate age in *S. plana* had already been experienced by several authors (Green 1957; Hughes 1970; Bachelet 1981). Alternatively, the counts of internal growth lines in shell cross-sections are commonly used to estimate age (e.g., MacDonald and Thomas 1980; Richardson and Walker 1991). In our study, the oxygen profiles have an overall good correlation with internal lines (except for shell 1243), especially for the more recently deposited portion of the shell. However, the absence of identifiable internal lines in the older portions of some shells would lead to an underestimation of age and overestimation of growth rates, making this an unreliable method, as well. Other studies also established the unreliability of using internal lines for age estimations in bivalves, namely freshwater mussels (Kesler and Downing 1997; Versteegh et al. 2009), and suggested that analysis of $\delta^{18}O_{SHELL}$ records is a more reliable method (Versteegh et al. 2009).

Conclusion

The bivalve *S. plana* precipitates its shell in near isotopic equilibrium with the ambient water. However, the seasonal growth of *S. plana*, suggested by the truncated sinusoidal pattern of $\delta^{18}O$ profiles, implies that caution is required when interpreting environmental data. Winter temperatures will not be represented in the shell, due to growth cessation at temperatures <12°C, and any reconstructions of seawater temperatures from *S. plana* shells should take this into account. Nevertheless, shells of *S. plana* preserve environmental records as isotopic variation that can then be related to growth patterns, namely periods and temperatures of growth cessation. As for the $\delta^{13}C_{SHELL}$ values, the overall trend suggests that $\delta^{13}C_{SHELL}$ of *S. plana* is at least partially influenced by seasonal processes. However, $\delta^{13}C_{SHELL}$ values of *S. plana* do not directly respond to $\delta^{13}C_{DIC}$ values, which can be explained by kinetic and/or metabolic effects. As kinetic effects explained, at best, 24% of the observed variation in one shell, departure from equilibrium is most likely due to the incorporation of metabolic carbon in the shell.

Regarding age determination, counting external or internal lines does not provide an accurate estimate of age. The analysis of external lines leads to an overestimation of age as disturbance lines in the shell surface are often identified as annual growth lines. In our data, age estimations based on the counts of external lines always resulted in an error, varying from one to four years. As for the internal lines, the error associated with this method was considerably smaller (±1 year). Nevertheless, in most cases, it also resulted in a wrong estimate of age. Since most studies on the growth of *S. plana* rely on the use of external lines for age estimations, one must be careful when considering such data. The same may be true for other bivalves, making species-specific age validation essential. We stress the need to use isotope sclerochronology to identify true growth lines and to more accurately estimate age and growth rates in *S. plana*, although it is not practical when analyzing large samples.

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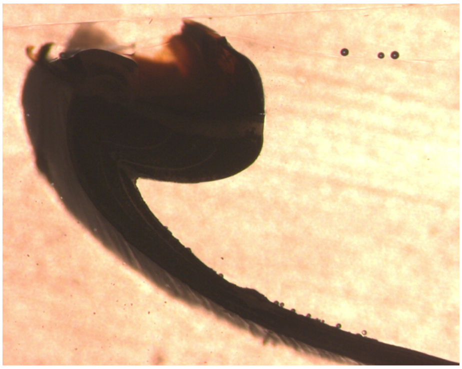
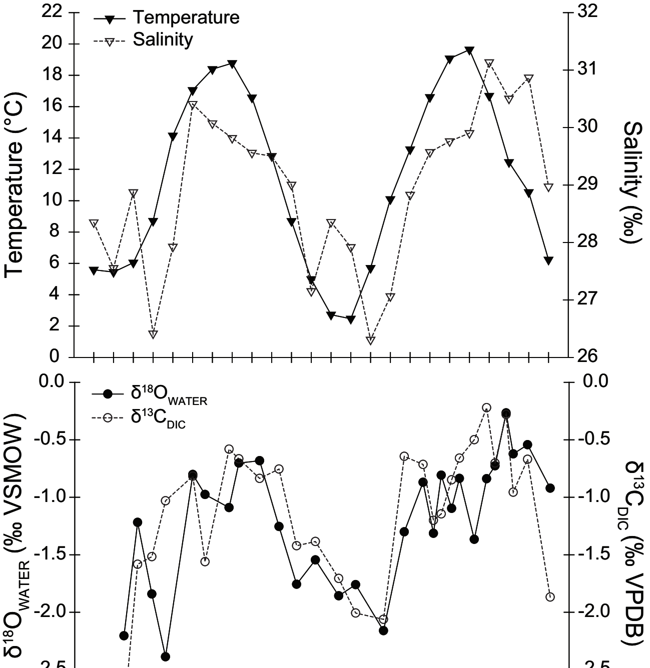
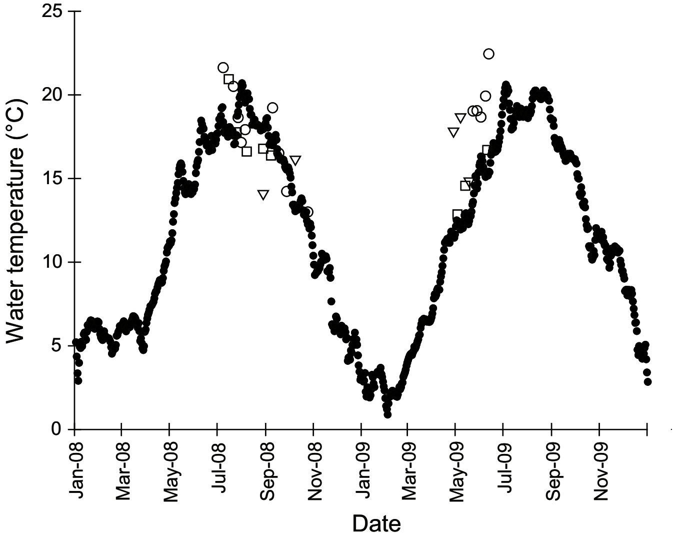
Appendix 4.1. Cross-section of *Scrobicularia plana* stained with Feigl's solution. The black color is indicative that aragonite is the main component of the shell.



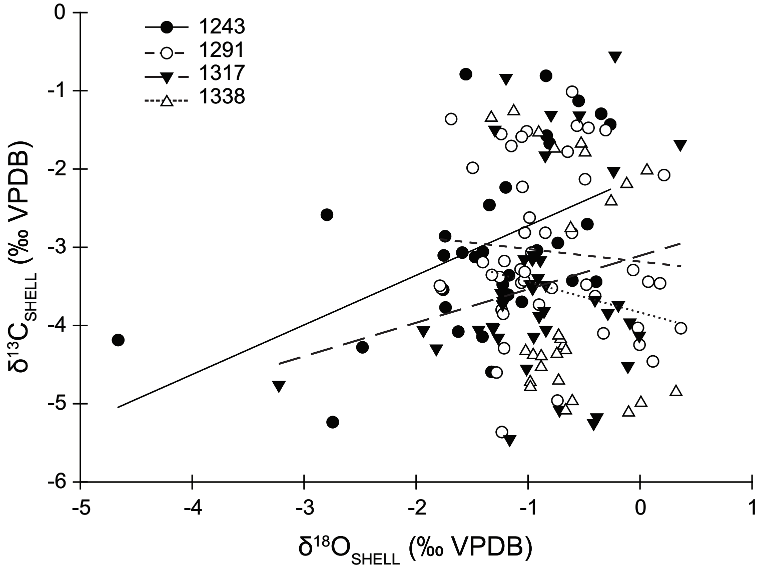
Appendix 4.2. Mean monthly water temperature, mean monthly salinity, $\delta^{18}O_{WATER}$ and $\delta^{13}C_{WATER}$ values for 2008-2009, measured near experimental site (Marsdiep, western Dutch Wadden Sea).



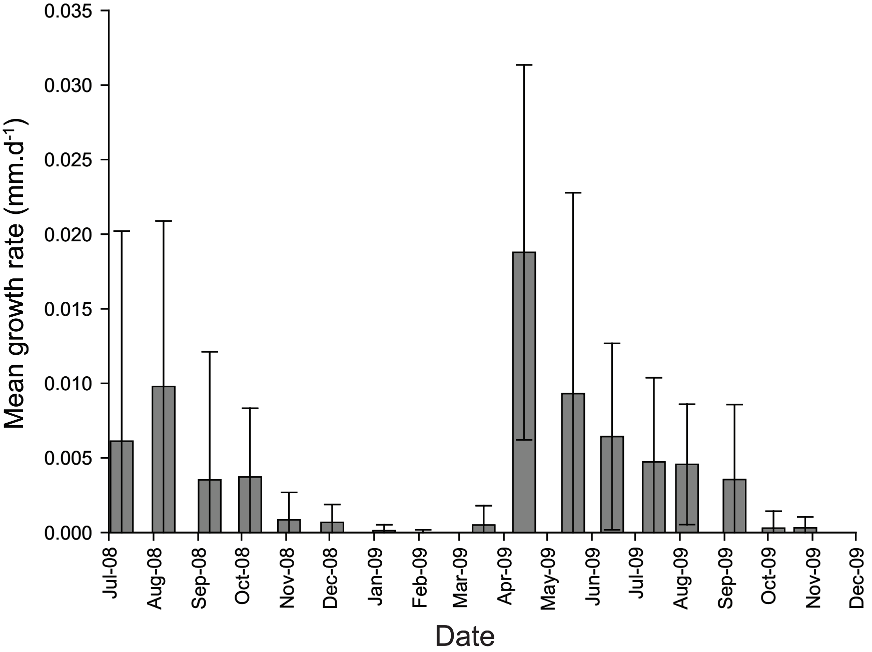
Appendix 4.3. Daily seawater temperatures (open circles) measured close to experimental site. Estimated water temperatures based on $\delta^{18}O_{SHELL}$ and $\delta^{18}O_{WATER}$ are represented for shells 1291 (open circles), 1317 (open triangles) and 1338 (open squares).



Appendix 4.4. Covariation of $\delta^{18}O_{SHELL}$ and $\delta^{13}C_{SHELL}$ values of four *Scrobicularia plana* shells. Correlation was determined by linear regression (shell 1243: $r^2 = 0.24$, $F1,30 = 9.69$, $p = 0.004$; shell 1291: $r^2 = 0.01$, $F1,45 = 0.31$, $p = 0.58$; shell 1317: $r^2 = 0.05$, $F1,39 = 1.96$, $p = 0.17$; shell 1317: $r^2 = 0.01$, $F1,25 = 0.31$, $p = 0.58$).



Appendix 4.5. Shell growth of *Scrobicularia plana* during experimental period. Only animals measured monthly that survived the first winter, in a total of 25 animals, were considered.



Chapter 5

Isolation by distance and low connectivity in the peppery furrow shell *Scrobicularia plana* (Bivalvia) (da Costa, 1778)

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Abstract

Scrobicularia plana, a commercially important bivalve species in southern Europe, is commonly found along the NE Atlantic and Mediterranean coasts. Like other intertidal mollusk species, it has a wide distributional range and high potential for larval dispersal. However, *S. plana* has a more patchy distribution than most co-distributed soft sediment bivalves of the intertidal which could lead to lower interpopulation connectivity and stronger population structure. We surveyed 18 locations from throughout the species' range to determine overall population structure, phylogeographic distribution and historical demography. We sequenced a portion of the mitochondrial cytochrome-c-oxidase I gene (COI) for 423 individuals. Three population clusters (Trondheim, Atlantic and Pisa) were identified on the basis of pairwise F_{ST}'s. Demographic parameters were analysed in a coalescence framework. Strong differentiation was found between most Atlantic locations and the single Mediterranean location (Pisa). Among Atlantic locations, differentiation was weak and non-significant; though significant isolation-by-distance was detected. A star-shaped phylogeny, with mostly 1-step mutations, was found. Although 65 haplotypes were detected, 50 were private. The higher diversity observed in southern Europe, Brittany and Norway was consistent with glacial refugia. Population expansion occurred recently with the oldest split taking place 0.3-1.1 million years ago (Mya), between all Atlantic groups and the Mediterranean group. Negative values for neutrality tests and the star-shaped haplotype network were also indicative of recent population expansion. An isolation-by-distance effect and absence of migration reveal low interpopulation connectivity, which is likely reinforced by the species' patchy spatial distribution.

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Introduction

During the Pleistocene, repeated episodes of glacial advance and retreat occurred, resulting in major transformations in the geography and climate of northern Europe (Kukla et al. 2002). At the last glacial maximum (LGM) some 18,000 years before present (BP), the European ice sheet extended south to 52°N, while the permafrost extended to 47°N (Bradwell et al. 2008). Intrusion of ice sheets and unfavorable climate conditions beyond species' tolerance ranges pushed species south (Wares & Cunningham 2001, Ilves et al. 2010). As the LGM ended, c. 10,000 BP (Dawson 1992), temperatures increased causing a northward expansion of many species from southern refugia (Hewitt 2000, Maggs et al. 2008). These shifts in latitudinal range affected population dynamics, with the present genetic structure of the species reflecting those past conditions (Hewitt 2004).

Recolonization pathways and the location of refugia can be inferred from population genetic and phylogeographic analyses of contemporary populations (Avise 2000). Numerous studies targeting the Atlantic and Mediterranean have been conducted for benthic intertidal organisms over the past 15 years, including a number of bivalves and gastropods [e.g., Luttikhuizen et al. 2003 (baltic tellin *Macoma balthica*), Baker et al. 2008 (northern quahog *Mercenaria mercenaria*), Riginos & Henzler 2008 (blue mussel *Mytilus edulis*), Rawson & Harper 2009 (blue mussel *Mytilus trossulus*), Strasser & Barber 2009 (softshell clam *Mya arenaria*), Tarnowska et al. 2010 (lagoon cockle *Cerastoderma glaucum*), Krakau et al. 2012 (common cockle *Cerastoderma edule*)]. In virtually all studies, the Atlantic and Mediterranean are well separated and a number of refugia have been identified in Northern Spain, Brittany, SW Ireland and the Lofoten area of Scandinavia (Coyer et al. 2011, *Fucus distichus*). Genetic diversity patterns typically follow the "southern richness, northern purity" (Hewitt 2000) model of allelic/haplotypic richness or modifications involving population admixtures (reviewed in Maggs et al. 2008). High allelic/haplotypic richness in northern latitudes has, so far, only been observed in the cockle, *C. edule* (Krakau et al. 2012) and the fucoid seaweed, *F. distichus* (Coyer et al. 2011).

In addition to historical factors, contemporary population dynamics also shape population genetic and phylogeographic structure. This is especially true in species whose life-history traits include planktonic larval stages of considerable duration, in which case demographic contact is expected to lead to "open" populations, successful gene flow and, ultimately, greater inter-population connectivity (Swearer et al. 2002, Selkoe & Toonen 2011). Examples of this are often found in fish (Riginos & Victor 2001, Hoarau et al. 2002, Bradbury & Bentzen 2007, Reece et al. 2011) mollusks (Becker et al. 2007, Crandall et al. 2010, Hoffman et al. 2011) and crustaceans (Palero et al. 2008, Domingues et al. 2010). More complex patterns can also be caused by regional to local oceanographic currents and their interactions with the topography and habitat (e.g., Barber et al. 2000, Shanks et al. 2003, Cowen et al. 2007, Galarza et al. 2009, White et al. 2010). In some cases retention zones may be formed (e.g., Bradbury et al. 2008, Cowen & Sponaugle 2009, Galindo et al. 2010, Small & Wares 2010). This suggests that the link between pelagic larval duration and connectivity in marine systems is not that straightforward (e.g., Lester et al. 2007, Weersing & Toonen 2009, Riginos et al. 2011). Analysis of the geographic pattern of genetic variation should allow, however, a better understanding of the relationship between the population structure of a species and its ecological and habitat characteristics.

The interest in molecular studies of bivalve species stems from the fact that these organisms play a central role in intertidal soft-sediment ecosystems (e.g., Thrush et al. 2006). Assessment of genetic variation and population differentiation throughout their geographical range is crucial for the preservation of natural populations. In intertidal soft-sediment areas of NW Europe, *Macoma balthica*, *Cerastoderma edule*, *Abra tenuis* and *Scrobicularia plana* are the four most common and abundant bivalve species (Bocher et al. 2007). High population subdivision has been observed for *M. balthica* in spite of the species' potential for high gene flow (e.g., Luttikhuizen et al. 2003, Väinölä 2003). Geographically structured populations were also found for *C. edule,* with a significant isolation-by-distance in northern populations (Krakau et al. 2012). A similar structure was observed for *A. tenuis*, which was, however, expected for this species since *A. tenuis* has a direct development, i.e., no planktonic larval stage (Holmes et al. 2004). As for *S. plana*, although a survey of population structure using allozymes found high within population variation (Skibinski et al, 1978), the authors did not address interpopulation connectivity. While all four species have a wide distributional range, *A. tenuis* and *S. plana* are characterized by a patchier spatial distribution (Bocher et al. 2007), which is expected to affect population connectivity and in *S. plana*'s case is not predicted from its life history, namely dispersal mode (Johnson et al. 2001).

Scrobicularia plana is a temperate species that occurs along the NE Atlantic coast, from the Norwegian Sea to Senegal, as well as in the Mediterranean Sea (Tebble 1976). The species inhabits intertidal areas with soft bottoms (sand, clay or mud), rich in organic matter, but has a clear preference for muddy sediments (Bocher et al. 2007). *S. plana* is an important component of shallow-water benthic communities and is of commercial importance in southern Europe (Tebble 1976, Keegan 1986). The species is gonochoristic, with planktotrophic development and an average pelagic life stage of two to four weeks (Frenkiel & Mouëza 1979). After hatching (and before the settling pediveliger stage) veligers dwell in a totally pelagic environment, a stage during which larvae are capable of dispersal (Frenkiel & Mouëza 1979).

The aim of the present study is to identify LGM refugia and to infer the historical demography of *Scrobicularia plana* using mitochondrial DNA data analyzed in a coalescence framework. Moreover, the genetic structure and connectivity of *S. plana* populations will be analyzed along the species' distributional range.

Material & Methods

Sampling

Scrobicularia plana adults were collected at 18 locations along its distributional range (from Baltic and North Sea to the Northeast Atlantic Ocean and Mediterranean Sea), during the period of 2007-2009 (Table 5.1). Animals were collected in intertidal areas during low tide, and were immediately preserved in 95% ethanol. Next, a tissue sample was removed from each animal and stored at -20°C, until DNA extraction.

Table 5.1. Sample locations and sample sizes (*n*) of *Scrobicularia plana*.

Location	**Latitude**	**Longitude**	**Sampling date**	**Code**	***n***
Trondheim, Norway	63°18’60.88’’N	10°11’01.74’’E	April 2008	TRO	29
Tjärnö, Sweden	58°53’32.94’’N	11°10’04.14’’E	October 2007	TJ	26
Killala Bay, Ireland	54°13’34.62’’N	09°12’43.14’’W	March 2008	KB	23
Wadden Sea, Germany	53°42’49’’N	07°48’04’’E	May 2007	WS	32
Balgzand, Netherlands	52°56’09’’N	04°48’07.20’’E	March 2008	BA	27
King’s Lynn, England	52°49’14.30’’N	00°17’16.60’’E	February 2008	KL	30
Clonakilty Estuary, Ireland	51°37’17.50’’N	08°52’40.39’’W	April 2008	CLO	29
Terneuzen, Netherlands	51°20’47.76’’N	03°47’44.52’’E	May 2008	TER	29
Plymouth, England	50°12’35’’N	05°05’27’’W	January 2008	PLY	20
Roscoff, France	48°40’18.83’’N	04°03’26.13’’W	April 2009	RO	25
Moeze plaisance, France	45°55’15.29’’N	01°04’31.11’’W	March 2008	MP	19
Astúrias, Spain	43°34’59.79”N	10°18’00.43”E	April 2007	AS	17
Pisa, Italy	43°28’00’’N	05°26’00’’W	July 2008	PI	30
Ponte Vedra, Galicia	42°28’00.57’’N	08°42’10.50’’W	April 2007	PV	9
Caminha, Portugal	41°53’08.00’’N	08°50’50.40’’W	February 2008	CA	26
Algarve, Portugal	37°07’37.60”N	07°36’36.40”W	April 2008	AL	24
Cádiz, Spain	36°52’31.50’’N	06°20’44’’W	May 2007	CZ	11
Agadir, Morocco	30°21’50.03”N	09°35’41.80”W	July 2006	AG	17

Molecular protocols

DNA was extracted using the GenEluteTM Mammalian Genomic DNA kit (SIGMA®) according to the Mammalian Tissue protocol (partB), provided by the manufacturer. Extracted DNA was visualized on 1% TBE agarose gels to assess quantity and quality.

Universal primers HCO2198 and LCO1490 (Folmer et al. 1994) were used to amplify a 710-bp fragment of the mitochondrial cytochrome-c-oxidase subunit I region (COI) gene. PCR amplifications were carried out in a 50 µl reaction containing 5 µl template DNA (1:10 dilution of DNA), 4.6 µl 10x reaction buffer (BiothermTM), 4.6 µl dNTPs (2.5 µmol), 0.3 µl of each primer (0.02 µmol) and 0.25 µl Taq polymerase (Biotherm PlusTM). The amplification reaction was performed with an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 94°C denaturation for 30 sec, 40°C annealing for 45 sec, 45 sec extension at 72°C, and a final 7 min extension at 72°C during the last cycle.

Twenty-three sequences were aligned and species-specific primers, CO_SCROB_F (5' TTGGGAGTCTTTATTTGTTTTAG 3') and CO_SCROB_R (5' AAGAAAGAAGTATTAAAATTACGATCA 3'), were designed using Primer3 (Rozen & Skaletsky 2000). The specific primers, used for the remaining sequences, amplified a 622-bp fragment. The PCR reaction was identical except for an annealing temperature of 50°C.

Amplifications were confirmed by 2% TBE agarose gel electrophoresis. In some cases, re-amplification was necessary and performed as follows: a small portion of the DNA band of the gel was collected and transferred into a 1.5 ml tube; 200 µl of sterile water was then added and the tube incubated for 2 min at 95°C; 1 µl of solution was used as template for the PCR reaction. After confirmation, fragments were purified directly, using QuickClean 5M PCR Purification Kit (GenScript).

Sequencing was carried out by Macrogen Inc. (Seoul, South Korea), using ABI-BigDyeTM terminator cycling conditions and sequenced on an ABI 3730XL Gene Analyzer.

Population genetic and phylogeographical analysis

Sequences were aligned with BioEdit v. 7.0.9.0 (Hall 1999) and reduced to a 507-bp length. All polymorphisms were double-checked on the chromatograms. Haplotypes were detected with the help of sequence analysis toolbox FaBox v. 1.35 (Villesen 2007). Genetic variation was estimated as haplotype diversity *h* (Nei 1987) and as nucleotide diversity π (Tajima 1983, Nei 1987) with Arlequin v. 3.1 (Excoffier et al. 2005). A minimum spanning network among the haplotypes was determined using Arlequin v. 3.1 (Excoffier et al. 2005).

Differentiation between sampling locations was estimated on the basis of pairwise F-statistics (F_{ST}) using the Kimura two-parameter model (K2P) (Kimura 1980), in Arlequin v. 3.1. The K2P model was the model implemented in Arlequin v. 3.1. that best fit our data according to the test performed in jModeltest v. 0.1.1 (Guindon & Gascuel 2003, Posada 2008). While conventional F_{ST} considers haplotype frequencies only, the K2P model takes genetic distances into account, allowing correction for multiple substitutions per site and different substitution rates between transitions and transversions. The significance of the statistics was computed using 10,000 permutations of the original data matrices to generate null distributions of pairwise F_{ST} values under the hypothesis of no difference between the populations. An analysis of molecular variance (AMOVA) based on the K2P model was also conducted within Arlequin v. 3.1 in order to estimate the degree of genetic structuring for mtDNA-COI sequences among and within populations or groups. To test the significance of covariance components and fixation indices, 10,000 permutations were performed. To address the problem of multiple comparisons, probability levels were adjusted using the False Discovery Rate (FDR) correction procedure (Benjamini & Hochberg 1995), which controls for the expected proportion of incorrectly rejected null hypotheses (type I errors).

To test for evidence of isolation-by-distance, a Mantel test was performed on genetic distances [F_{ST} / (1-F_{ST})] against (linear) geographic distance (minimum coastline distance) between all pairs of sampling locations, using IBDWS v. 3.21 (Jensen et al. 2005). In a one-dimensional habitat (considered as such given that differentiation occurs over spatial scales greater than the habitat width) a linear relationship between these two variables is expected (Rousset 1997). The significance of Mantel's Z test statistic was based on 30,000 permutations.

Population clusters were identified using pairwise F_{ST} values and a minimum spanning network in which groupings were formed based on the existence of significant differences between populations and geographically identifiable clusters. This was done in order to apply a population model for estimation of demographic parameters.

To infer historical demographic expansions, pairwise mismatch distributions were generated for the different groups, with DnaSP v. 5.10 (Librado & Rozas 2009), and the raggedness r index was calculated (Harpending 1994). This analysis tests a null hypothesis of population expansion with the failure to reject it (non-significant raggedness index) indicating lack of support for the alternative hypotheses of population stability. Departure from neutrality (as would be expected under population expansion) was also tested using R2 (Ramos-Onsins & Rozas 2002), in DnaSP v. 5.10, and Fu's Fs (Fu 1997) and Tajima's D (Tajima 1989), in Arlequin v. 3.1. by generating 10,000 simulated samples under the hypothesis of selective neutrality and stable population size.

Estimation of divergence time was obtained under an isolation-with-migration analytical model in IMa2 (Hey 2010). The program uses a Markov chain Monte Carlo (MCMC) simulation. The model assumes that an ancestral population of size θ_A split into two populations of sizes θ_1 and θ_2, t generations ago, and that after divergence the two populations exchanged migrants with rates m_1 and m_2. The demographic parameters estimated by IMa2 are scaled by mutation rate μ: $\theta = 4N_e\mu$ for nuclear genes, where Ne is the effective population size; $t = \mathrm{t}\mu$, where t is the time since splitting in units of years; and $m = \mathrm{m}/\mu$, where m is the rate per gene per generation in the coalescent. To convert the estimates of IMa2 into demographic units, an inheritance scalar of 0.25 for mitochondrial data (i.e. $\theta = N_e\mu$) and a generation time of two years were assumed. The mutation rate μ for COI was assumed to be between $0.355\mathrm{x}10^{-6}$ and $1.315\mathrm{x}10^{-6}$ substitutions per sequence per year, based on a molecular clock calibrated by Luttikhuizen et al. (2003). Divergence rates were calculated based on fossil record dates from three distinct sets: (i) the family Arcidae [*Arca* spp. vs. *Barbatia* spp. (Cox 1969)]; (ii) the mussel genus *Mytilus* [*M. californianus* vs. *M. edulis* species complex (Coan et al. 2000)]; and (iii) the Tellinacea [*Donax* spp. vs. *Macoma* spp. and *Sinonovacula constricta* vs. *Donax* spp./*Macoma* spp. (Pohlo 1982)]. For all IMa2 runs the HKY substitution model was used.

Pairs of populations were compared because it was not possible to fit a regular 3-population model to data from one single locus (due to the high number of demographic parameters). All individuals were included in the analysis. Upper limits to parameter priors were determined based on preliminary runs, to ensure that posterior distributions fell completely within the prior distributions. The analysis was performed using four independent runs with identical priors and number of coupled chains, but different random number seeds, for each pair of populations. Three shorter runs consisting of ten MCMC chains with geometric heating (h_1 = 0.99, h_2 = 0.75) of two million steps plus one longer run of ten million steps were sampled after an initial burn-in period of one million steps. To reduce the number of parameters in the model, a single migration parameter was estimated for each pair of populations (equal migration rate in both directions, -j2 option). To ensure convergence of parameter distributions, effective sample size (ESS) values, autocorrelation values and chain swapping were examined; trendline plots were checked for absence of trends, and parameter distributions were checked for unimodality. Genealogies from the four runs were then combined in a single L-mode run and the peaks of the marginal posterior distributions were taken as estimates of the parameters.

Results

Sequence data

A total of 423 individuals was sequenced and 65 different haplotypes were detected among the samples (Genbank accession numbers JN176805 – JN176869). Among the haplotypes, 58 of 507 sites were polymorphic (Appendix 5.1), with two different substitutions being observed in five of those sites adding up to a total of 63 substitutions, of which 48 were transitions and 15 were transversions (TI/TV = 3.2). Of the 63 substitutions, 17 were nonsynonymous replacement substitutions while the remaining 46 were synonymous substitutions (Ka/Ks = 0.37).

Population genetic analysis

The frequency of haplotypes, as well as nucleotide and gene diversities and their standard deviations per sample are given in Appendix 5.2. Haplotype h01 was clearly the most common haplotype (67.1% of the individuals analyzed) and the only one present at all sampling sites. The minimum spanning network showed only one main clade based on the presence of h01 (Fig. 5.1). To connect all observed haplotypes to the network, the number of necessary substitutions varied between one and five. The geographical distribution of all haplotypes is shown in Fig. 5.2. The number of observed haplotypes within populations ranged from 3 (PV) to 14 (AL). The majority of haplotypes (50/65) were private alleles, i.e., observed only in one population, of which 47 were single occurrences. Haplotype diversity (*h*) ranged from 0.32 in Ireland (CLO) to 0.86 in South of Portugal (AL) and with an overall average of 0.52 (Appendix 5.2). The lowest (0.0007) values of nucleotide diversity (π) were observed in Sweden (TJ) and Ireland (CLO), while the highest (0.0029) value was detected in the South of Portugal (AL). Mean nucleotide diversity among all locations was 0.0016 indicating that, on average, individuals differed by less than 0.2% per base pair. No significant correlation was observed between latitude and either haplotype diversity (p = 0.25) or nucleotide diversity (p = 0.33).

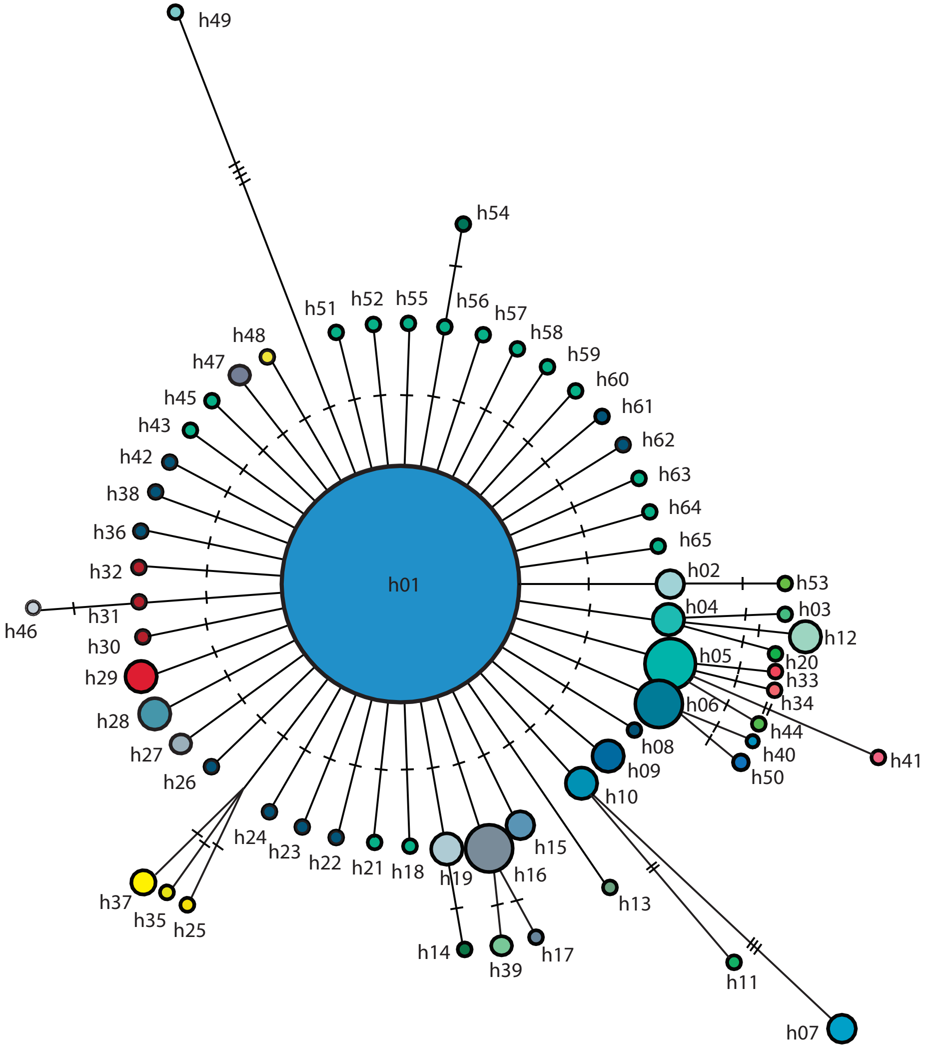

Fig. 5.1. Minimum spanning network among haplotypes of *Scrobicularia plana*, where each circle represents a haplotype; size of circles is proportional to haplotype and slash marks indicate the number of substitutions. Three groups are represented by different colors: Trondheim (yellow), Atlantic (blue/ green) and Pisa (red).

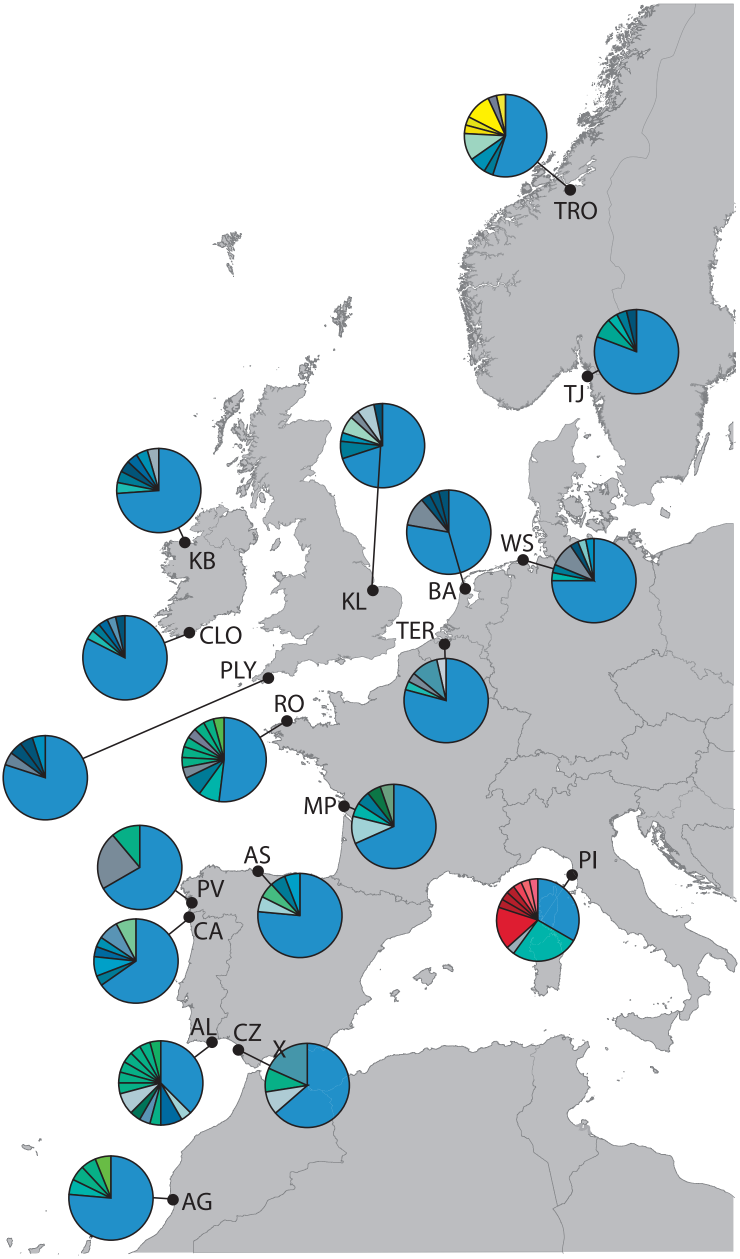

Fig. 5.2. Distribution of COI haplotypes for *Scrobicularia plana*, across Northeastern Atlantic coast and Mediterranean Sea. Different colors represent haplotypes that are only found in the respective groups: Trondheim (yellow) and Pisa (red); the Atlantic group (blue/green) includes all remaining haplotypes. Letters indicate sample code (see Table 5.1 for more information on sample locations).

Pairwise F_{ST} values after FDR correction for multiple testing are shown for all samples in Table 5.2. Statistically significant differences were observed in 15 of the 153 pairwise population comparisons. The AMOVA showed that the global F_{ST} value across all samples amounted to 0.036, with 96.44% of the variation being explained by differences within populations, while only a small percentage (3.56%) resulted from differences among populations. The correlation between genetic [F_{ST} / (1-F_{ST})] and geographic distances is presented in Fig. 5.3. The Mantel test showed a highly significant positive correlation between genetic divergence of populations and linear geographic distances among all samples (Z = 11909.48, r = 0.63; null hypothesis of r <= 0: one sided $p = 0.0001$ from 30,000 randomizations; slope with a value of 3.91×10^{-5} and R^2 of 0.40). A significant isolation-by-distance effect was also detected when analyzing the Atlantic cluster only ($p = 0.041$).

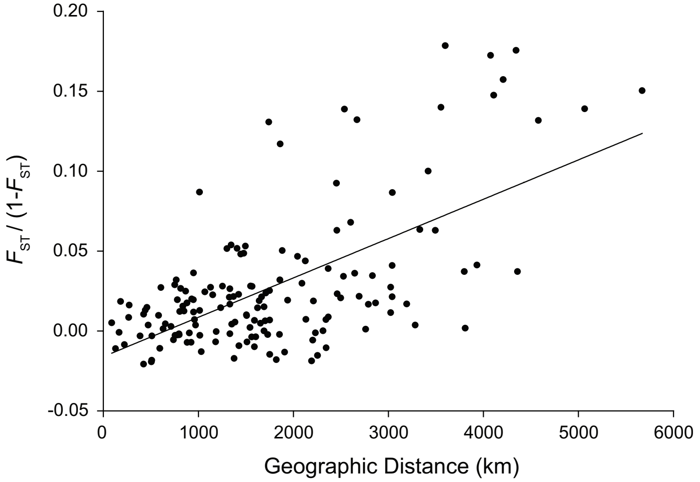

Fig. 5.3. Isolation-by-distance for *Scrobicularia plana*. Genetic distances [F_{ST} / (1-F_{ST})] were plotted against geographical distance (minimal coastline distance) between all sites. Mantel test for matrix correlation: Z = 11909.48, r = 0.63; null hypothesis of r <= 0: one sided $p = 0.0001$ from 30,000 randomizations. The slope had a value of 3.91×10^{-5} and R^2 of 0.40.

Tests to detect additional geographic structure were performed by grouping the samples in to three clusters: Trondheim (TRO), Atlantic (TJ, WS, BA, TER, KB, CLO, PLY, KL, RO, MP, AS, PV, CA, AL, CZ, AG) and Pisa (PI). Pisa was considered a different group given the significant differences in pairwise F_{ST} values between Pisa and 11 other populations. As for the Trondheim cluster, its placement in a distinct group was justified by significant pairwise F_{ST} values (Table 5.2) and the identifiable cluster formed by three of its private alleles (Fig.5.1) which constitutes the only geographically restricted clade observed in the haplotype network. Results from the AMOVA analysis revealed evidence of differentiation among the three groups ($F_{CT} = 0.111$, $p = 0.019$) as well as significant variance among populations within groups ($F_{SC} = 0.003$, $p < 0.001$) and among individuals within populations (F_{ST} =

0.114, $p < 0.001$). The percentage of total molecular variation was 88.57% within groupings, 0.31% among samples within groups and 11.12% among groups, indicating that the majority of variation observed was explained by differences within populations.

Historical demography

Significant departure from neutrality, as determined by R2 and Fu's FS tests was observed for all three groups (Table 5.3). Similar results were obtained with the Tajima's D test, although the Trondheim sample was non-significant in this case, which may be due to higher power of R2 and Fu's FS tests (Ramos-Onsins & Rozas 2002). Mismatch distributions of the three groups are shown in Fig. 5.4. Two of the groups were characterized by a clear unimodal distribution, while Trondheim seems to also have a unimodal distribution that has apparently been under sampled. Visually, the Atlantic group fit a typical L-shaped curve, while Trondheim and Pisa were closer to a bell-shaped curve. All groups were characterized by low and non-significant raggedness values (not shown).

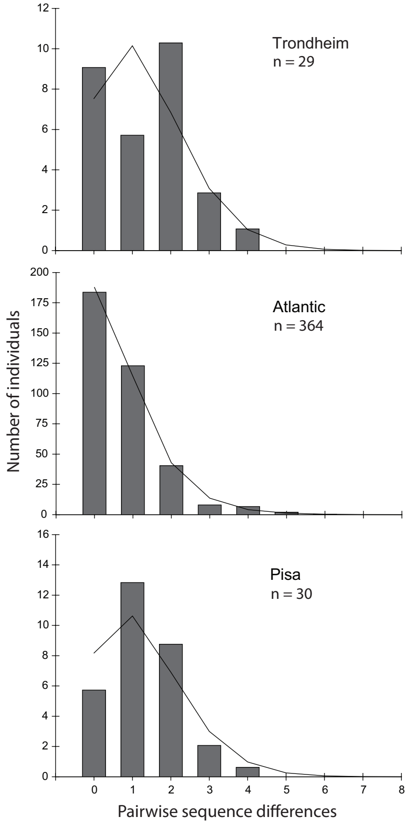

Fig. 5.4. Mismatch distribution of COI sequences of *Scrobicularia plana*. Gray bars represent observed distribution of pairwise differences among individuals from each group while black line corresponds to expected distribution under a model of sudden expansion.

Table 5.2. Pairwise population comparisons. Below diagonal are pairwise F_{ST}'s based on Kimura two-parameters distances between haplotypes. Above diagonal are adjusted p-values after False Discovery Rate correction. In bold are values significantly different from zero (p-level 0.05), while in italics are values also significant at the Bonferroni corrected level (p-level 0.0003268 of uncorrected values).

Site	TRO	TJ	WS	BA	TER	RI	CLO	PLY	KL	RO	MP	AS	PV	CA	AL	CZ	AG	PI
TRO	*	0.260	**0.009**	0.060	0.060	0.260	0.080	0.250	0.263	0.062	0.214	0.357	0.354	0.089	0.075	0.250	0.250	***0.000***
TJ	0.03	*	0.501	0.250	0.443	0.870	0.784	0.741	0.699	0.461	0.495	0.824	0.260	0.214	**0.009**	0.214	0.586	***0.005***
WS	0.05	0.00	*	0.756	0.355	0.641	0.525	0.850	0.505	0.617	0.453	0.588	0.581	0.260	**0.027**	0.260	0.517	***0.004***
BA	0.05	0.03	-0.01	*	0.354	0.280	0.250	0.517	0.453	0.260	0.235	0.250	0.517	0.280	**0.037**	0.185	0.260	***0.002***
TER	0.05	0.01	0.01	0.02	*	0.365	0.463	0.372	0.315	0.080	0.214	0.354	0.311	0.250	0.137	0.641	0.260	***0.000***
RI	0.03	-0.02	0.00	0.02	0.01	*	0.928	0.784	0.756	0.534	0.461	0.916	0.281	0.461	0.688	0.250	0.517	**0.008**
CLO	0.04	-0.01	0.01	0.02	0.00	-0.02	*	0.641	0.617	0.238	0.311	0.617	0.224	0.260	0.372	0.214	0.432	***0.000***
PLY	0.03	-0.01	-0.01	0.00	0.01	-0.01	0.00	*	0.617	0.609	0.517	0.748	0.443	0.437	0.354	0.260	0.628	**0.009**
KL	0.02	-0.01	0.00	0.01	0.02	-0.01	0.00	0.00	*	0.321	0.617	0.748	0.573	0.260	0.357	0.354	0.386	***0.000***
RO	0.04	0.00	0.00	0.02	0.03	0.01	0.01	0.00	0.01		0.617	0.771	0.372	0.280	0.214	0.260	0.654	0.080
MP	0.03	0.01	0.01	0.03	0.03	0.00	0.01	0.00	0.00	0.00	*	0.924	0.381	0.280	0.684	0.461	0.942	0.060
AS	0.02	-0.02	0.00	0.02	0.01	-0.02	0.00	-0.01	-0.01	-0.01	-0.02	*	0.539	0.751	0.756	0.389	0.870	0.078
PV	0.03	0.06	0.00	0.00	0.05	0.05	0.08	0.02	0.00	0.02	0.03	0.01	*	0.447	0.617	0.453	0.234	0.080
CA	0.04	0.03	0.02	0.02	0.03	0.01	0.02	0.01	0.02	0.02	0.02	-0.02	0.01	*	0.517	0.372	0.354	***0.000***
AL	0.04	0.02	0.02	0.02	0.02	0.00	0.01	0.01	0.01	0.01	0.00	-0.01	0.00	0.00	*	0.770	0.680	***0.000***
CZ	0.04	0.06	0.03	0.06	-0.01	0.04	0.05	0.03	0.02	0.02	0.01	0.02	0.03	0.02	-0.01	*	0.260	0.060
AG	0.04	0.00	0.00	0.02	0.02	0.00	0.01	0.00	0.01	-0.01	-0.02	-0.02	0.05	0.02	0.00	0.03	*	0.078
PI	0.13	0.12	0.12	0.15	0.15	0.13	0.15	0.12	0.14	0.06	0.09	0.08	0.12	0.12	0.10	0.12	0.08	*

Table 5.3. Tests of neutrality within the four groups of *Scrobicularia plana*.

Groups	**Fu's FS (*p*-value)**	**Tajima's D (*p*-value)**	**R2 (*p*-value)**
Trondheim	-3.953 (0.007)	-1.500 (0.059)	0.063 (0.001)
Atlantic	-3.403×10^{38} (0.000)	-2.555 (0.000)	0.009 (0.010)
Pisa	-5.357 (0.001)	-1.535 (0.046)	0.064 (0.006)

Population size (θ), migration (*m*) and splitting time (*t*) parameters were estimated for Trondheim, Atlantic and Pisa groups (Table 5.4). Although we were unable to obtain high ESS values, the lack of a pattern in the trend line plots, the high swapping rates, low autocorrelations and similarity of posterior estimates generated from different runs suggested convergence in most parameters (Appendix 5.3). The splits between the Mediterranean and all other Atlantic groups appear to be the oldest at between 0.30 and 1.10 million years ago (Mya). The splitting time between Trondheim and Atlantic is estimated to have been more recent, occurring 0.10-0.37 Mya. Migration rates were estimated to be extremely low among all three groups.

Discussion

Population structure

Our analysis of mitochondrial sequences of *Scrobicularia plana* revealed weak population structure along the species distributional range. Low levels of differentiation were indicated by low F_{ST} values between populations and groupings. Minimal population structure can be attributed to both a recent expansion and high gene flow. Negative values of neutrality tests point to an excess of low frequency polymorphisms indicating population expansion following a bottleneck or selective sweep (Tajima 1989). This fits well with the topology of the haplotype network, which is dominated by a single, high frequency, ancestral haplotype and many 1-step peripheral ones (Slatkin & Hudson 1991). Demographic events can also be inferred from the mismatch analysis, as the distribution of pairwise differences is shaped by episodes of population growth or decline (Rogers & Harpending 1992). Under population growth or directional selection, the mismatch distribution is smooth and unimodal with low raggedness values, whereas ragged multimodal distributions result from long-term stationary populations (Harpending 1994). The unimodal distributions of the all groups are indicative of recent population expansion with the L-shaped distributions of the Atlantic group suggesting a recent genetic bottleneck, with the subsequent rapid population expansion creating a "wave" in the distribution that will shift to the right over time (Rogers & Harpending 1992). The non-significant raggedness values observed for all groups are also indicative of expanding populations.

Table 5.4. Coalescent estimates of population size parameter (θ) of current populations (1 and 2) and their ancestral population (A), migration parameter (*m*; equal migration rate in both directions was assumed) and the splitting time parameter (*t*) between populations (1) and (2) were derived from combined parameter distributions of four IMa runs. Effective population size (N_e), time since divergence (t) were estimated based on a mutation rate μ of 0.355×10^{-6} to 1.315×10^{-6} substitutions per sequence per year, assuming a generation time of two years. Error ranges are described by the 95% highest posterior density (HPD).

Model	θ_1	θ_2	θ_A	$N_{e(1)}$, millions of individuals	$N_{e(2)}$, millions of individuals	$N_{e(A)}$, millions of individuals	$m_{1<>2}$	migration rate per year	t	t, milion years
Trondheim (1) vs. Atlantic (2)										
Marginal peak location	14.3	2327.6	15.1	1.36 - 5.02	221.25 - 819.56	1.44 - 5.33	0.388	1.38×10^{-7} - 5.10×10^{-7}	0.13	0.10 - 0.37
Lower 95% HPD	4.5	85.5	0	0.43 - 1.58	8.13 - 30.11	0	0	0	0.06	0.04 - 0.16
Upper 95% HPD	76.5	2999	43.5	7.27 - 26.94	285.08 - 1055.99	4.13 - 15.32	3.75	1.33×10^{-6} - 4.93×10^{-6}	0.39	0.29 - 1.09
Atlantic (1) vs. Pisa (2)										
Marginal peak location	146.2	14.4	0.8	13.89 - 51.47	1.37 - 5.06	0.08 - 0.28	0.021	7.46×10^{-9} - 2.76×10^{-8}	0.34	0.26 - 0.96
Lower 95% HPD	52.5	4.5	0	4.99 - 18.49	0.43 - 1.58	0	0	0	0.11	0.08 - 0.30
Upper 95% HPD	1238	73.5	31.5	177.68 - 435.92	6.99 - 25.88	2.99 - 11.09	0.83	2.95×10^{-7} - 1.09×10^{-6}	0.57	0.43 - 1.59
Trondheim (1) vs. Pisa (2)										
Marginal peak location	14.3	19.8	2.2	1.36 - 5.04	1.88 - 6.95	0.21 - 0.78	0	0	0.39	0.30 - 1.10
Lower 95% HPD	0	4.5	0	0	0.43 - 1.58	0	0	0	0.12	0.09 - 0.34
Upper 95% HPD	106.5	2735	1544	10.12 - 37.50	259.98 - 963.03	146.77 - 543.66	7.51	2.67×10^{-6} - 9.88×10^{-6}	4.00	3.04 - 11.26

Species with a pelagic larval life stage, such as *Scrobicularia plana*, are expected to show high gene flow between populations as a consequence of their high dispersal potential in combination with the apparent absence of geographical barriers (e.g., Hellberg et al. 2002, Carr et al. 2003, Palumbi 2003). However, there is little direct evidence that marine populations are demographically open and broadly connected over large spatial scales with several studies highlighting the heterogeneity in dispersal scale among marine species (e.g., Kinlan & Gaines 2003, Kinlan et al. 2005, Bowen et al. 2006, Cowen et al. 2007, Weersing & Toonen 2009, Riginos et al. 2011). Our study indicates restricted gene flow between populations, suggested by the observed isolation-by-distance effect. Geographical distance explained almost 40% of the genetic variation along the distribution range. This suggests that populations of S. plana are self-recruiting. Local retention has been observed in several marine populations (e.g., Swearer et al. 2002, Jones et al. 2005, Almany et al. 2007, Bradbury et al. 2008, Galindo et al. 2010) and it may be related to the species' ecological and habitat characteristics (Swearer et al. 2002, Cowen & Sponaugle 2009). Moreover, although *S. plana* populations show high haplotype diversity, the observed values are relatively low when compared to other marine bivalves (Appendix 5.4) which may also be an effect of local retention. In this sense, isolation may be associated with *S. plana*'s habitat-related, patchy spatial distribution. The low levels of genetic differentiation observed between groups may then not be a result of high gene flow but instead of large effective population sizes, in combination with the recent population expansion. The low estimated migration rates between groups constitute further evidence of contemporary isolation and ongoing divergence.

In the absence of gene flow, selection and genetic drift will be the initial driving forces of interpopulation differentiation by acting on existing genetic variation (Hellberg et al. 2002). Patterns of reduced neutral variation can be produced by both selective sweeps (Smith & Haigh 1974, Kaplan et al. 1989) and background selection (Charlesworth et al. 1993, Hudson & Kaplan 1995). As selective sweep is a process by which a selected mutations reduce variability in linked neutral sites as it increases in frequency in the population (Nielsen 2005). Background selection can occur when deleterious alleles are maintained by recurrent mutation (Charlesworth et al. 1993), which also reduces neutral diversity since the elimination of a deleterious mutation lowers the frequencies of any associated neutral or nearly neutral variants (Kreitman & Akashi 1995). The frequency distribution of segregating mutations may help distinguish these two processes. While a selective sweep will cause an excess of private alleles leading to a star-like genealogy of the marker considered, this is not the case in background selection (Slatkin 1985, Charlesworth et al. 1993, Kreitman & Akashi 1995). The star-shaped network observed for *Scrobicularia plana* suggests that background selection is not the cause of the low levels of differentiation. A similar population structure could, however, be observed due to demographic causes such as the recovery of variation following a population bottleneck. The reduction in population size is likely to eliminate many rare variants, reducing polymorphism. As the population recovers, a rapid expansion leads to new (rare) mutations which results in an apparent excess in allelic diversity (Maruyama & Fuerst 1985, Simonsen et al. 1995). Comparisons of other species from the same region can help to distinguish between potential biogeographical scenarios (Avise 2000) and mechanisms. The population structure of *S. plana* is distinct from what has been observed for either *Macoma balthica* (Luttikhuizen et al. 2003) or *Cerastoderma edule* (Krakau et al. 2012) from the same area. *M. balthica* consists of deeply diverged lineages which may be explained by considerably longer divergence times. Divergence time does not, however, seem to explain the differences between *S. plana* and *C. edule* with the later showing a more structured minimum spanning network despite similar divergence times. It is thus likely that the two species coped with the LGM differently through differential habitat selection. At present, population genetic surveys are still mainly conducted with markers assumed to be neutral so that the effects of selection cannot be discerned. To distinguish between demographic and selective causes of a recent reduction of genetic variability, different loci need to be analyzed since demographic events apply to the whole genome while selective events affect only distinct regions of the genome (Galtier et al. 2000). Note, however, that if selection is operating, the molecular clock hypothesis may not hold (Tajima 1993). In addition, rates of molecular evolution may vary considerably, both through time and among lineages (Smith & Peterson 2002) which emphasizes once again the need to analyze other genetic markers and to interpret molecular clock estimates with due caution.

The last glacial maximum

During the LGM, the extension of ice sheets as far south as the northern coast of the Iberian Peninsula (Frenzel et al. 1992) caused *Scrobicularia plana*'s northern edge of distribution to likely contract to that area. As the ice retracted, new habitats became available which would have allowed the northern spread from its periglacial refugia. This postglacial recolonization would have led to a loss of genetic diversity along the leading edge, as a consequence of random genetic drift , resulting in a gradient of decreasing genetic variation with increasing latitude (Hewitt 2004).

Although individual populations of *Scrobicularia plana* have considerable variation in haplotype diversity, a general decreasing trend with increasing latitude (although non-significant) is observed. Four locations, however, stand out due to their higher haplotype diversity and presence of private haplotypes: Trondheim, Roscoff, Algarve and Pisa. It is probable that these locations served as refugia.

The Iberian Peninsula and the Mediterranean Sea are widely recognized glacial refugia as several marine species were confined to those areas as ice sheets expanded south (e.g. Consuegra et al. 2002, Gysels et al. 2004, Sá-Pinto et al. 2005, Chevolot et al. 2006, Hoarau et al. 2007, Mäkinen & Merilä 2008). The high haplotype diversity observed for the populations of Pisa and Algarve and the high level of differentiation of the Mediterranean population do suggest that these locations served as refugia for Scrobicularia plana.

The Brittany/English Channel area is also a well-known refuge for marine species (Coyer et al. 2003, Provan et al. 2005, Chevolot et al. 2006, Hoarau et al. 2007, Remerie et al. 2009, Campo et al. 2010, Olsen et al. 2010, Krakau et al. 2012). *Scrobicularia plana* also appears to have had a refuge population in this area as suggested by the high haplotype diversity of the

Brittany sample, with only two southern populations presenting higher values. The presence of this refugial area could be explained by the drop in sea-level during the LGM (Frenzel et al. 1992) which exposed an ice-free terrestrial depression, in the nascent English Channel, known as the Hurd Deep (Lericolais et al. 1995, Lericolais et al. 2003). This depression may have persisted as a marine lake in which marine organisms were able to survive until sea levels began to rise after the LGM (Provan et al. 2005).

Northern Norway, on the other hand, is not commonly recognized as a marine refuge. Although ice free areas in Scandinavia (Sutherland 1984, Vorren et al. 1988, Svendsen et al. 2004) suggest that this area may also have served as a northern refuge, evidence remains scant [*Cerastoderma edule* (Krakau et al. 2012) and the fucoid macroalga *Fucus distichus* (Coyer et al. 2011)]. The high haplotype diversity observed for the Norwegian sample, including four private alleles, is consistent with a northern refugium for *Scrobicularia plana*. Moreover, as the splitting of the Trondheim group precedes the LGM (see Table 5.4), the high variability of the Norwegian population is most likely not a result of a recolonization event but of the presence of a glacial refugium.

In summary, genetic diversity patterns are commonly used to determine range expansion routes. In our study, the high genetic diversity and heterogeneity of Scrobicularia plana populations in glaciated areas (Brittany Peninsula and Scandinavia) suggest a colonization of these areas prior to the LGM and survival in refugial areas. Several marine species follow this pre-LGM expansion model (e.g. Luttikhuizen et al. 2003, Gysels et al. 2004, Provan et al. 2005, Chevolot et al. 2006, Hoarau et al. 2007, Campo et al. 2010), with their ability to persist in glaciated areas depending on the species' biological and ecological constraints.

Conclusion

The present study revealed a weak population genetic structure of the bivalve *Scrobicularia plana* but a significant isolation-by-distance effect, with the species' patchy spatial distribution likely contributing to low connectivity. *S. plana* has undergone recent expansion between 0.30-1.10 Mya, which in combination with the high population sizes, explains the weak geographic pattern of genetic variation. During the LGM, *S. plana* would have retreated to southern Europe, although some populations likely survived in ice-free areas along the Brittany Peninsula and Scandinavia, the latter only recently recognized as a glacial refuge for marine species (Coyer et al. 2011, Krakau et al. 2012). Results were, however, obtained using only mitochondrial COI data. Since different genetic markers can reflect different aspects of population biology and history, only by combining data from several markers is it possible to fully understand the complex demographic history of a species (Eytan & Hellberg 2010).

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Appendix 5.1. Polymorphic sites in COI haplotypes identified for *Scrobicularia plana*.

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	1	1	2	3	5	5	5	5	8	9	0	1	1	2	2	2	2	3	5	6	6	7	7	8	9	0	0	1	1	1	1	3	7	8	9	9	0	1	2	3	6	7	8	9	1	1	2	3	3	4	6	6	7	8	8	9	9	9
haplotype	1	7	6	8	0	3	6	8	6	3	4	0	9	1	5	8	9	4	8	4	7	0	9	5	4	0	7	0	2	5	8	9	7	7	0	6	2	1	3	8	2	4	9	8	0	6	8	0	2	3	6	8	9	3	8	2	5	9
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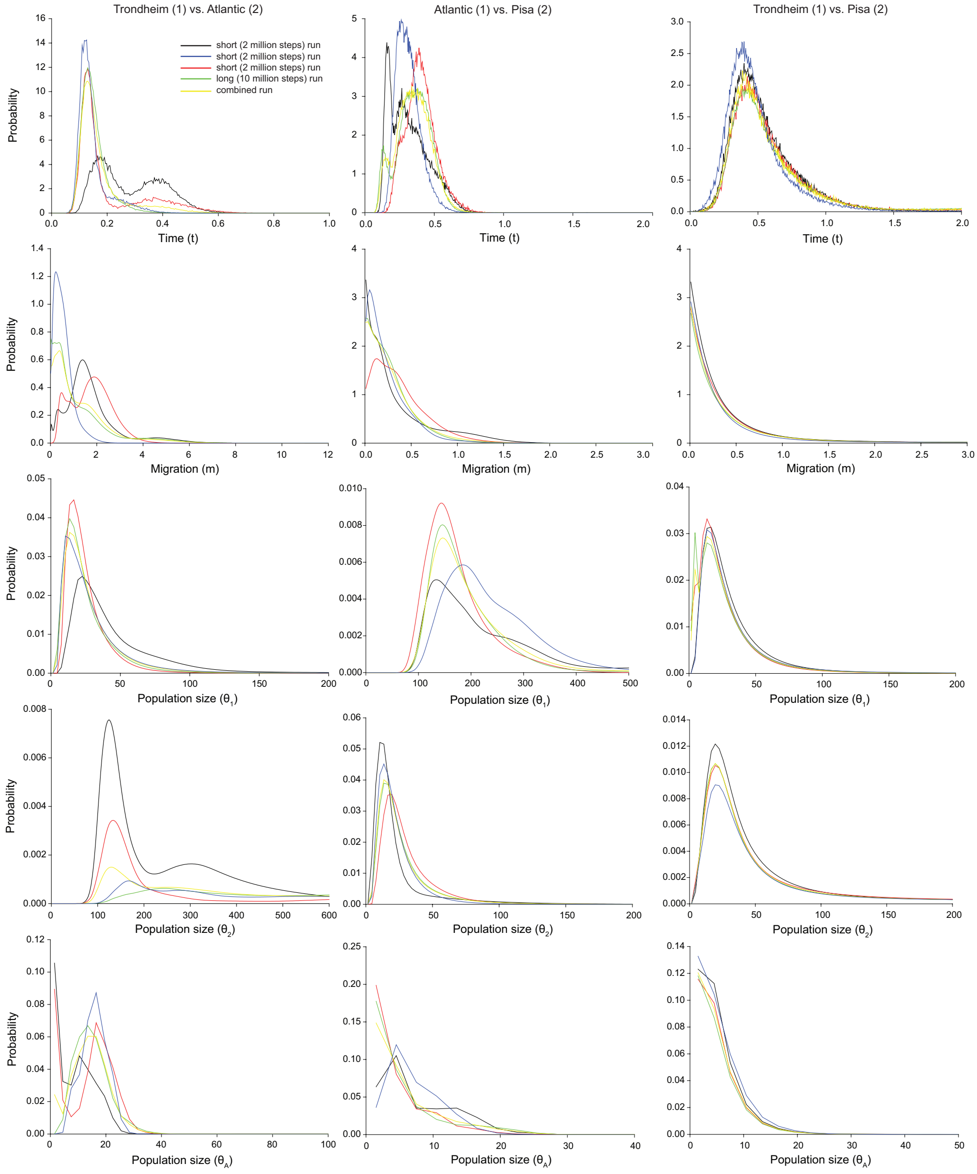
h 63	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
h 64	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
h 65	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Appendix 5.2. Haplotype occurrence, nucleotide diversity (π) and haplotype diversity (h) per sampled site, with corresponding standard deviation (stdev).

Code	Site	h 01	h 02	h 03	h 04	h 05	h 06	h 07	h 08	h 09	h 10	h 11	h 12	h 13	h 14	h 15	h 16	h 17	h 18	h 19	h 20	h 21	h 22	h 23	h 24	h 25	h 26	h 27	h 28	h 29	h 30	h 31	h 32	h 33	h 34	h 35	h 36	h 37	h 38
TRO	Trondheim, Norway	16	0	0	0	0	1	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	3	0
TJ	Tjärnö, Sweden	21	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WS	German Wadden Sea	24	0	0	0	1	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
BA	Balgzand, Netherlands	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TER	Terneuzen, Netherlands	23	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
KB	Killala Bay, Ireland	17	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
CLO	Clonakilty Estuary, Ireland	24	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
PLY	Plymouth, England	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
KL	King's Lynn, England	21	0	0	0	0	2	0	0	0	1	0	2	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RO	Roscoff, France	13	0	0	0	2	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MP	Moeze Plaisance, France	13	2	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AS	Astúrias, Spain	13	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PV	Ponte Vedra, Galicia, Spain	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CA	Caminha, Portugal	17	0	0	0	0	1	2	0	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AL	Tavira, Algarve, Portugal	9	1	0	0	0	0	0	0	2	0	1	0	0	0	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CZ	Cádiz, Spain	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
AG	Agadir,Morocco	13	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PI	Pisa, Italy	10	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5	1	1	1	1	1	0	0	0	0
	sum	284	4	1	5	14	12	3	1	5	5	1	5	1	1	4	11	1	1	5	1	1	1	1	1	1	1	2	5	5	1	1	1	1	1	1	1	3	1

Code	Site	h39	h40	h41	h42	h43	h44	h45	h46	h47	h48	h49	h50	h51	h52	h53	h54	h55	h56	h57	h58	h59	h60	h61	h62	h63	h64	h65	sum	π (stdev)	*h* (stdev)
TRO	Trondheim, Norway	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0.0027 (0.0019)	0.6872 (0.0905)
TJ	Tjärnö, Sweden	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0.0007 (0.0008)	0.3508 (0.1172)
WS	German Wadden Sea	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0.0015 (0.0012)	0.4375 (0.1072)
BA	Balgzand, Netherlands	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	27	0.0008 (0.0009)	0.3932 (0.1136)
TER	Terneuzen, Netherlands	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0.0009 (0.0009)	0.3695 (0.1097)
KB	Killala Bay, Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0.0010 (0.0010)	0.4625 (0.1283)
CLO	Clonakilty Estuary, Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0.0007 (0.0008)	0.3202 (0.1116)
PLY	Plymouth, England	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0.0012 (0.0011)	0.3684 (0.1351)
KL	King's Lynn, England	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0.0014 (0.0012)	0.5103 (0.1087)
RO	Roscoff, France	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	25	0.0020 (0.0015)	0.7333 (0.0952)
MP	Moeze Plaisance, France	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0.0016 (0.0014)	0.5380 (0.1330)
AS	Astúrias, Spain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	0.0019 (0.0015)	0.4837 (0.1382)
PV	Ponte Vedra, Galicia, Spain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0.0016 (0.0015)	0.5556 (0.1653)
CA	Caminha, Portugal	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0.0025 (0.0018)	0.5723 (0.1114)
AL	Tavira, Algarve, Portugal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	24	0.0029 (0.0020)	0.8623 (0.0666)
CZ	Cádiz, Spain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0.0014 (0.0013)	0.6000 (0.1539)
AG	Agadir,Morocco	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	17	0.0012 (0.0011)	0.4265 (0.1468)
PI	Pisa, Italy	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0.0026 (0.0018)	0.8092 (0.4610)
	sum	2	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	423		

Appendix 5.3. Marginal posterior probability distributions for isolation with migration analytic (IMa) model parameters of three distinct models: Trondheim vs. Atlantic, Atlantic vs. Pisa and Trondheim vs. Pisa. For each model, four IMa independent runs (black, blue and red represent the three 2 million steps runs while green corresponds to one 10 million step run) and combined run (yellow) are represented. A = ancestral population. Note that values correspond to model parameter estimates. For corresponding demographic parameters estimates check Table 4.



Appendix 5.4. Haplotype diversity (h) in the mtDNA COI gene for several marine bivalve species.

Species	**Geographic region**	**Mean h (range)**	**Reference**
Anomalocardia brasiliana	NW Atlantic Ocean	0.67 (0.45-0.85)	Arruda et al. 2009
Austrovenus stutchbur	SW Pacific Ocean	0.85 (0.58-0.97)	Ross et al. 2012
Brachidontes spp.	Indian Ocean, Pacific Ocean and Mediterranean/Red Sea	0.98 (0.97-1.00)	Terranova et al. 2007
Brachidontes exustus	Gulf of Mexico and NW Atlantic Ocean	0.88 (0.55-0.98)	Lee & Foighil 2004
Cerastoderma edule	NE Atlantic Ocean	0.63 (0.14-0.93)	Krakau et al. 2012
Cerastoderma glaucum	NE Atlantic Ocean and Southern Europe	0.63 (0.21-0.91)	Tarnowska et al. 2010
Mercenaria mercenaria	NW Atlantic Ocean	0.84 (0.86-0.95)	Baker et al. 2008
Mytilopsis sallei	Indian Ocean and Pacific Ocean	0.69 (0.52-0.79)	Wong et al. 2011
Mytilus coruscus	NW Pacific Ocean	0.87 (0.72-0.92)	Shen et al. 2009
Mya arenaria	NW Atlantic Ocean, NE Pacific Ocean and North Sea	0.39 (0.00-0.65)	Strasser & Barber 2009
Pinna nobilis	Mediterranean Sea	0.67 (0.40-0.81)	Rabaoui et al. 2011
Ruditapes decussatus	Mediterranean Sea	0.49 (0.17-0.76)	Gharbi et al. 2010
Ruditapes philippinarum	NW Pacific Ocean	0.96 (0.80-1.00)	Mao et al. 2011
Scrobicularia plana	NE Atlantic Ocean and Southern Europe	0.52 (0.32-0.86)	present study
Spisula spp.	NW Atlantic Ocean	0.73 (0.42-0.86)	Hare & Weinberg 2005
Tridacna crocea	Indo-West Pacific Oceans	0.91 (0.80-1.00)	DeBoer et al. 2008
Tridacna maxima	Indo-Malay Archipelago and Red Sea	0.94 (0.59-1.00)	Nuryanto & Kochzius 2009

Chapter 6

Spatial autocorrelation analysis of the distribution of the bivalve *Scrobicularia plana* along the European coast: a species or site specific trait?

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Abstract

The spatial scales across which organisms interact with each other and the structuring influence of their environments can be determined by analyzing species' distribution patterns. Here, the spatial variation in the distribution of *Scrobicularia plana* is described for four intertidal areas along the species' distributional range. Spatial autocorrelation correlograms based on Moran's coefficient reveal that, while the Trondheim (Norway) population was randomly distributed, at Minho (Portugal), the Westerschelde and the Wadden Sea (both in the Netherlands) populations were aggregated. Patch diameter varied from 150 to 1250 m, in Minho and Westerschelde, respectively; while in the Wadden Sea, patches of 4-10 km were detected. Comparisons of spatial patterns with those of other co-occurring bivalve species (*Abra tenuis, Cerastoderma edule* and *Macoma balthica*) revealed that *S. plana*'s distribution was generally patchier. The distribution of *S. plana* was found to be correlated with sediment type at Westerschelde and Trondheim, but not Minho. The observed differences in distribution patterns and their correlation with environmental factors reveal that spatial patterns of *S. plana* are site-specific rather than species-specific.

Introduction

Intertidal mudflats are valuable ecological entities that support large populations of birds (e.g. Beukema et al. 1993, Zwarts & Wanink 1993, Ens et al. 1994, Leguerrier et al. 2003, van de Kam et al. 2004), and act as nursery and feeding areas for fish and crustaceans (Martinho et al. 2007, Campos et al. 2010). Macrobenthic communities are key elements of intertidal ecosystems, playing an important role in the food web and system dynamics (Herman et al. 1999). To help understand the factors and processes shaping these soft-sediment communities, identification and quantification of spatial patterns is essential.

The spatial pattern of a population is the result of a balance between dispersal and aggregation (Heip 1975, Meire et al. 1989, Folmer et al. 2010). Spatial patterns can be characterized by two aspects: intensity and form (Andrew & Mapstone 1987). Intensity, which relies on the distribution of density estimates, sorts distributions into three types: uniform, random and aggregated (Meire et al. 1989, Thrush et al. 1989, Thrush 1991). A uniform pattern means that individuals are arranged in a regular way, likely due to intraspecific competition; while in the random pattern the space occupied by an individual is independent of the space occupied by others. In the aggregated pattern, individuals are clumped together as a result of environmental heterogeneity, predation pressure, competition or reproductive behavior (Heip 1975, Meire et al. 1989). As for the form of the spatial pattern, it corresponds to the size of patches and can be assessed with, e.g., spatial autocorrelation which analyzes the degree of dependency among observations in relation to the arrangement of individuals in space (Thrush et al. 1989, Thrush 1991).

Since the majority of natural environments are patchy and environmental heterogeneity tends to cause aggregated distributions, most populations are aggregated to some extent (Levinton 1972, Heip 1975, Barry & Dayton 1991, Kraan et al. 2009). Spatial aggregation can occur on different scales, e.g. from the geographical distribution of a species to the physical structure of the sediment (de Wolf 1989, Underwood & Chapman 1996, Kraan et al. 2010) and, as a consequence, ecological variables and processes tend to be scale-dependent (Legendre & Fortin 1989). By analysing local spatial patterns of intertidal macrobenthic assemblages, the proportion of the variation that is attributable to each spatial or temporal scale can be quantified.

Highly patchy spatial distributions are typical for many hard substrate invertebrates and also, for example, reef forming bivalves such as mussels; while soft bottom infauna is generally more evenly distributed (Meire et al. 1989). Nevertheless, a patchy distribution has been recorded in several co-distributed soft bottom bivalves such as, e.g., *Mya arenaria* (Strasser et al. 1999, Bocher et al. 2007), *Macoma balthica* and *Cerastoderma edule* (Meire et al. 1989, Kraan et al. 2009), and *Scrobicularia plana* (Hughes 1970, Langston 1986). Local distribution patterns of intertidal molluscs, however, may vary between sites and only by comparing the spatial patterns from different areas can we determine if patterns are species or site specific (Bocher et al. 2007).

Here, we focus on the peppery furrow shell, *Scrobicularia plana*, a common species in European intertidal soft-sediment communities (Bocher et al. 2007). *S. plana* seems to be characterized by an overall patchily distribution (Hughes 1970, Langston 1986), which appears to be determined by the existence of specific environmental conditions during settlement (Santos et al. 2011b). The species is an important food source for avifauna and ichtyofauna (e.g. Hughes 1970, Moreira 1997), and economically valuable, especially in southern Portugal and Spain (Langston et al. 2007). The species is present along the NE Atlantic coast, from the Norwegian and Baltic Seas in the north to Senegal in the south, and in the Mediterranean Sea (Tebble 1976). Although it can be found in areas of sand, clay or mud, rich in organic matter, it has a clear preference for muddy sediments (Casagranda & Boudouresque 2005, Bocher et al. 2007).

In this study, we analyzed the spatial distribution of *Scrobicularia plana*, as well as several co-distributed bivalve species, at four sites and two spatial scales.

Material & Methods

Field sampling

Distributions and densities of benthic bivalves were determined for three smaller-scale intertidal areas along the European coast: Minho, Portugal (N41°51-52’, E08°49-50’); Westerschelde, The Netherlands (N51°20-21’, E03°42-44’); and Trondheim, Norway (N63°18-19’, E10°09-11’); as well as, one larger scale area, the Dutch Wadden Sea (Fig. 6.1). In order to estimate temporal changes and spatial differences in abundance, while accurately estimating model autocorrelation parameters, the most effective sampling design was a grid with additional random samples placed on the gridlines (Bijleveld et al. 2012). The distance between points varied between locations according to the total area of the sampling sites. In addition to the regular grid, ~10% (Wadden Sea) to 25% (remaining three locations) extra cores were collected at random positions to account for autocorrelations at smaller distances (for the rationale of this approach, see Bijleveld et al. 2012). Description of the dimensions and number of points sampled in each grid can be found in Table 6.1.

At each sample point, identified using GPS coordinates, a sediment core (15 cm diameter, to a depth of 20-25 cm) was taken. The samples were sieved, on site, over a 1 mm mesh and all organisms were collected and stored at -20°C for later analysis in the lab. A second core (6 cm diameter, to a depth of 5 mm) was taken in order to determine grain size composition of the sediment surface layer. Samples were stored at -20°C until further analysis.

Laboratory analyses

All bivalves collected were identified and counted. Sediment samples were freeze-dried (Christ Alpha 1-4 LD plus) and then treated to remove organic matter and carbonate material, as described by Van der Bergh et al. (2003). Analysis of median particle size and the percentage of silt (fraction < 63 µm) of sediment samples was performed using a Coulter LS 13 320 particle size analyzer and autosampler.

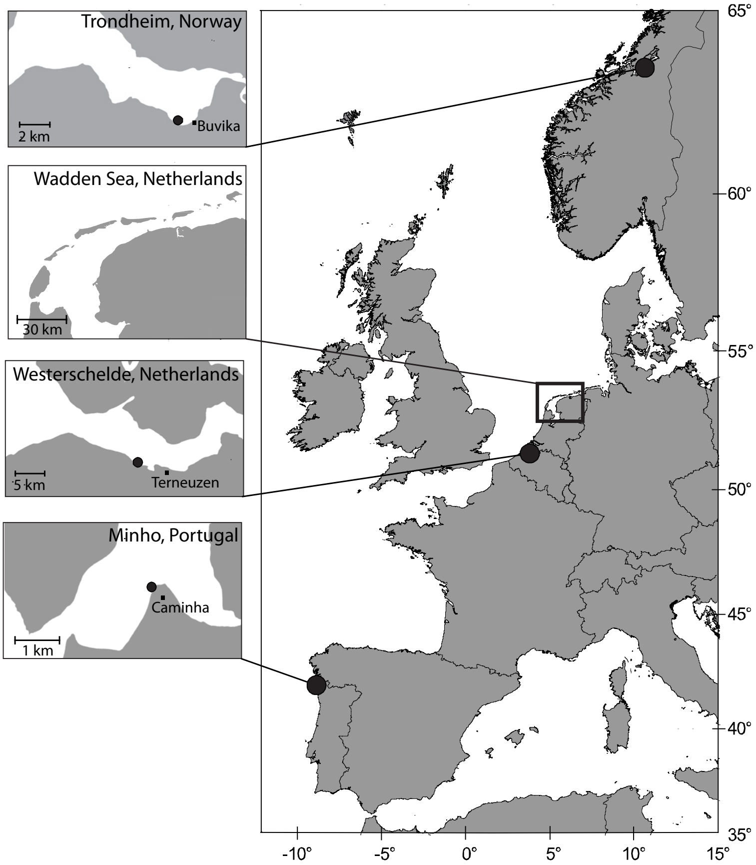

Fig. 6.1. Sampling locations of *Scrobicularia plana* along the European coast.

Statistical analyses

The analyses were performed in two steps. Small-scale patchiness of *Scrobicularia plana* and a number of co-occurring bivalve species was analyzed for Minho, Westerschelde and Trondheim. A larger-scale analysis for *S. plana* was conducted in the Dutch Wadden Sea.

Distribution and density were plotted using the ggplot2 Package (Wickham 2009), in R v. 2.13.1. Spatial autocorrelation (SAC) between abundance and distance was calculated for *Abra tenuis, Cerastoderma edule, Macoma balthica, Mya arenaria* and *Scrobicularia plana* using Moran's index (I), also in R. This index compares geographic neighbors in terms of their deviation from the mean of all observations, estimating an autocorrelation coefficient that varies between -1.0 (negative autocorrelation) and 1.0 (positive autocorrelation), with values near zero under the null hypothesis of no spatial autocorrelation. Where correlations are found, the cross point of the zero line is considered a rough measure of patch diameter.

Table 6.1. Description of dimensions and number of points sampled in each sampling grid. Number of sample points corresponds to total number of points sampled, i.e. including regular grid points as well as ~10% (Wadden Sea) to 25 % (remaining three locations) of randomly placed points along the gridlines. * Each point was sampled at a distance of 50 m northward and 100 m eastward.

Location	**Year**	**Grid length (easting, km)**	**Grid width (northing, km)**	**Total area (km^2)**	**Distance between points (m)**	**# sample points**
Minho, Portugal	2009	1.6	1.6	2.6	50	137
Westerschelde, The Netherlands	2009	3.2	1.0	3.2	100	161
Wadden Sea, The Netherlands	2008	-	-	1500	500	4376
	2009	-	-	1500	500	4771
Trondheim, Norway	2008	1.3	0.7	0.9	50*100	79

Partitioning geographic distances into discrete a priori classes must be evaluated. Classes containing too few pairs of locations will produce less reliable Moran's I values, whereas coarser classes (encompassing a larger increment of geographic distance), while statistically reliable will sacrifice resolution of the actual distance (patch) size. The number of distance classes (or lags) was calculated using Sturges' rule (Sturges 1926). The width of distance classes was determined as the maximum geographic distance divided by two (Rossi et al. 1992) and then subdivided by the number of distance classes. Once distance classes were determined, significance was tested by a Monte Carlo randomization test with 999 permutations, under a one-tailed test for positive autocorrelation (Legendre & Legendre 1998), using the spdep R Package (Bivand et al. 2011). As the pattern of a correlogram may be affected by the lag distances selected, the consistency of patterns was tested by altering the lag distance.

Correlograms, in which Moran's I coefficients are plotted against spatial distances, were generated in the ncf R package (Bjornstad 2009). Correlograms were globally significant only when at least one of its Moran's I values was significant at the Bonferroni corrected level (Oden 1984, Legendre & Legendre 1998). Within each correlogram, the significance of Moran's I for each distance class was tested using Holm's correction for multiple comparisons (Holm 1979). A requirement for this analysis is second-order stationarity, i.e. the mean and variance are constant and the autocorrelation function depends only on the distance between observations (Legendre & Legendre 1998). Therefore, in order to stabilize the mean and variance and avoid deviations that may result in violation of stationarity, frequency data were log-transformed $x' = \log(x + 1)$ prior to analysis (Zar 1996).

The relationship between sediment type, i.e., median particle size and the percentage of silt (fraction <63 μm), and the distribution of *Scrobicularia plana* was determined with a partial Mantel test (Mantel 1967) implemented in the cluster (Maechler et al. 2011) and vegan (Oksanen et al. 2011) R packages. Significance was tested using 10,000 permutations. Probability levels were adjusted using the False Discovery Rate (FDR) correction procedure (Benjamini & Hochberg 1995), which controls the expected proportion of incorrectly rejected null hypotheses (type I errors).

Results

Distribution and spatial autocorrelation in Scrobicularia plana

The numerical distribution of *Scrobicularia plana* over the three smaller-scale sampling sites (Fig. 6.2) shows that mean densities varied between 43 ind m^{-2} (SE = 5.49), in Minho, to 72 ind m^{-2} (SE = 13.81), in Trondheim; while a value of 56 ind m^{-2} (SE = 6.87) was registered for the Westerschelde.

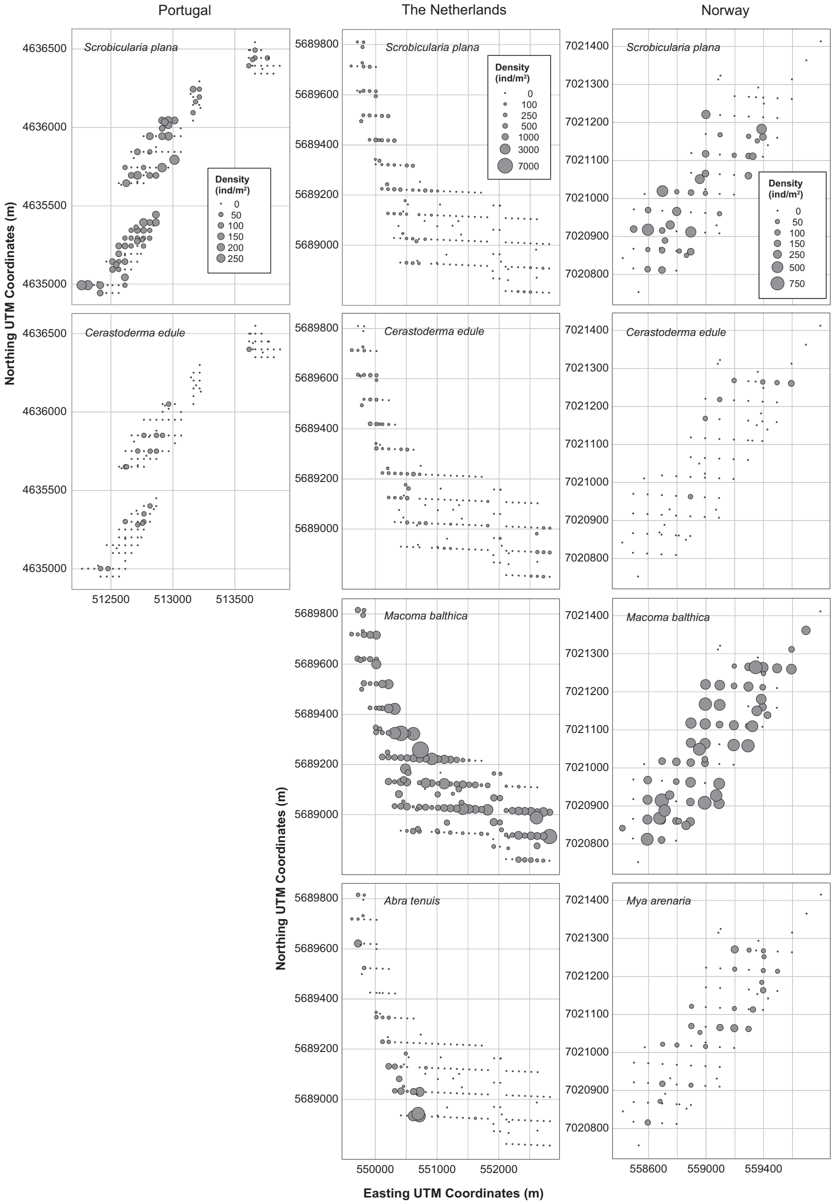

Fig. 6.2. Numerical distribution of *Abra tenuis*, *Cerastoderma edule*, *Macoma balthica*, *Mya arenaria* and *Scrobicularia plana* (whenever present) at three smaller-scale sampling sites: Minho estuary, Portugal; Westerschelde, Netherlands; and Trondheim fjord, Norway. Each dot corresponds to a sample point. Density scale is the same for all sites. Coordinates are associated with the Universal Transverse Mercator coordinate system (UTM), in which the term easting refers to the eastward-measured distance (longitudinal distance), while northing refers to the northward-measured distance (latitudinal distance), both displayed in meters (true scale).

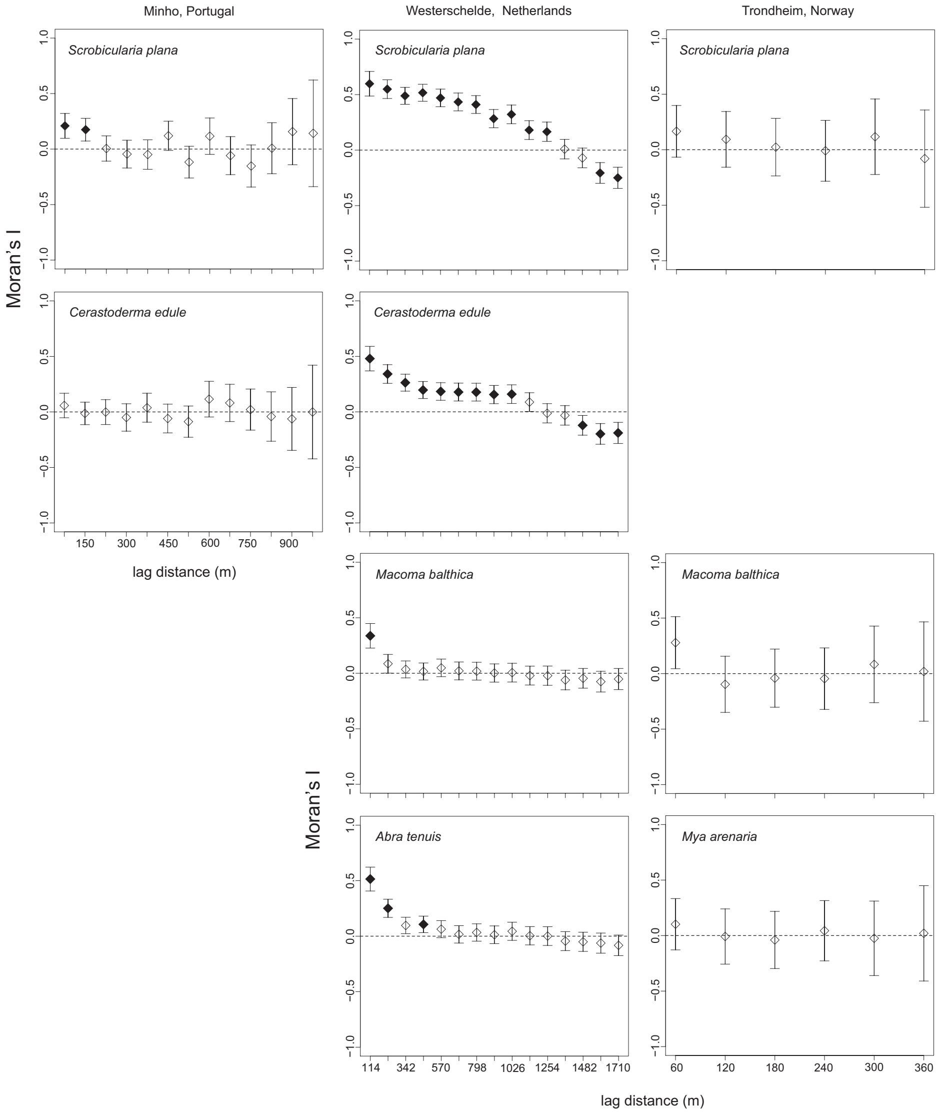

Fig. 6.3. Spatial correlograms of *Abra tenuis*, *Cerastoderma edule*, *Macoma balthica*, *Mya arenaria* and *Scrobicularia plana* at three smaller-scale sampling sites: Minho estuary, Portugal; Westerschelde, Netherlands; and Trondheim fjord, Norway. Full symbols represent statistically significant correlations, while open symbols indicate non-significant values. Please not that distance class scales are different for all locations.

Regarding the SAC analysis, the number of distance classes calculated for each sampling site varied only slightly, with 13 in Trondheim, 14 in Minho and 15 in the Westerschelde. Lag distance was defined as 75 m, 114 m and 60 m, for Minho, Westerschelde and Trondheim, respectively. A distance-based, spatial weights matrix was created for each location, using the defined lag distance as the upper distance bound. Only lags including more than 30 pairs of points were considered (Rossi et al. 1992) and, as a result, while all 15 lags were included in the analysis at Westerschelde; lags 14, for Minho, and 7-13, for Trondheim, were not analyzed. Tests of significance of the Moran's I for each lag revealed that, for *Scrobicularia plana*, lags 1, 2, 6, 8, 12 and 13, from Minho, had a significant positive correlation coefficient. For the Westerschelde, lags 1-11 were significantly correlated while, in Trondheim, only lag 5 was significant.

Correlograms of *Scrobicularia plana* (Fig. 6.3) were globally significant at the Bonferroni corrected level for Minho and Westerschelde, which indicates that the species is patchily distributed at these locations, but not for the Trondheim sampling site. Indeed, densities showed positive autocorrelations at the smallest distance-classes, followed by random oscillations around zero and, in some cases, significantly negative correlation (i.e., dissimilar values appear in close association). *S. plana* in Minho was spatially correlated up to a distance of 150 m. In the Westerschelde, positive spatial correlation was observed for distances up to ~1250 m, while significantly negative correlation was detected at distances > ~1600 m. No spatial correlation was found for *S. plana* in Trondheim. Lag distances of 50 and 100 m for Minho and Trondheim, and of 100, 150 and 200 m for Westerschelde were tested, with no changes on the patterns in the correlograms.

The larger-scale analysis of the Wadden Sea data provided information on SAC of *Scrobicularia plana*; while the comparative sampling over two years also provided an indication of pattern stability and uniformity (Fig. 6.4). Twenty-four lags were defined at 1-km distances. Moran's I values were statistically significant for lags 1-6, 8, 10-11 and 15-24, in 2008; and only lags 1-3, in 2009. Correlograms were globally significant at the Bonferroni corrected level (Fig. 6.5). After Holm's correction, positive correlation was observed for distances of up to 6 km, with a second patch between 10-11 km, in 2008; and at distances <4 km as well as at 24 km, in 2009. However, patterns were not stable when lag distances were changed. Considering a lag distance of 2 km, a single patch was detected in 2008, at distances of up to 10 km; while for 2009, two patches were detected at up to 4 km and 14 km, respectively.

Distribution and spatial autocorrelation of other species

SAC analyses (Fig. 6.3) of the distribution of co-occurring bivalve species (Fig. 6.2) revealed that *Abra tenuis*, which was only found in the Westerschelde, showed a significant correlation coefficient for lags 1-2. For *Cerastoderma edule*, lag 8 and lags 1-10 were significant in Minho and the Westerschelde, respectively. *C. edule* was also found in Trondheim but was not included in the analysis given the extremely low densities. *Macoma balthica* was present in the Westerschelde where lags 1-3 were significant, and in Trondheim where it showed no correlation at all. Finally, no significant correlations were found for *Mya arenaria* in Trondheim. In Trondheim (*M. balthica* and *M. arenaria*) and in Minho (*C. edule*), correlograms were non-significant which indicates a random distribution. In contrast, all bivalve species in the Westerschelde showed globally significant correlograms. For these, a patch size of 1000 m was observed for *C. edule* while smaller patches of 115 m and up to 230 m were observed for *M. balthica* and *A. tenuis*, respectively.

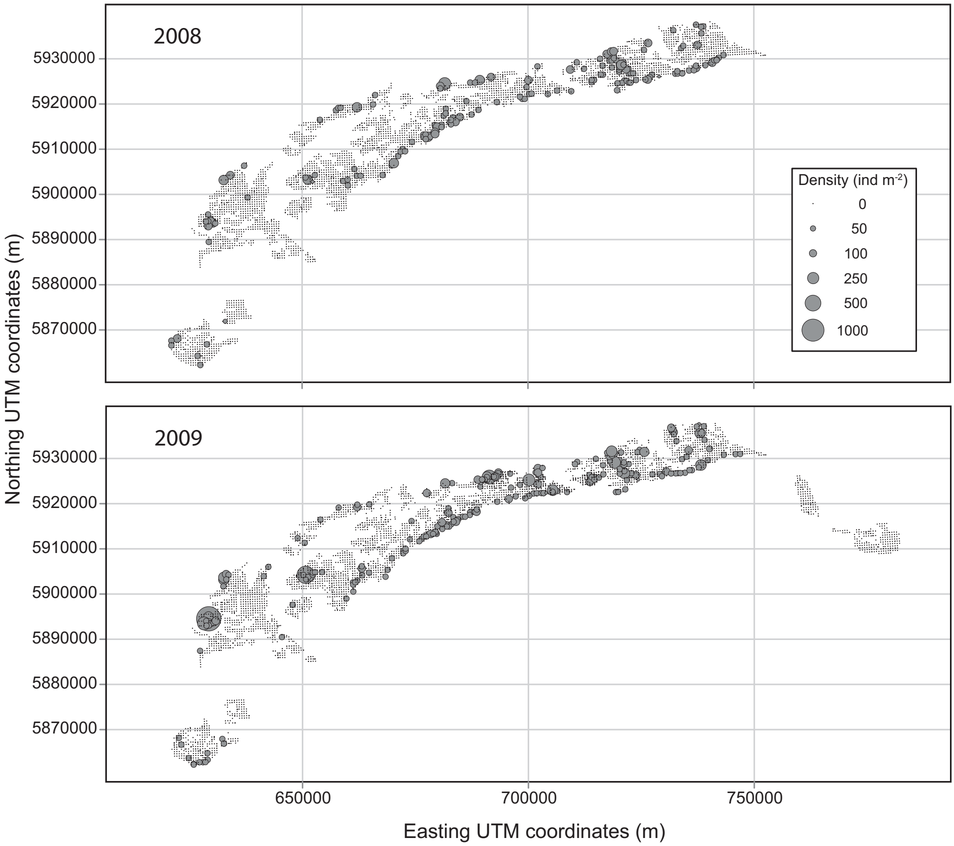

Fig. 6.4. Numerical distribution of *Scrobicularia plana* in the Dutch Wadden Sea. Each dot corresponds to a sample point. Density scale is the same for both years. Please see caption of Fig. 6.2 for explanation on UTM coordinates.

Relation with environmental factors

No significant correlation was observed between sediment type and the spatial distribution of *Scrobicularia plana* in Minho. Significant values were found with both median particle size and percentage of silt in Westerschelde, and for percentage of silt in Trondheim (Table 6.2).

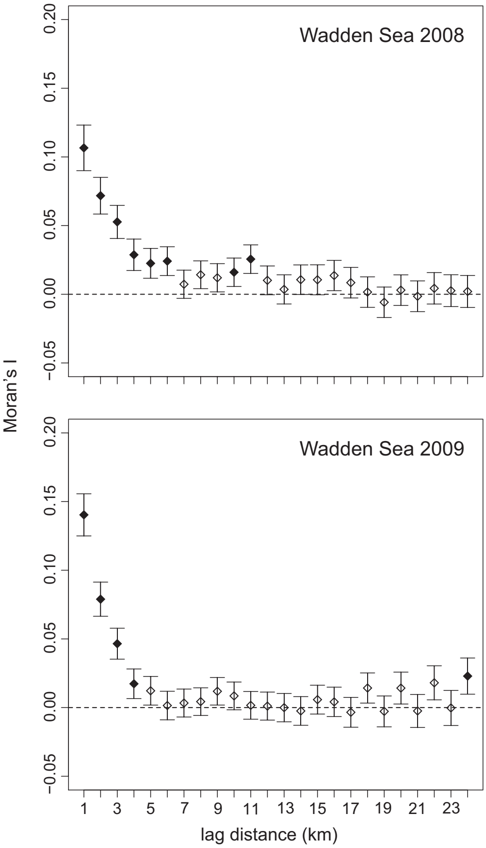

Fig. 6.5. Spatial correlograms of *Scrobicularia plana* in the Dutch Wadden Sea. Full symbols represent statistically significant correlations, while open symbols indicate non-significant values.

Table 6.2. Partial Mantel test correlation coefficients (*r*) between sediment variables and frequency of *Scrobicularia plana* at three small-scale sampling locations. Probabilities have been FDR corrected and are expressed as: $***p < 0.0003$; $**p < 0.0033$; $*p < 0.0167$.

Location	Sediment variable	*r*
Minho, Portugal	Median particle size	0.028
	Percentage of silt	-0.058
Westerschelde, The Netherlands	Median particle size	0.176***
	Percentage of silt	0.191***
Trondheim, Norway	Median particle size	-0.019
	Percentage of silt	0.228**

Discussion

This study has provided insight into the small to large scale spatial patterns of *Scrobicularia plana* at four different sites along the species' distributional range. While in Minho and Westerschelde, individuals showed an aggregated pattern, the Trondheim population was randomly distributed. These findings confirm previous statements (Hughes 1970, Langston 1986) that *S. plana* is characterized by patchy distributions.

A key point was to analyze the shape of the correlogram since it is associated with particular types of spatial structures (Legendre & Fortin 1989). Results demonstrated that the spatial distribution of *Scrobicularia plana* varied between locations. The random pattern observed for the Trondheim population has also been detected in a previous study in the Oosterschelde, The Netherlands (Meire et al. 1989). However, most populations appear to have some degree of aggregation. In Minho, positive autocorrelations at the smallest distances, with non-significant random variation from ~200 m onwards, suggested the presence of differently sized random patches (Kraan et al. 2009). This pattern is similar to the one detected in a population in North Wales (Hughes 1970); as well as the one inferred from the Westerschelde correlogram, with the difference being that negative values were observed at greater distances. The later is the most commonly observed pattern for natural populations in which nearby sites have similar values and that the most dissimilar localities are farthest apart (Sokal & Oden 1978). However, when increasing lag distance to 250 m, which allowed the coverage of a much larger area, values at the farthest distances became non-significant. The shape of the correlogram is, therefore, indicative of a single large patch as opposed to a gradient (Kraan et al. 2009).

Different spatial arrangements are expected to have major effects on species performance and population dynamics. For example, when conspecifics are aggregated, strong competitors perform poorly which can slow competitive exclusion, facilitating coexistence (Hart & Marshall 2009). On the other hand, if intraspecific competition is strong relative to interspecific competition, most species are likely to benefit from being randomly distributed (Turnbull et al. 2007). An aggregated distribution can also be disadvantageous if energy is a limiting factor (Heip 1975). This may help explain the random distribution of the Trondheim population since food availability is generally lower at northern latitudes

As most natural populations are, to some extent, patchy in their spatial pattern (Levinton 1972, Heip 1975, Barry & Dayton 1991), it is more important to determine the size of patches. Differences in patch size were observed between sites indicating that patchiness occurs at different spatial scales. Analysis of the Wadden Sea data also provided information about the effects of temporal scale stability. Density and SAC were similar in both years but size varied between 2008 and 2009. Variation in patch size may affect recruitment success and, ultimately, population dynamics, depending on the location of patches, as well as the dispersal ability of a species. If populations are self-recruiting from within patches, then larger patches should promote recruitment success.

Are distribution patterns consistent between species?

Comparison of spatial patterns of co-occurring bivalve species allowed us to determine if distribution patterns differ (and to what extent) between species within the same area. All bivalve species from Trondheim showed a random distribution. It is possible however, that aggregation at an even smaller spatial scale went undetected because samples were collected at distances > 50 m. Similar to *Scrobicularia plana*, *Cerastoderma edule* showed a highly aggregated pattern in both the Westerschelde (this study) and the Wadden Sea (Kraan et al. 2009) although with a somewhat smaller patch size. Note, however, that Kraan et al. (2009) used a cut-off point in the Moran's I graphs of 0.1 instead of 0 and patch sizes of both species are very similar if a single cut-off point (0) is used. In contrast, in Minho, *C. edule* was characterized by a random distribution. Although both bivalve species are characteristic of the mid-tidal zone of the mudflats (Eltringham 1971), and are often found together in the same area (e.g. Ysebaert & Herman 2002, Dolbeth et al. 2003), *C. edule* is more common in sandy areas (Bocher et al. 2007), which could help explain the observed differences. The similar distribution patterns of *A. tenuis* and *S. plana* in the Westerschelde (although at different scales) can also be related to the occupation of similar niches, with both species being more abundant at southern sites and with a clear preference for muddy sediments (Bocher et al. 2007). Finally, *Macoma balthica* in the Wadden Sea (Kraan et al. 2009) showed a very similar pattern to the one observed in the present study for *S. plana,* but a smaller degree of aggregation in the Westerschelde. Differences may be explained by density-dependent processes as *M. balthica* shows higher densities at higher latitudes, while *S. plana* is more abundant in southern areas (Bocher et al. 2007).

Species composition differed between the sites studied, which probably affects *Scrobicularia plana*'s functional role in the macrobenthic community; in particular, density dependent processes related to predator-prey interactions and intraspecific competition. For example, the oystercatcher *Haematopus ostralegus*, one of the main predators of *S. plana* (Hughes 1970), also feeds on other bivalves, such as *Cerastoderma edule*, *Macoma balthica* and *Mya arenaria* (Zwarts & Wanink 1993, Wanink & Zwarts 2001). If these species are absent from the community, the predation pressure on *S. plana* can increase leading to higher mortality (Holt 1977). Prey switching in oystercatchers as a response to changes in abundance has been observed (Zwarts & Wanink 1993). In contrast, the absence of other bivalve species may have a positive effect on S. plana, as the intraspecific competition for food and space can result in a decrease in growth and the overall condition of the animals (e.g., Kamermans et al. 1992). However, as body condition of *S. plana* in Westerschelde is better than in Minho, and similar to that of Trondheim (Santos et al. 2011a) and the larger patch size and higher species diversity in Westerschelde would suggest higher interspecific competition, this does not seem to be the case.

Not only do the spatial patterns of *Scrobicularia plana* differ among sites but also those of the other co-occurring bivalve species. This suggests that the spatial distributions of all the species are site-specific, which is in agreement with a previous study from Bocher et al. (2007). Nevertheless, *S. plana* appears, overall, to be more aggregated than other co-distributed bivalve species.

Relating distribution patterns to environmental factors

Sediment composition is one of the factors that has been shown to influence estuarine benthic assemblage structure and species distribution at local (smaller) spatial scales (Warwick et al. 1991, Ysebaert & Herman 2002, Bocher et al. 2007, Kraan et al. 2010). In our study, the distribution of *Scrobicularia plana* in the Westerschelde and in Trondheim was correlated with median particle size (Table 6.2) confirming that a muddy sediment type is an important environmental factor determining the species' settlement patterns and subsequent survival, as shown on previous studies (Casagranda & Boudouresque 2005, Bocher et al. 2007, Compton et al. 2009). For deposit-feeders, such as *S. plana*, the higher content of organic matter in muddy sediments (as compared to sandy sediments), is expected to provide higher quality habitat (Levinton 1972). Therefore, a coupling of feeding mode and sediment type is expected. If this is indeed the case, it suggests active habitat selection at the time of settlement with larvae actively settling in areas where the adults will do well in terms of feeding performance. Such argument is strengthen by the observation that the spatial distributions of juvenile *S. plana* match those of the adults (Compton et al. 2009).

Active patch/habitat selection is expected when patches differ in "quality" (Wiens 1976). The limited dispersal of adult *Scrobicularia plana* (Hughes 1970) shows, however, that the observed spatial patterns are unlikely to be a result of aggregative behavior of adults, but instead caused by larval settling and post-settlement behavior (Underwood & Chapman 1996). Despite the limited mobility of adult bivalves, during the pelagic larval phase, individuals are free to drift with currents. Pelagic larval duration is a very important process in shaping species distribution patterns (e.g. Johnson et al. 2001). Species with no (or short) pelagic stages tend to have strongly aggregated distributions as a result of low mobility, while a long pelagic phase allows the population to be distributed along a larger area (Johnson et al. 2001, Shanks et al. 2003). As the pelagic stage of *S. plana* can take several weeks (Frenkiel & Mouëza 1979), larvae should be able to disperse for distances of tens of km (Shanks et al. 2003) hence connecting the different patches. However, evidence is mounting that larval dispersal in marine populations is often limited (Cowen et al. 2000). As larvae are difficult to track directly, the extent of interpopulation connectivity can only be indirectly estimated using genetic methods. We have shown low interpopulation connectivity for *S. plana* (Chapter 5 of this thesis), a strong indication of local larval retention.

In conclusion, the spatial distribution of *Scrobicularia plana* is site-specific. Larval retention within sites appears to be the rule. At the local scale, aggregation patterns are greatly determined by abiotic factors, particularly sediment type which acts as a cue for larval settlement. At the very small scale, biotic interactions (e.g., predator-prey interactions and intra and interspecific competition for food and space) will have a stronger role.

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Chapter 7

Synthesis

Synthesis

Scrobicularia plana is among the most abundant bivalve species found along European sandy-muddy shores (Bocher et al., 2007). It plays a key role in the soft-sediment ecosystem mainly as a deposit feeder within the food web as it feeds on benthic diatoms and marine phytoplankton (Riera et al., 1999), which will affect primary production; and as prey for many species of shore birds (Moreira, 1997). In southern Europe it is also harvested for human consumption (Langston et al., 2007). In order to identify gaps in our general knowledge and to better evaluate *S. plana*'s potential vulnerability in response to, e.g., climate change, habitat fragmentation and commercial harvesting, we started with a comprehensive review that examined numerous aspects of *S. plana*'s natural history (**Chapter 2**). Our qualitative meta-analysis revealed no consistent combinations of factors that could explain, e.g., settlement patterns and the observed aggregations that are so characteristic of *S. plana*. Next we took an empirical, analytical approach with a focus on spatial and temporal patterns and their relation to population dynamics and population structure at three spatial scales: the European latitudinal gradient; within the Wadden Sea; and at the level of specific areas of <3 km^2. Together these spatial-temporal patterns helped us to assess at what level particular attributes inform adaptation potential, dispersal, effects of habitat fragmentation/isolation and the trajectory that management must adopt in order to preserve population sustainability.

Population ecology

Life history variation and phenological characteristics (**Chapter 3**) across the species' latitudinal range were investigated at three sites: southern (Portugal), middle (Netherlands) and northern (Norway) Europe. Seasonal cycles of body mass (BMI), somatic mass (SMI) and gonadal mass (GMI) were compared. As expected, temperature and food availability affected the timing and values of the three indices. Portugal led the spawning season, which continued throughout the summer. Start of the spawning season was progressively later northwards with decreasing reproductive periods and outputs. Individuals spawned completely, i.e. all energy of gonadal mass was released in the form of gametes, similar to what has been observed for *Cerastoderma edule* (Cardoso et al., 2009). Other bivalves, such as *Mya arenaria*, have a different allocation strategy in which the gonad is used not only for reproduction but also for storage (Cardoso et al., 2009). This constitutes an advantage under unfavorable conditions and suggests that *M. arenaria* would be less vulnerable to environmental changes as compared with *S. plana* and *C. edule*. BMI and SMI were lower in Portugal, which may reflect smaller overall size in relation to fishing pressure. Commercial fishing size in Portugal is 25 mm (Coelho et al., 2006), which corresponds to the smallest reproductive individual observed. It is possible that, in some areas, a major fraction of the reproductive population of this species is being harvested for human consumption, which may lead to a decline, and eventually disappearance, of those populations. Temperature and plankton availability were strongly correlated with reproductive output and somatic mass cycles indicating that (like many other species), changes in coastal temperatures and timing of phytoplankton blooms will affect range shifts as well as phenological shifts in life history traits. It is possible that temperature shifts will lead to an earlier spawning in the spring in *S. plana* which may well result in decreased recruitment success and increased predation pressure, as observed for *Macoma balthica* (Philippart et al., 2003). As a result, it is concluded that cold-water species, such as *M. balthica*, will further decline while warm-water species will take over. *S. plana* is already "replacing" *M. balthica* and *C. edule* in more southern latitudes (Bocher et al., 2007). It is likely that, with increasing temperatures, the species will shift its distribution to more northern latitudes which may affect the commercial importance of the species in Southern European countries. Our findings also suggest considerable phenotypic plasticity in *S. plana*, which although energetically costly, constitutes a selective advantage for the species over all (Jokela and Mutikainen, 1995; Kingsolver and Huey, 1998), as it allows flexibility in the responses to environmental changes.

Genetic indicators of population-level health and fitness show that high genetic diversity and large population sizes are characteristic in *S. plana* (Reed and Frankham, 2003; Reed, 2005; Gamfeldt and Källström, 2007). It should be noted, however, that there is only a weak correlation between diversity at presumably neutral markers (such as mtDNA) and specific quantitative phenotypic traits directly related to fitness (McKay and Latta, 2002; Holderegger et al., 2006). In *S. plana*, quantitative traits of interest include, for example, growth, reproductive effort, age-specific fecundity and age and size at maturity. Nevertheless, neutral genetic diversity enhances community biodiversity and its resilience following disturbance (Hughes et al., 2008). From the neutral diversity perspective then, *S. plana* is not at risk of local or regional extinction. This result is particularly important for commercially exploited species, where excessive harvesting from particular areas can result in a decrease in local diversity (Luttikhuizen et al., 2003b). Again, in *S. plana*'s case, this does not yet appear to be a problem as intraspecific diversity is higher overall in southern latitudes, where the species is commercially exploited. However, individual sizes are notably smaller in harvested areas as the larger individuals are naturally preferred. The long term concern of smaller size classes is a shift downward in age of first reproduction which often correlates with lower fecundity and overall lower fitness on the medium term (e.g., in almost all commercially harvested fish). Alternatively, fisheries selection on size might result in an overall decrease in size and fecundity independent of age. This is something that can now be examined using the sclerochronological validation method developed in **Chapter 4**.

Sexual maturity of *S. plana i*s a function of age and not size. The same has been observed for other bivalve species such as e.g., *Donax trunculus* (Gaspar et al., 1999), *Mytilus edulis* (Seed, 1969), *Spisula solida* (Gaspar and Monteiro, 1999), and *Venus striatula* and *Ensis siliqua* (Gaspar and Monteiro, 1998). Age at first reproduction is expected to be a balance between the advantages of short generation time, which increases the probability of reproducing before death, and the advantages of a larger size, which is related to higher fecundity and the ability for deeper burial, which reduces predation risk (Stearns and Koella, 1986; Kozlowski, 1992; Zaklan and Ydenberg, 1997). Reproduction in *S. plana* occurs in individuals above 2 years old (Paes-da Franca, 1956; Sola, 1997; Guerreiro, 1998). In other commonly co-occurring species in European estuaries, namely *M. balthica* and *M. arenaria*, for which sexual maturity appears to be a function of size, individuals can attain first reproduction as early as in their first year of life (Brousseau and Baglivo, 1988; Harvey and Vincent, 1989). As maturity is a function of size, increased growth rates can lead to an even earlier first reproduction. Earlier maturity usually provides a higher adaptability to changes in habitat conditions, conferring an advantage to *M. balthica* and *M. arenaria*.

Age at maturity needs to be determined for each area where the species is exploited in order to guide management for sustainability and stock health. For *S. plana* age estimation has been traditionally based on growth lines or rings on the shell. Given the importance of accurate age estimations, we decided to assess the reliability of different ageing methods (**Chapter 4**) for this species by comparing external and internal growth-line counts with stable isotope ($\delta^{18}O$ and $\delta^{13}C$) profiles from the shells of *S. plana*. Results revealed that the analysis of $\delta^{18}O$ profiles provides an accurate estimate of age in *S. plana*. In addition, we found that estimations based on external lines were unreliable, resulting in overestimation of age and underestimation of growth rates; the latter as a result of so-called "disturbance bands" being wrongly identified as annual bands. Analysis of internal lines was also not reliable, particularly in older portions of the shell, leading to over- or underestimation of age. Given that the majority of studies on growth and population dynamics of *S. plana* (Green, 1957; Hughes, 1970; Bachelet, 1981; Sola, 1997; Guerreiro, 1998), and most bivalve species, rely on the use of external lines for age estimations (individuals scored as older, when in fact they are younger), estimates in the literature are not reliable. Although isotope sclerochronology is accurate, it is also expensive. Alternatively, trace elements analysis of shells could be performed, as it has been shown that Sr/Ca ratios allow the identification of annual growth lines in *M. balthica* (Cardoso et al., unpubl.), although such method is also expensive and time-consuming. No other alternative methodologies seem feasible at the present time.

Spatial patterns

The distribution patterns of *S. plana* were analyzed at the phylogeographic, as well as at two habitat scales. Large-scale patterns provide an evolutionary perspective on paleoclimatic episodes (notably the last ices ages) that have shaped biogeographic distributions throughout northern Europe. In contrast, smaller scale patterns at the level of estuaries and bays provides insights about dispersal, population connectivity and possible metapopulation structure. Taken together, spatial scale integration provides a snapshot of what has happened in the past and what might be projected (as opposed to predicted) in the future.

As discussed in **Chapter 3**, seasonal and latitudinal differences in annual cycles of somatic and gonadal mass at different locations were attributed to varying temperature and food availability. The observed relationship between the species' large scale patterns and temperature suggests that changes in climate conditions will almost certainly cause a shift in *S. plana*'s distribution, with possible extinction of some southern, trailing edge populations (affecting *S. plan*a fisheries), as mentioned above. However, there may also be the possibility for rapid adaptation through strong selection acting on pre-adapted genotypes that are certainly to be found in the genetically diverse, large population clusters.

The phylogeographic view of *S. plana* presented in **Chapter 5** captures the shaping effects of both the last glacial maximum (20,000 years ago) and the earlier Pleiocene. Such changes can have dramatic effects on the present genetic structure of the species (Hewitt, 2004). Phylogeographic patterns of *S. plana* suggest a recent demographic expansion and large effective population sizes with very low migration rates. The star shaped haplotype network, i.e. the presence of one, widely distributed and abundant haplotype, and a large number of recently derived and closely related haplotypes are typical of species that experienced ancient bottlenecks and subsequently recovered. We estimate that this expansion occurred between 0.3-1.1 million years ago. During this period the species has been subjected to repeated episodes of glacial advance and retreat that resulted in major transformations in the geography and climate of northern Europe (Kukla et al., 2002). Relatively strong isolation by distance and low migration at the basin scale further indicate that populations are locally distinct, panmictic units despite their theoretical potential for long distance dispersal through the pelagic larval phase. Oceanographic entrainment may be responsible for this, while smaller-scale, weak population differentiation may reflect connectivity at a metapopulation scale consistent with *S. plana*'s patchy distribution, e.g., within the Wadden Sea. The observed patterns are also suggestive of possible local adaptation, though this could not be tested using neutral markers. This pattern contrasts strongly with *Macoma balthica,* which is comprised of deeply diverged lineages (Luttikhuizen et al., 2003a) and strong differentiation. *M. balthica* populations may be less physiologically plastic suggesting, once again, that *S. plana* may replace it in the face of increased temperatures. This is not the case for *C. edule* which shows similar patterns (although a somewhat more structured minimum spanning network) and similar haplotype diversity values (Krakau et al., 2012). Therefore, like *S. plana*, *C. edule* may be more able to adapt to local environmental changes by shifting its distribution northwards. These foreseen changes have strong implications for altered ecosystem functioning.

The habitat view of spatial patchiness and aggregation of *S. plana* that was analyzed in **Chapter 6** revealed that these patterns were site-specific characteristics rather than species-specific. Using mapping and spatial autocorrelation analysis, aggregated patterns were found to be statistically significant at three of the four locations investigated and ranged in scale from 150-1250 m to 4-10 km. Variation at the scale of ten of meters is probably mediated by density-dependent ecological processes such as inter- and intraspecific competition for food and space, as well as predator-prey dynamics. At the scale of hundreds to thousands of meters aggregation may be related to habitat selection and recruitment (and/or mortality) combined with restricted local dispersal (Underwood and Chapman, 1996).

No discernible relation was found between the spatial patterns of *S. plana* and the presence, as well as densities, of other bivalve species belonging to similar niches. The absence of such species may have a positive effect on *S. plana*, as the intraspecific competition for food and space can result in a decrease in growth and the overall condition of the animals (e.g., Kamermans et al., 1992). However, the better condition of *S. plana* in Westerschelde

(The Netherlands), where the highest species diversity was observed, suggests that this is not the case. Prey switching has been observed in wading birds as a response to changes in abundance (Zwarts & Wanink, 1993). The absence of prey species such as *C. edule*, *M. balthica* and *M. arenaria* can then lead to increased predation pressure on *S. plana* and, consequently, to higher mortality and reduced densities. In Southern countries, however, where other bivalves taken by birds in North European estuaries are absent and *S. plana* constitutes the main prey species of bird communities (Moreira, 1997), densities are typically higher (Bachelet, 1979; Sola, 1997; Bazaïri et al., 2003; Verdelhos et al., 2005; Bocher et al., 2007). This seems to rule out the effect of biotic factors, although confirmation would only be possible if controlled habitat experiments (in which species diversity and abundances are manipulated, as well as predator exclusion experiments) were performed.

Significant correlations were found between sediment type and the distribution of *S. plana,* although not for all locations. This suggests that at the kilometer scales hydrological features and sediment type are probably the main shapers. For example, in the Wadden Sea, patch sizes are dynamic (6 km scale in 2008; 10-11 km scale in 2009). Scaling patterns of *S. plana* were also compared with other co-occurring species, namely *Abra tenuis*, *C. edule*, *M. balthica* and *M. arenaria*. Again, both random and aggregated patterns were found for each species, depending on the specific location. The Westerschelde (Netherlands), with four species, was consistently patchy with patch size varying between species. There was no apparent relationship between density and aggregation. At the Trondheim (Norway) site, in contrast, only three species were present with a random distribution found for all three. Finally, Minho (Portugal) had only two species with *S. plana* being minimally aggregated while *C. edule* was randomly distributed. These results support the greater role for local conditions over biological interactions.

The distances between suitable habitat patches can act as barriers to movement. Although the long pelagic stage of *S. plana* should allow larvae to be dispersed over distances of up to tens of km (Gilg and Hilbish, 2003; Shanks et al., 2003), the actual extent of larval dispersal is unknown. Oceanographic currents and their interactions with the topography and habitat can lead to more complex patterns where pelagic larval dispersal is decoupled from Euclidean distance (e.g., Cowen et al., 2007; Galarza et al., 2009; Weersing and Toonen, 2009; White et al., 2010). In some cases, retention zones may be formed (e.g., Bradbury et al., 2008; Cowen and Sponaugle, 2009; Galindo et al., 2010; Small and Wares, 2010). We observed a significant isolation-by-distance effect (**Chapter 6**) suggesting that populations of *S. plana* are self-recruiting. As local retention is often related to the species' ecological and habitat characteristics (Swearer et al., 2002; Cowen and Sponaugle, 2009), in *S. plana*'s case it is most likely associated with its habitat-related, patchy spatial distribution. *Cerastoderma edule* also exhibited isolation-by-distance but only for northern sampling sites (Krakau et al., 2012) where the species is more abundant and characterized by less intertidal zonation (Bocher et al., 2007), which strengthens the relationship between spatial patterns and population structure. The low interpopulation connectivity observed for *S. plana* may decrease metapopulation viability, leading to increased local extinction risk (Lecomte et al., 2004).

Conclusion

The main aim of this thesis was to study the temporal and spatial patterns of population structure and population dynamics in *Scrobicularia plana*, one of the most common and abundant bivalves in soft-sediment intertidal systems throughout Europe. Paleoclimatic effects have resulted in a relatively recent expansion of the species along European shores over roughly the past 300,000 years. Populations are large and genetically diverse with regional population structure characterized by strong, probably oceanographic, entrainment.

S. plana's wide latitudinal distribution suggests both local adaptation and broad physiological plasticity. Temperature drives the distribution and phenology with the reproductive period commencing in spring in Portugal and progressively starting later, and with a shorter duration, as one moves to Norway. The degree to which the biogeographic distribution of *S. plana* will be affected under climate change scenarios is probably minimal in light of the fact that trailing-edge, genetic diversity is high suggesting that local extinctions may not be significant as the potential for in situ rapid adaptation is present. Nevertheless, the species may shift its distribution northwards replacing other key species of intertidal communities such as *M. balthica* which seems to have a lower adaptive potential. Such a shift would disrupt the species' critical role in southern European countries, where *S. plana* is not only the main prey of birds but also economically valuable.

Growth in *S. plana* is strongly shaped by food availability with peaks in somatic mass coinciding with the phytoplankton blooms. Changes in temperature regimes will lead to changes in the timing, duration and composition of the blooms which will affect not only growth but also reproduction and, ultimately, survival. Determination of growth parameters was not possible since accurate age estimates in *S. plana* are only obtained using isotope sclerochronology, a methodology that is prohibitively expensive when analyzing such large datasets.

Sexual maturity in *S. plana* is reached at two years rather than when individuals reach a threshold size. However, in areas where the species is harvested, strong selection on size may drive age of first spawning lower and ultimately reduce fitness. Investigating this possibility is now possible with the ability to more accurately determine individual age. At present, there is no direct evidence for natural or fisheries pressure being a threat to the sustainability of *S. plana* though vigilance is required.

A patchy distribution at the local level are characteristic of *S. plana* and other co-occurring bivalves. Hydrographic features and sediment quality shape patches and their size on a site-specific basis, as opposed to biotic interactions related to competition and predation. The species' spatial pattern is likely to be a factor leading to the self-recruitment of local populations and consequent low interpopulation connectivity.

To conclude, the intertidal is by its very nature a dynamic area in which stochastic events play a strong role in shaping the relative importance of particular environmental factors that interact in a complex fashion, yet the overall patterns that result are not random. Although our ability to model complex biotic and abiotic interactions, and to identify thresholds of transition to other states is improving; the overall conclusion of this thesis is that *S. plana* is doing well at all of its spatial scales. However, low interpopulation connectivity will, most likely, lead to latitudinally disjunctive populations decreasing the ability for future populations to adapt to environmental changes.

Synthese

Langs de Europese kusten met zandig tot modderig sediment is *Scrobicularia plana* (de platte slijkgaper) een van de meest voorkomende tweekleppige schelpdieren (Bocher et al., 2007). De soort speelt een belangrijke rol in ecosystemen met zachte sedimenten als consument van vooral diatomeeën op de bodem maar ook van fytoplankton (Riera et al., 1999). Daarmee is hij van invloed op de primaire productie. Daarnaast is de platte slijkgaper prooi voor vele soorten wadvogels (Moreira, 1997). In Zuid-Europa wordt hij ook bevist voor menselijke consumptie (Langston et al., 2007). Om de gaten in onze algemene kennis over *S. plana* te vinden en diens mogelijke kwetsbaarheid voor bijvoorbeeld klimaatverandering, habitatfragmentatie en bevissing in te schatten, hebben we de beschikbare kennis over de natuurlijke historie van *S. plana* gebundeld in een overzicht (**Hoofdstuk 2**). Onze kwalitatieve meta-analyse liet geen consistente combinatie van factoren zien die de voor *S. plana* zo karakteristieke aggregatie zou kunnen verklaren. Vervolgens namen we een empirische, analytische aanpak met de nadruk op ruimtelijke en temporele patronen en hun relatie tot populatiedynamica en populatiestructuur op drie ruimtelijke schalen: de Europese breedtegraad-gradiënt; binnen de Waddenzee; en op het niveau van specifieke gebieden <3 km^2. Samen helpen deze ruimtelijk-temporele patronen ons om te ontdekken op welk niveau bepaalde kenmerken hun weerslag hebben op aanpassingsmogelijkheden, dispersie, effecten van habitatfragmentatie/isolatie, alsmede het traject wat het beheer zou moeten volgen om duurzaamheid te garanderen.

Populatie-ecologie

Variatie in levensloop- en fenologische eigenschappen (**Hoofdstuk 3**) langs de breedtegraad-gradiënt van de soort zijn onderzocht in drie gebieden: zuidelijk (Portugal), midden (Nederland) en noordelijk (Noorwegen) Europa. Seizoenale cycli in lichaamsmassa (BMI), somatische massa (SMI) en gonadenmassa (GMI) werden vergeleken. Zoals verwacht beïnvloedden temperatuur en voedselbeschikbaarheid de timing en amplitude van de drie indices. In Portugal, waar het paaiseizoen het vroegst begon, duurde het reproductieve seizoen de hele zomer. Het paaien begon steeds later naar het noorden toe met afnemende paaiseizoenlengtes en in totaal geproduceerde gonadenmassa. Individuen paaiden compleet, dat wil zeggen dat alle energie van de gonaden vrijkwam in de vorm van gameten, vergelijkbaar met hoe het bijvoorbeeld bij kokkels *Cerastoderma edule* gaat (Cardoso et al., 2009). Andere schelpdieren, zoals bijvoorbeeld *Mya arenaria*, hebben een andere strategie, waarbij de gonaden niet alleen functioneren voor reproductie maar ook voor opslag (Cardoso et al., 2009). Dat kan een voordeel zijn bij nadelige omstandigheden en het suggereert dat *M. arenaria* minder gevoelig zou kunnen zijn voor milieuveranderingen dan *S. plana* en *C. edule.* BMI en SMI waren over het algemeen lager (amplitude) in Portugal, wat mogelijk te maken zou kunnen hebben met door de bank genomen kleinere afmetingen vanwege de visserij daar. De commerciële ondergrens voor visserij is 25 mm in Portugal (Coelho et al., 2006), wat overeenkomt met het kleinste gonaden producerende individu dat wij waargenomen hebben. Mogelijk wordt dus in sommige gebieden een groot deel van de reproducerende populatie geoogst, wat zou kunnen leiden tot een afname, en uiteindelijk verdwijnen, van deze populaties. Temperatuur en planktonbeschikbaarheid waren sterk gecorreleerd met de productie van gonaden en de cycli in somatisch gewicht, wat aangeeft dat (net als bij vele andere soorten) veranderingen in kustwatertemperaturen en timing van fytoplanktonbloei zouden kunnen leiden tot verschuivingen in het voorkomen van de soort en in veranderingen in fenologie van levensloopkenmerken. Mogelijk zal de huidige trend van temperatuursverhoging leiden tot eerder paaien in het voorjaar bij *S. plana*, wat zou kunnen resulteren in verlaagd recruteringssucces en een hogere predatiedruk, zoals gerapporteerd voor *Macoma balthica* (Philippart et al., 2003). Zodoende zou gesteld kunnen worden dat koudwatersoorten als *M. balthica* verder af zullen gaan nemen, terwijl warmwatersoorten hun plek zullen innemen. Er zijn al indicaties dat *S. plana* in zuidelijke gebieden *M. balthica* en *C. edule* 'vervangt' (Bocher et al., 2007). Het is verder te verwachten dat het verspreidingsgebied van *S. plana* naar het noorden zal verschuiven, wat van negatieve invloed zal zijn op het commerciële belang van de soort in zuideuropese landen. Onze bevindingen suggereren ook dat *S. plana* behoorlijk plastisch is in fenotype, wat, hoewel het energetisch kostbaar mag worden geacht, over het geheel genomen een selectief voordeel betekent (Jokela en Mutikainen, 1995; Kingsolver en Huey, 1998), omdat het flexibiliteit in respons op omgevingsveranderingen mogelijk maakt.

Genetische indicatoren van gezonde populaties en fitness laten zien dat een hoge genetische diversiteit en grote populaties karakteristiek zijn voor *S. plana* (Reed en Frankham, 2003; Reed, 2005; Gamfeldt en Källström, 2007). Er is echter slechts een zwakke correlatie tussen diversiteit in verondersteld neutrale merkers (zoals mitochondriaal DNA) en specifieke kwantitatieve, fenotypische kenmerken die direct gerelateerd zijn aan fitness (McKay en Latta, 2002; Holderegger et al., 2006). Bij *S. plana* zijn belangrijke kwantitatieve kenmerken bijvoorbeeld groei, reproductieve inspanning, leeftijds-afhankelijke fecunditeit, en leeftijd en grootte bij volwassenheid. Desondanks heeft genetische diversiteit positieve effecten op de diversiteit op levensgemeenschapsniveau en op veerkracht na een verstoring (Hughes et al., 2008). Vanuit het perspectief van neutrale diversiteit geldt voor *S. plana* geen verhoogd risico op lokaal of regionaal uitsterven. Dat is een belangrijk gegeven voor commerciële soorten, waarbij overmatig oogsten in bepaalde gebieden kan leiden tot een afname van lokale genetische diversiteit (Luttikhuizen et al., 2003b). In *S. plana* lijkt dit (nog) geen probleem te zijn, mede doordat intraspecifieke diversiteit het hoogst is in de zuidelijker gebieden, waar de soort bevist wordt. Echter, de schelpdieren zijn gemiddeld wel kleiner in de beviste gebieden, wat waarschijnlijk een gevolg is van het vissen op de grotere dieren. Op lange termijn zou dit kunnen leiden tot een verlaging van de leeftijd waarop reproductie begint - vaak correleert dit met een lagere fecunditeit en overall fitness op de middellange termijn (bijvoorbeeld in veel commerciële vissoorten). Een ander gevolg van visserijselectie op grootte zou kunnen zijn dat grootte en fecunditeit afnemen onafhankelijk van leeftijd. Dit is iets wat in de toekomst onderzocht kan worden met behulp van de sclerochronologische validatiemethode die beschreven wordt in **Hoofdstuk 4**.

Seksuele volwassenheid bij *S. plana* is een functie van leeftijd en niet van grootte. Hetzelfde is beschreven voor andere tweekleppigen, zoals bijvoorbeeld *Donax trunculus* (Gaspar et al., 1999), *Mytilus edulis* (Seed, 1969), *Spisula solida* (Gaspar en Monteiro, 1999), en *Venus striatula* en *Ensis siliqua* (Gaspar en Monteiro, 1998). De leeftijd waarbij voor het eerst gereproduceerd wordt zou het resultaat moeten zijn van de voordelen van een korte generatieduur aan de ene kant en de voordelen van een grote afmeting aan de andere kant (Stearns en Koella, 1986; Kozlowski, 1992; Zaklan en Ydenberg, 1997). Het voordeel van een korte generatieduur is dat de kans te reproduceren voor de dood groter is. De voordelen van een grote afmeting zijn de ermee gepaard gaande hogere fecunditeit en de mogelijkheid zich dieper in te graven en aan predatie te ontsnappen. Geslachtelijke voortplanting gebeurt bij *S. plana* in individuen die ouder dan twee jaar zijn (Paes-da-Franca, 1956; Sola, 1997; Guerreiro, 1998). In andere algemene, met *S. plana* samen in Europese estuaria voorkomende schelpdieren, namelijk *M. balthica* en *M. arenaria*, lijkt seksuele volwassenheid een functie van grootte te zijn en kunnen individuën al voor het eerst reproduceren in hun eerste levensjaar (Brousseau en Baglivo, 1988; Harvey en Vincent, 1989). Wanneer volwassenheid een kwestie van afmeting is, kan een hogere groeisnelheid resulteren in een vervroegde reproductie. Vervroegde volwassenheid geeft doorgaans een betere aanpassingsmogelijkheid aan veranderingen in habitat, wat wellicht een voordeel voor *M. balthica* en *M. arenaria* betekent.

Leeftijd bij volwassenheid moet apart bepaald worden voor elk gebied waar een soort geëxploiteerd wordt, om het beheer af te stemmen op duurzaamheid en de gezondheid van het bestand. Voor *S. plana* werd leeftijdsbepaling gedaan aan de hand van de lijnen of ringen op de schelp. Vanwege het grote belang van accurate leeftijdsbepaling hebben we besloten om de betrouwbaarheid van verschillende leeftijdsbepalende methoden te onderzoeken voor deze soort (**Hoofdstuk 4**). Onderzocht zijn externe schelpgroeilijnen, interne schelpgroeilijnen en stabiele isotopenprofielen ($\delta^{18}O$ en $\delta^{13}C$). De resultaten lieten zien dat $\delta^{18}O$ profielen een betrouwbare schatting van de leeftijd van *S. plana* oplevert. Daarnaast vonden we dat schattingen op basis van externe schelpgroeilijnen onbetrouwbaar zijn, en resulteren in een overschatting van de leeftijd en daarmee een onderschatting van groeisnelheid. De oorzaak hiervan is het voorkomen van zogenaamde 'verstoringslijnen' die foutief geïnterpreteerd worden als jaarlijnen. Analyse van interne schelpgroeilijnen is ook niet betrouwbaar, vooral niet in de oudere delen van de schelp, en kan leiden tot zowel een over- als een onderschatting van de leeftijd. Omdat de meeste onderzoeken naar groei en populatiedynamiek van *S. plana*, en in feite van de meeste schelpdiersoorten, gebaseerd zijn op externe schelpgroeilijnen voor leeftijdsbepaling (en individuen dus te oud worden ingeschat), zijn resultaten in literatuur niet betrouwbaar. Hoewel isotopensclerochronologie accuraat is, is het ook duur. Als een alternatief zou de bruikbaarheid van sporenelementen onderzocht kunnen worden. Eerder is namelijk aangetoond dat Sr/Ca ratios schelpgroeilijnen in *M. balthica* kunnen identificeren (Cardoso et al., unpubl.), hoewel zulke methoden ook duur en tijdrovend zijn. Geen andere methoden lijken haalbaar op dit moment.

Ruimtelijke patronen

De verspreidingspatronen van *S. plana* zijn geanalyseerd op zowel de schaal van fylogeografie als op twee habitatsschalen. Grootschalige patronen geven een evolutionair perspectief op paleoklimaatsepisodes (in het bijzonder de laatste ijstijden) die de biogeografische verspreidingen in noord-Europa hebben gevormd. Patronen op kleinere schaal, daarentegen, zoals op het niveau van estuariën en baaien, levert inzicht in dispersie, populatieconnectiviteit en mogelijk metapopulatiestructuur. Samen geven patronen op kleine en grote schaal een korte-termijnbeeld van wat in het verleden gebeurd is en kan worden geprojecteerd op de toekomst.

Zoals bediscussieerd in **Hoofdstuk 3** hebben we seizoenale en breedtegraadsvariatie in jaarlijke cycli in somatische en gonadenmassa gerelateerd aan verschillen in temperatuur en voedselbeschikbaarheid. De waargenomen relatie tussen de patronen op grote schaal en temperatuur suggereert dat klimaatverandering vrijwel zeker voor een verschuiving zal zorgen in het voorkomen van *S. plana*, waarbij niet uitgesloten moet worden dat de meest zuidelijke populaties aan de rand van de verspreiding zouden kunnen uitsterven. Met, zoals besproken, gevolgen voor de visserij. Het is echter ook mogelijk dat een snelle aanpassing plaatsvindt door sterke selectiedruk op preadaptatie van reeds aanwezige genotypes die zeker verwacht mogen worden in genetisch diverse, grote populatieclusters.

De fylogeografische blik op *S. plana* zoals gegeven in **Hoofdstuk 5** vangt zowel de vormende effecten van het laatste glaciale maximum (20.000 jaar geleden) als die van het vroegere Pleistoceen. Zulke gebeurtenissen kunnen dramatische effecten hebben voor de hedendaagse populatiegenetische structuur van een soort (Hewitt, 2004). Fylogeografische patronen van *S. plana* impliceren een recente demografische expansie en enorme effectieve populatiegroottes met erg lage migratie. De stervorm van het haplotypennetwerk, dat wil zeggen een wijd verspreid en algemeen centraal haplotype met grote aantallen zeldzame, recent afgeleide haplotypen, is typerend voor soorten die in hun geschiedenis extreem in aantal gekelderd zijn geweest en vervolgens weer hersteld. We schatten dat deze expansie 0,3 tot 1,1 millioen jaar geleden heeft plaatsgevonden. Gedurende deze periode is de soort ten prooi gevallen aan een serie episodes van glaciale opmars en terugtocht. Deze hebben gezorgd voor grote transformaties in geografie en klimaat van noord-Europa (Kukla et al., 2002). Relatief sterke isolatie-met-afstand en lage migratie op de schaal van zeebasins geven ook aan dat populaties gescheiden, lokaal panmictische eenheden zijn. Dit is ondanks het feit dat *S. plana* in potentie een lange-afstandsmigrant zou kunnen zijn vanwege de vrijzwemmende larven. Oceanografische insluiting zou hiervoor verantwoordelijk kunnen zijn, terwijl zwakkere populatiedifferentiatie op kleinere schaal het gevolg zou kunnen zijn van connectiviteit op metapopulatieniveau, consistent met de fragmentarische verspreiding van *S. plana*, zoals bijvoorbeeld in de Waddenzee. De waargenomen patronen suggereren ook de mogelijkheid van lokale adaptatie, alhoewel dit niet met zekerheid kan worden vastgesteld aan de hand van alleen neutraal veronderstelde genetische merkers. Dit patroon contrasteert sterk met *M. balthica*, die bestaat uit een aantal diepe (oude) afstammingslijnen (Luttikhuizen et al., 2003a) en sterke populatiedifferentiatie. Populaties van *M. balthica* vertonen misschien minder fenotypische plasticiteit wat zou betekenen, andermaal, dat *S. plana M. balthica* op termijn zou kunnen gaan verdringen wanneer de temperatuur verder gaat stijgen. Dit is niet het geval voor *C. edule*, die een populatiegenetisch patroon laat zien dat vergelijkbaar is met *S. plana* (hoewel met een meer gestructureerd haplotypennetwerk), inclusief vergelijkbare haplotypendiversiteiten (Krakau et al., 2012). Om die reden zou *C. edule*, net als *S. plana*, beter uitgerust kunnen zijn om zich aan te passen aan lokaal veranderende omstandigheden door naar het noorden te verschuiven. Deze voorpellingen hebben belangrijke implicaties voor een veranderd functioneren van ecosystemen.

Hoofdstuk 6 neemt een habitatvisie op ruimtelijke fragmentatie en aggregatie van *S. plana* en laat zien dat deze patronen specifiek zijn voor een gebied en niet voor de soort. Met gebruik van kartering en ruimtelijke autocorrelatie werden geaggregeerde patronen gevonden die statistisch significant waren in drie van de vier bestudeerde locaties. De schaal van deze aggregaties variëerde van 150-1250 m tot 4-10 km. Variatie op een schaal van tientallen meters wordt waarschijnlijk gevormd door dichtheidsafhankelijke ecologische processen zoals inter- en intraspecifieke competitie om voedsel en ruimte en predator-prooi dynamiek. Op een schaal van honderden tot duizenden meters kan aggregatie gerelateerd zijn aan habitatkeuze tijdens recrutering en/of mortaliteit in combinatie met beperkte lokale verspreiding (Underwood en Chapman, 1996).

Tussen de ruimtelijke patronen van *S. plana* en aanwezigheid en dichtheid van andere schelpdiersoorten van vergelijkbare niche werd geen relatie gevonden. De afwezigheid van zulke soorten zou desalniettemin een positief effect op *S. plana* kunnen hebben, omdat intraspecifieke competitie om voedsel en ruimte kan leiden tot een afname in groei en algehele conditie van de dieren (zie bijvoorbeeld Kamermans et al., 1992). Wat hier echter niet voor pleit is dat de hoogste soortendiversiteit in de Westerschelde werd waargenomen, waar *S. plana* ook de beste lichaamsconditie bereikte. Wisseling van prooi is gezien in steltlopers als reactie op veranderingen in mate van voorkomen (Zwarts en Wanink, 1993). De afwezigheid van prooisoorten als *C. edule, M. balthica* en *M. arenaria* kan dan leiden tot het toenemen van de predatiedruk op *S. plana*, en, daardoor, een hogere mortaliteit en lagere dichtheden. In zuidelijke landen echter, waar andere in het noorden veel gegeten schelpdiersoorten ontbreken en *S. plana* de voornaamste prooisoort vormt van vogelgemeenschappen (Moreira, 1997), zijn de dichtheden doorgaans hoger (Bachelet, 1979; Sola, 1997; Bazaïri et al., 2003; Verdelhos et al., 2005; Bocher et al., 2007). Dit lijkt een effect van biotische factoren uit te sluiten, hoewel een hard bewijs daarvan niet geleverd kan worden zonder gecontroleerde experimenten, met manipulatie van soortendiversiteit, dichtheden en buitensluiten van predatoren.

Significante correlaties zijn gevonden tussen type sediment en het voorkomen van *S. plana*, hoewel niet op alle locaties. Dit impliceert dat op een schaal van kilometers de voornaamste vormende krachten hydrologie en sediment zijn. In de Waddenzee bijvoorbeeld, zijn de *Scrobicularia*-plekken veranderlijk in grootte (6 km in 2008; 10-11 km in 2009). Patronen van schaal zijn vergeleken met andere, samen met *S. plana* voorkomende soorten: *Abra tenuis*, *C. edule*, *M. balthica* en *M. arenaria*. Andermaal werden, voor alle soorten, zowel willekeurige en geaggregeerde patronen gevonden, afhankelijk van de specifieke locatie. De Westerschelde, met vier soorten, was consistent geaggregeerd met een aggregatiegrootte variërend tussen de soorten. Een relatie tussen dichtheid en aggregatie was afwezig. Bij Trondheim in Noorwegen waren alle drie de daar voorkomende soorten daarentegen willekeurig verspreid. Bij Minho in Portugal, ten slotte, waren twee soorten matig geaggregeerd en de derde, *C. edule*, willekeurig verspreid. Deze resultaten onderstrepen de grote rol van lokale condities boven biologische interacties.

De afstand tussen plekken geschikt habitat kan fungeren als een barrière. Hoewel het lange pelagische levensstadium van *S. plana* larven zou moeten toestaan om grote afstanden af te leggen, tot tientallen kilometers (Gilg en Hilbish, 2003; Shanks et al., 2003), is de gerealiseerde larvale dispersie-afstand onbekend. Oceanografische stromingen en hun interacties met topografie en habitat kunnen leiden tot complexe patronen waarbij larvale dispersie is losgekoppeld van Euclidische afstand (bv. Cowen et al., 2007; Galarza et al., 2009; Weersing en Toonen, 2009; White et al., 2010). In sommige gevallen worden retentiezones gevormd (bv. Bradbury et al., 2008; Cowen en Sponaugle, 2009; Galindo et al., 2010; Small en Wares, 2010). Wij namen een significant isolatie-met-afstandseffect waar (**Hoofdstuk 6**) wat suggereert dat populaties *S. plana* zelf-recruterend zijn. Omdat lokale retentie vaak gerelateerd is aan soortspecieke ecologie en habitat (Swearer et al., 2002; Cowen en Sponaugle, 2009), mogen we aannemen dat het in het geval van *S. plana* te maken heeft met diens habitat-gerelateerde, ruimtelijk geaggregeerde verspreiding. *Cerastoderma edule* liet eerder ook isolatie-met-afstand zien, maar alleen voor noordelijke onderzoekslocaties (Krakau et al., 2012), waar de soort algemener is en minder gezoneerd (Bocher et al., 2007), wat de relatie tussen ruimtelijke patronen en populatiegenetische structuur versterkt. De lage mate van connectiviteit tussen populaties zoals hier afgeleid voor *S. plana* zou de levensvatbaarheid van de metapopulatie kunnen verlagen en de kans op lokaal uitsterven vergroten (Lecomte et al., 2004).

Conclusie

Het hoofddoel van dit proefschrift was om patronen in ruimte en tijd van populatiestructuur en populatiedynamics te bestuderen van *Scrobicularia plana*, een van de meest voorkomende schelpdiersoorten in Europa's getijdezones met zacht sediment. Effecten van het paleoklimaat hebben ertoe geleid dat de soort zich uitgebreid heeft langs de Europese kusten gedurende de afgelopen 300.000 jaar. De populaties zijn groot en over het algemeen genetisch divers met een regionale populatiegenetische structuur gekarakteriseerd door sterke, waarschijnlijk oceanografische insluiting.

Scrobicularia plana komt voor over een enorme breedtegraadgradient, wat suggereert dat de soort zowel lokaal aangepast als fenotypisch plastisch zal zijn. Temperatuur stuurt de verspreiding en fenologie, en de aanvang van het paaiseizoen in het voorjaar. Het paaiseizoen begint het vroegst en duurt het langst in Portugal en begint steeds later en duurt korter naar het noorden toe. De mate waarin de geografische verspreiding van *S. plana* beïnvloed zal worden door klimaarverandering is waarschijnlijk minimaal vanwege het feit dat de genetische diversiteit zo hoog is, wat lokaal uitsterven onwaarschijnlijk maakt gezien de potentie voor in situ aanpassing. Desalniettemin zou de soort naar het noorden kunnen verschuiven en daar andere soorten van het getijdegebied vervangen, zoals bijvoorbeeld *M. balthica*, die een lager aanpassingsvermogen zou kunnen hebben. Zo'n verschuiving zou de belangrijke rol van de soort kunnen verstoren in zuideuropese landen, waar deze niet alleen de belangrijkste prooisoort is voor vogels maar ook van economische waarde is.

Groei wordt bij *S. plana* sterk beïnvloed door voedselbeschikbaarheid; de pieken in somatische massa vallen samen met fytoplanktonbloei. Veranderingen in temperatuurregimes zullen leiden tot veranderde timing, duur en samenstelling van de fytoplanktonbloei, wat niet alleen groei maar ook voortplanting en uiteindelijk overleving zal treffen. Het vaststellen van groeiparameters was niet mogelijk omdat nauwkeurige leeftijdsbepaling van *S. plana* slechts mogelijk zijn met behulp van isotopensclerochronologie, een methode die onmogelijk kostbaar en daardoor niet geschikt voor toepassing op grote datasets.

Seksuele volwassenheid ontwikkelt bij *S. plana* bij een leeftijd van twee jaar, en dus niet wanneer de dieren een bepaalde minimale afmeting bereiken. In gebieden waar de soort bevist wordt echter, zou sterke selectie op grootte de leeftijd waarop voor het eerst gepaaid wordt uiteindelijk kunnen vervroegen en/of fitness verlagen. Deze mogelijkheid kan onderzocht worden nu de mogelijkheid bestaat om de leeftijd meer nauwkeurig de bepalen. Op dit moment is er geen direct bewijs dat natuurlijke of visserijgerelateerde stress een bedreiging is voor de duurzaamheid van *S. plana*, hoewel waakzaamheid geboden is.

Aggregatie in verspreiding en de ruimtelijke schaal ervan op lokaal niveau zijn karakteristiek voor *S. plana* en andere ermee samen voorkomende soorten tweekleppige schelpdieren. Hydrografie en sedimentsamenstelling zorgen samen voor de aggregatie en de afmeting van deze aggregatie, op een manier die per locatie verschilt; er zijn geen aanwijzingen dat ook biotische interacties zoals competitie of predatie ook een rol spelen. Het ruimtelijke patroon van de soort is waarschijnlijk een factor die zorgt voor zelf-recrutering van lokale populaties en daardoor voor lage connectiviteit tussen populaties onderling.

Concluderend is het getijdegebied van nature dynamisch en speelt daar stochastiek een grote rol bij het bepalen van het relatieve belang van bepaalde omgevingsfactoren, die op een complexe manier samenhangen - toch zijn de resulterende patronen niet geheel willekeurig. Hoewel het nog altijd een uitdaging is om deze complexe biotische en abiotische interacties te modelleren, en de drempelwaarden van transities naar alternative evenwichten te identificeren, is de conclusie van dit proefschrift dat *S. plana* goed fictioneert op alle ruimtelijke schalen. De lage connectiviteit tussen populaties betekent echter wel over de noord-zuid gradiënt geïsoleerde populaties, wat grenzen stelt aan het vermogen van toekomstige populaties om zich aan te passen aan veranderende omstandigheden.

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Síntese

A lambujinha *Scrobicularia plana* é uma das mais abundantes espécies de bivalves ao longo da costa Europeia (Bocher et al., 2007). Esta espécie tem um papel chave nos ecossistemas bentónicos, nomeadamente ao nível da cadeia alimentar. Além de afectar a produção primária através do consumo de diatomáceas e fitoplâncton marinho (Riera et al., 1999), serve também de alimento para diversas espécies de aves marinhas (Moreira, 1997). No sul da Europa tem ainda uma importância acrescida dada a sua comercialização para consumo humano (Langston et al., 2007). De modo a identificar as lacunas no conhecimento geral acerca da espécie e melhor avaliar como esta responderá a, por exemplo, alterações ambientais, fragmentação do habitat e exploração comercial, foi efectuada uma revisão exaustiva da literatura abordando diversos aspectos da história natural da lambujinha (**Capítulo 2**). Esta análise qualitativa revelou não existir uma combinação específica de factores que consiga explicar, por exemplo, os padrões de assentamento larvar ou de agregação espacial tão característicos desta espécie. Seguidamente, foi efectuada uma análise empírica focada nos padrões espaciais e temporais e nos seus efeitos na dinâmica e estrutura das populações. Para isso foram analisadas três escalas espaciais: a larga escala - gradiente latitudinal ao longo da costa Europeia; a média escala - Mar de Wadden; e a pequena escala (inferior a 3 km^2) - três áreas específicas em Portugal, Holanda e Noruega. Estes padrões temporais e espaciais permitiram determinar de que forma características específicas da espécie dão informação sobre o potencial de adaptação, dispersão, efeitos da fragmentação de habitat/isolamento e sobre as políticas de gestão a seguir de forma a preservar a sustentabilidade das populações.

Ecologia populacional

A dinâmica populacional e fenológica de *S. plana* (**Capítulo 3**) foram analisadas em três áreas ao longo da distribuição latitudinal da espécie: no sul da Europa (Portugal), a uma latitude intermédia (Holanda) e no norte da Europa (Noruega). Nestas zonas, os ciclos sazonais de massa corporal (BMI), massa somática (SMI) e massa da gónada (GMI) foram comparados. Como já era esperado, a temperatura e a disponibilidade de alimento afectaram o ciclo sazonal dos três índices. O período reprodutivo iniciou-se primeiro em Portugal, na Primavera, continuando ao longo do Verão. Nas latitudes mais altas, o início do período reprodutivo foi progressivamente retardado, os períodos reprodutivos foram mais curtos e o investimento individual na reprodução (em termos de tamanho na gónada) foram menores. No final do período reprodutivo, em todos os locais, a desova foi completa, à semelhança do que já foi observado para o berbigão *Cerastoderma edule* (Cardoso et al., 2009). Outros bivalves, como *Mya arenaria*, têm uma estratégia reprodutiva diferente em que a gónada é usada não só para fins reprodutivos mas também para armazenamento de energia (Cardoso et al., 2009). Isto pode constituir uma vantagem quando os animais estão sujeitos a condições desfavoráveis, e sugere que *M. arenaria* será menos susceptível a alterações ambientais do que *S. plana* e *C. edule*. Quer a massa corporal quer a massa somática apresentaram valores menores em Portugal, o que poderá ser devido ao menor tamanho dos animais como consequência da exploração comercial. O tamanho comercial em Portugal é de 25 mm (Coelho et al., 2006), um tamanho idêntico ao do animal mais pequeno que estava sexualmente maduro neste estudo. Assim sendo, é possível que, nalgumas áreas, uma grande proporção da população reprodutiva esteja a ser apanhada para consumo humano, o que poderá resultar no declínio, ou até mesmo no desaparecimento dessas populações. Verificou-se que a temperatura e a abundância de plâncton estão fortemente correlacionadas com o investimento reprodutivo individual e com os ciclos de massa somática indicando que, tal como em muitas outras espécies, alterações nas temperaturas costeiras bem como na altura dos picos de plâncton afectarão a distribuição e fenologia da espécie. É então possível que uma alteração das temperaturas leve à antecipação da desova em *S. plana*, o que poderá resultar numa diminuição do recrutamento e num aumento da predação, como já foi observado em *Macoma balthica* (Philippart et al., 2003). Espera-se portanto que em espécies mais adaptadas a climas frios, como *M. balthica*, ocorra um declínio das populações enquanto espécies de climas mais quentes ocuparão o seu lugar. *S. plana* já “ocupa” o lugar de *M. balthica* e *C. edule* noutras áreas, a latitudes mais baixas (Bocher et al., 2007). É esperado que, com um aumento das temperaturas, *S. plana* mude a sua distribuição para norte o que poderá afectar negativamente a sua comercialização no sul da Europa. Os resultados desta tese também revelam que *S. plana* tem uma considerável plasticidade fenotípica o que, embora seja energeticamente dispendioso, constitui uma vantagem selectiva para a espécie (Jokela and Mutikainen, 1995; Kingsolver and Huey, 1998), pois permite uma maior flexibilidade na resposta a alterações ambientais.

Os indicadores genéticos de viabilidade populacional (fitness) revelam que *S. plana* tem geralmente populações com um elevado número de indivíduos e elevada diversidade genética (Reed and Frankham, 2003; Reed, 2005; Gamfeldt and Källström, 2007). Convém contudo referir que existe uma correlação fraca entre os marcadores genéticos teoricamente neutrais (tais como o ADN mitocondrial) e os atributos fenotípicos quantitativos relacionados com a viabilidade populacional (McKay and Latta, 2002; Holderegger et al., 2006). Em *S. plana*, tais atributos incluem por exemplo o crescimento, o esforço reprodutivo, a fecundidade e a idade e tamanho aquando da primeira maturação. Contudo, a diversidade genética neutral não deixa de levar a um aumento da biodiversidade dentro das comunidades e da sua resistência a distúrbios ambientais (Hughes et al., 2008). Assim sendo, de uma perspectiva neutral, *S. plana* não aparenta estar em risco de sofrer extinções locais ou regionais. Esta conclusão é especialmente importante no caso de espécies comercializadas, uma vez que a sobre exploração pode resultar na perda de diversidade local (Luttikhuizen et al., 2003b). Mais uma vez este não parece ser o caso em *S. plana* dado que a diversidade genética é superior a latitudes mais baixas, onde a espécie é explorada para fins comerciais. Contudo, dada a natural preferência por animais de maior dimensão, nas áreas exploradas os tamanhos individuais são mais reduzidos. Isto poderá resultar numa predominância de classes de tamanho inferior causando uma diminuição da idade a que *S. plana* atinge a maturidade e, consequentemente, resultando numa menor fecundidade e viabilidade a médio termo (como já é verificado em quase todas as espécies de peixe comercializadas). Alternativamente, a selecção de indivíduos maiores poderá levar a uma redução do tamanho e da fecundidade, independentemente da idade. Tal hipótese poderá ser agora verificada através da análise de isótopos estáveis nas conchas (**Capítulo 4**).

A maturação sexual em *S. plana* está relacionada com a idade e não com o tamanho do indivíduo. O mesmo foi observado para outras espécies de bivalves como *Donax trunculus* (Gaspar et al., 1999), *Mytilus edulis* (Seed, 1969), *Spisula solida* (Gaspar and Monteiro, 1999), e *Venus striatula* e *Ensis siliqua* (Gaspar and Monteiro, 1998). Presume-se que a idade de maturação sexual resulte da tentativa de obter um equilíbrio entre as vantagens de um período de vida curto e as desvantagens resultantes de um menor crescimento. Enquanto um período de vida mais curto aumenta a possibilidade de um indivíduo reproduzir antes da sua morte; um tamanho maior resulta numa maior fecundidade e na possibilidade de os indivíduos se enterrarem a maiores profundidades reduzindo assim o risco de predação (Stearns and Koella, 1986; Kozlowski, 1992; Zaklan and Ydenberg, 1997). A reprodução em *S. plana* ocorre em indivíduos com idades iguais ou superiores a dois anos de vida (Paes-da Franca, 1956; Sola, 1997; Guerreiro, 1998). Contudo, noutras espécies de bivalves também presentes nos estuários Europeus, nomeadamente *M. balthica* e *M. arenaria*, para as quais a maturação sexual é uma função do tamanho, os indivíduos podem atingir a maturidade no primeiro ano de vida (Brousseau and Baglivo, 1988; Harvey and Vincent, 1989). Sendo a maturidade uma função do tamanho, um aumento das taxas de crescimento podem levar a que a maturidade seja atingida ainda mais cedo. Dado que uma maturação sexual antecipada permite uma melhor adaptação na resposta a mudanças das condições ambientais, *M. balthica* e *M. arenaria* têm assim uma estratégia mais vantajosa.

A idade de maturação deve ser determinada para todas as áreas onde a espécie é explorada de forma a manter a sustentabilidade e condição do stock. Na *S. plana*, a determinação da idade baseia-se, tradicionalmente, na análise dos anéis de crescimento na superfície da concha. Uma vez que é importante determinar correctamente a idade de um indivíduo, diferentes métodos de determinação da idade em *S. plana* foram usados para verificar qual deles resulta em estimativas mais correctas (**Capítulo 4**). Assim sendo, contagens de anéis externos e internos foram comparadas com os perfis de isótopos estáveis ($\delta^{18}O$ e $\delta^{13}C$) nas conchas de *S. plana*. Os resultados revelaram que a análise de perfis de $\delta^{18}O$ proporciona uma estimativa correcta da idade de *S. plana*. Adicionalmente, verificámos que as análises baseadas em anéis externos não são de confiança, resultando numa sobrestimação da idade e subestimação das taxas de crescimento; estas últimas devido à ocorrência de falsos anéis de crescimento. A análise de anéis internos também se revelou pouco precisa, particularmente nas zonas mais velhas da concha. Uma vez que a maioria dos estudos sobre crescimento e dinâmica das populações de *S. plana* (Green, 1957; Hughes, 1970; Bachelet, 1981; Sola, 1997; Guerreiro, 1998), bem como da maioria das espécies de bivalves, se baseiam na análise de anéis externos para determinação de idades, os seus resultados terão que ser considerados com bastantes reservas. Embora a análise de perfis de isótopos estáveis seja um método preciso, é também bastante dispendioso. Como alternativa, a análise de elementos químicos na concha poderá ser utilizada dado que se verificou que a proporção Sr/Ca permite a identificação de anéis anuais de crescimento em *M. balthica* (Cardoso et al., unpubl.). Contudo, este método é igualmente dispendioso e moroso. No momento, não parecem existir alternativas mais práticas.

Padrões espaciais

Os padrões de distribuição de *S. plana* foram analisados à escala filogeográfica bem como a duas escalas mais reduzidas. Os padrões a larga escala proporcionaram uma perspectiva evolucionária dos episódios paleoclimáticos (especialmente das últimas glaciações) que moldaram as distribuições biogeográficas de *S. plana*, ao longo do norte da Europa. Por outro lado, os padrões a escalas mais reduzidas, nomeadamente ao nível dos estuários e baías, permitiram obter informação sobre a dispersão, a conectividade das populações e a estrutura das metapopulações. Juntos, estes padrões permitiram determinar o que aconteceu às populações de *S. plana* no passado e, simultaneamente, o que é esperado ocorrer no futuro.

Tal como foi discutido no **Capítulo 3**, diferenças sazonais e latitudinais nos ciclos de massa somática e da gónada em diferentes áreas foram atribuídos a variações de temperatura e disponibilidade de alimento. A relação entre os padrões da espécie em larga escala e a temperatura sugere que alterações climáticas levarão, muito provavelmente, a uma deslocação da distribuição de *S. plana* para norte, com a possível extinção de algumas populações do sul da Europa (o que por sua vez afectará a comercialização da espécie). Contudo, também se poderá verificar uma rápida adaptação da espécie através de uma forte selecção sobre os genótipos pré-adaptados que estarão certamente presentes em populações numerosas e com elevada diversidade genética, como é o caso de *S. plana*.

A perspectiva filogeográfica apresentada no **Capítulo 5** capta os efeitos modeladores do último máximo glacial (20,000 anos atrás) e do início do Pleioceno. Estes terão um efeito dramático na actual estrutura genética das espécies (Hewitt, 2004). Os padrões filogeográficos de *S. plana*, em conjunto com o elevado número de indivíduos em cada população e as taxas de migração reduzidas sugerem uma expansão demográfica recente. A rede (*network*) de haplótipos em forma de estrela (isto é, a presença de um haplótipo principal, abundante e amplamente distribuído, com uma série de haplótipos que divergiram recentemente e que estão fortemente relacionados com o haplótipo principal) é típica de espécies que sofreram uma rápida diminuição populacional (*bottleneck*) e, subsequentemente, recuperaram. Neste estudo estimámos que tal expansão terá ocorrido há cerca de 0.3-1.1 milhões de anos atrás. Durante este período, a espécie esteve sujeita a uma série de episódios glaciares que resultaram em enormes alterações na geografia e clima da Europa do Norte (Kukla et al., 2002). O elevado isolamento geográfico e as reduzidas taxas de migração verificadas, indicam que as populações estão localmente isoladas apesar do potencial teórico para uma elevada dispersão larvar durante a fase pelágica. As condições oceanográficas poderão ser responsáveis pelo padrão observado, enquanto a fraca diferenciação populacional a escalas mais reduzidas poderá reflectir conectividade ao nível meta-populacional, o que seria consistente com a distribuição agregada de *S. plana*, por exemplo, no Mar de Wadden. Os padrões observados também sugerem adaptação local, o que, contudo, não poderá ser testado usando marcadores neutrais. O bivalve *M. balthica* apresenta um padrão bastante diferente, com populações geneticamente muito distintas e que divergiram há consideravelmente mais tempo (Luttikhuizen et al., 2003a). Populações de *M. balthica* poderão revelar então uma menor plasticidade fisiológica o que sugere, mais uma vez, que a espécie poderá ser substituída por *S. plana* no caso de um aumento da temperatura. Por sua vez, apesar de uma maior estruturação da rede de haplótipos, *C. edule* apresenta um padrão, bem como valores de diversidade haplotípica, semelhantes aos de *S. plana* (Krakau et al., 2012). Assim sendo, tal como *S. plana*, espera-se que *C. edule* se adapte mais facilmente a alterações ambientais, deslocando a sua distribuição para norte. Estas previsões terão uma enorme influência ao nível do funcionamento dos ecossistemas.

A agregação espacial de *S. plana* a uma escala local, analisada no **Capítulo 6**, revelou que tais padrões são característicos da área e não da espécie. Através da análise de autocorrelações espaciais, uma agregação significativa estatisticamente foi verificada em três das quatro áreas investigadas, com o tamanho das agregações (*patches*) variando entre 150-1250 m e entre 4-10 km. As variações à escala de dezenas de metros resultam, provavelmente, de processos ecológicos dependentes das densidades tais como a competição por alimento e espaço, e as dinâmicas predador-presa. A escalas de centenas a milhares de metros, a agregação espacial poderá estar associada à selecção de habitat e ao recrutamento (e/ou mortalidade) em combinação com uma dispersão local reduzida (Underwood and Chapman, 1996).

Não foi possível identificar uma relação entre os padrões espaciais de *S. plana* e a presença, bem como com a densidade, de outras espécies de bivalves pertencentes a nichos ecológicos semelhantes. A ausência de tais espécies poderá ter um efeito positivo uma vez que a competição entre espécies por alimento e espaço pode levar a uma redução do crescimento e da condição geral dos animais (e.g., Kamermans et al., 1992). Contudo, *S. plana* apresenta uma melhor condição em Westerschelde (Holanda), onde foi observada uma maior diversidade específica, o que contraria essa hipótese. Sabe-se que as aves marinhas podem alterar a sua dieta em resposta a variações de abundância de diferentes grupos de presas (Zwarts & Wanink, 1993). A ausência de presas como *C. edule*, *M. balthica* e *M. arenaria* poderá então levar a aumento da pressão de predação sobre *S. plana* e, consequentemente, a um aumento da mortalidade e a uma redução da densidade da espécie. No Sul da Europa, contudo, onde outras espécies de bivalves normalmente consumidas em estuários da Europa do Norte estão ausentes e *S. plana* é uma das principais presas das comunidades de aves marinhas (Moreira, 1997), as densidades são tipicamente mais elevadas (Bachelet, 1979; Sola, 1997; Bazaïri et al., 2003; Verdelhos et al., 2005; Bocher et al., 2007). Isto parece excluir o efeito de factores abióticos na definição dos padrões espaciais de *S. plana*, no entanto, experiências em condições controladas (através da manipulação da abundância e diversidade específica, bem como da exclusão de predadores) têm que ser feitas para confirmar esta hipótese.

Foi também verificada uma correlação significativa entre o tipo de sedimento e a distribuição de *S. plana*, mas não para todas as áreas. Isto sugere que, a escalas de milhares de metros, as condições hidrográficas e o tipo de sedimento serão os principais modeladores dos padrões espaciais. Por exemplo, no Mar de Wadden, o tamanho das agregações é temporalmente variável (6 km em 2008; 10-11 km em 2009). Os padrões de *S. plana* foram também comparados com os de outras espécies que ocorrem nas mesmas áreas, nomeadamente *Abra tenuis*, *C. edule*, *M. balthica* e *M. arenaria*. Novamente, quer padrões aleatórios como agregados foram observados para as diferentes espécies, dependendo da área em estudo. No Westerschelde (Holanda), as quatro espécies revelaram um padrão agregado com o tamanho das agregações variando entre espécies. Não se observou uma relação clara entre densidade e agregação. Contrariamente, em Trondheim (Noruega), as três espécies presentes apresentaram uma distribuição aleatória. Finalmente, no Minho (Portugal) onde apenas duas espécies estão presentes *S. plana* demonstrou um padrão agregado e *C. edule* um padrão aleatório. Estes resultados são outra indicação de uma maior influência de condições locais do que de interacções biológicas nos padrões de distribuição.

A distância entre agregações pode funcionar como uma barreira ao movimento. Apesar da longa fase pelágica de *S. plana* permitir que as larvas, teoricamente, se dispersem por dezenas de km (Gilg and Hilbish, 2003; Shanks et al., 2003), o nível real de dispersão é desconhecido. As correntes oceanográficas e a sua interacção com a topografia e com o habitat podem resultar em padrões complexos em que a dispersão larvar é independente da distância geográfica (e.g., Cowen et al., 2007; Galarza et al., 2009; Weersing and Toonen, 2009; White et al., 2010). Nalguns casos, poderão formar-se zonas de retenção (e.g., Bradbury et al., 2008; Cowen and Sponaugle, 2009; Galindo et al., 2010; Small and Wares, 2010). Nós observamos um efeito significativo do isolamento-por-distância (*isolation-by-distance*) (**Capítulo 6**), o que sugere a ocorrência de recrutamento a nível local. Dado que este fenómeno se encontra, na maioria das vezes, relacionado com as características ecológicas e do habitat das espécies (Swearer et al., 2002; Cowen and Sponaugle, 2009), no caso de *S. plana* é então provável que esteja relacionado com a distribuição agregada da espécie, que por sua vez é definida por factores locais. *Cerastoderma edule* também exibe um efeito significativo do isolamento-por-distância na variação genética mas apenas no norte da Europa (Krakau et al., 2012) onde a espécie é mais abundante e caracterizada por uma zonação, relativamente ao nível do mar, menos marcada (Bocher et al., 2007), o que fortalece a relação entre os padrões espaciais e a estrutura das populações. A baixa conectividade observada entre populações de *S. plana* poderá levar a uma diminuição da viabilidade das metapopulações, resultando num aumento do risco de extinções a nível local (Lecomte et al., 2004).

Conclusão

O objectivo principal desta tese foi estudar os padrões espaciais e temporais da estrutura e dinâmica das populações de *Scrobicularia plana*, um dos bivalves mais comuns e abundantes nos ecossistemas estuarinos europeus. Efeitos paleoclimáticos resultaram numa expansão (relativamente) recente da espécie ao longo da costa europeia, nos últimos 300,000 anos. As populações são numerosas e geneticamente diversas com a estrutura populacional a nível regional a resultar, muito provavelmente, da retenção oceanográfica.

A vasta distribuição latitudinal de *S. plana* sugere uma elevada adaptação local bem como plasticidade fisiológica. A temperatura molda a distribuição e fenologia da espécie, com o período reprodutivo a ter início na Primavera em Portugal e sendo, progressivamente, mais tardio e curto à medida que avançamos para latitudes mais elevadas. Num cenário de alterações climáticas, o impacto das alterações ambientais, n a distribuição biogeográfica de *S. plana* será mínimo uma vez que a diversidade genética é elevada, sugerindo que extinções locais podem não ter um grande efeito negativo uma vez que o potencial de adaptação da espécie é elevado. Ainda assim, é possível que a espécie desloque a sua distribuição para áreas mais a norte substituindo outras espécies chaves das comunidades intertidais como, por exemplo *M. balthica* dado esta possuir um menor potencial adaptativo. Tais alterações irão afectar o papel chave de *S. plana* nos estuários do sul da Europa, não só como a principal presa das comunidades de aves marinhas mas também como espécie de elevado interesse económico.

O crescimento de *S. plana* é fortemente moldado pela disponibilidade de alimento, com os picos dos ciclos de massa somática a corresponder a picos na abundância do fitoplâncton. Mudanças de temperatura levarão a alterações do momento, duração e composição dos picos de fitoplâncton o que afectará não só o crescimento mas também a reprodução e, até mesmo, a sobrevivência da espécie. A determinação de parâmetros de crescimento não foi possível dado que estimativas correctas da idade em *S. plana* só são obtidas através da análise de isótopos estáveis na concha, um método economicamente incomportável aquando da análise de um número tão grande de amostras.

A maturação sexual em *S. plana* é atingida aos dois anos de idade e não quando os animais atingem um tamanho específico. Contudo, em áreas onde a espécie é explorada, uma forte selecção sobre o tamanho dos organismos poderá levar a uma diminuição da idade a que a maturidade é atingida, e pode reduzir a viabilidade das populações. Esta hipótese pode agora ser testada dada a possibilidade de obter estimativas rigorosas da idade. De momento, não existem indicações concretas de que a exploração comercial seja uma ameaça á sustentabilidade das populações mas vigilância é necessária.

Os padrões de distribuição agregados, ao nível local, são característicos de *S. plana* bem como de outras espécies de bivalves que ocorrem nas mesmas áreas. As condições hidrográficas e o tipo de sedimento, mais do que as interacções biológicas relacionadas com a competição e a predação, moldam a forma e o tamanho das agregações a nível local. O padrão espacial da espécie será, pelo menos parcialmente, responsável pelo recrutamento local e, consequentemente, pela baixa conectividade entre populações.

Em conclusão, as zonas intertidais são por natureza áreas dinâmicas onde processos imprevisíveis têm um papel importante na determinação da importância de factores ambientais específicos que interagem de forma complexa, resultando em padrões não aleatórios. Embora a nossa capacidade para compreender as complexas interacções bióticas e abióticas, e identificar o limiar da transição entre diferentes estados, está a melhorar, a conclusão geral da tese é que *S. plana* tem um bom desempenho ao nível de todas as escalas analisadas. Contudo, a baixa conectividade inter-populacional levará, certamente, a populações latitudinalmente desconectadas o que diminuirá a capacidade para, no futuro, as populações se adaptarem a alterações ambientais.

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Curriculum vitae

Sílvia Santos was born on the 6th of May 1982 in Ribeirão, Portugal. In 2000 she started studying Biology at the Faculty of Sciences of the University of Porto, from which she graduated in 2004, with a specialization in Applied Animal Biology. During the following three years, she participated in a series of projects which provided her with considerable experience in the area of benthic ecology. Soon after graduation she started a 5 month internship at CIIMAR where she provided assistance to ongoing projects, helping with field work, and sorting and analyses of biological samples. This was followed by an 8 month period (April to November 2005) at Bodø University College, in Norway, where she studied different components of intertidal benthic communities, from growth and reproduction in bivalves and crustaceans, to predation pressure of different fish species, and abundance of marine birds. In February 2006, she started a 4 month internship at the molecular laboratory at NIOZ to help investigate the phylogeography of the common shrimp *Crangon crangon* across its distribution range, as part of a project from Dr. Joana Campos. From June to July 2006, she trained undergraduate students from a university summer course in the area of Marine Biology by preparing and giving lectures, while also setting-up field sampling and laboratory experiments. All periods in between internships, she was volunteering at CIIMAR where she provided assistance to ongoing projects at Minho estuary. In April 2007, she was granted a 4-year scholarship from the Portuguese Science Foundation to do her PhD at NIOZ. The results from that project are documented in this thesis.

Publications

Santos, S., Cruzeiro, C., Olsen, J.L., van der Veer, H.W., Luttikhuizen, P.C., *in press*. Isolation by distance and low connectivity in the peppery furrow shell *Scrobicularia plana* (Bivalvia) (da Costa, 1778). Mar. Ecol. Prog. Ser.

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