

**Biogeographic patterns of the marine
bivalve *Cerastoderma edule* along
European Atlantic coasts**

DISSERTATION

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CONTENTS

SUMMARY	I
ZUSAMMENFASSUNG	III
GENERAL INTRODUCTION	1
CHAPTER 1: Shell forms of the intertidal bivalve <i>Cerastoderma edule</i> L. from Africa to the Arctic	9
CHAPTER 2: Cockle parasites across biogeographic provinces	36
CHAPTER 3: Genetic diversity in high latitudes – an intertidal bivalve contradicts a common pattern	59
GENERAL DISCUSSION	89
REFERENCES	95
APPENDIX	X1
ACKNOWLEDGEMENTS/DANKSAGUNG	

SUMMARY

The cockle *Cerastoderma edule* is a common bivalve that inhabits the marine soft-bottom intertidal along European shores. This invertebrate plays a key role in coastal food webs of the Northeast Atlantic coasts due of its high abundances. I studied cockles from 19 sites along the distribution range with the aim to describe the variation of geographic population structures on different analytical levels. Cockles from the Barents Sea to the African Atlantic coast were analysed with respect to their geographical differentiation in shell morphology, parasite load and population genetics.

Morphological characteristics were analysed for a potential geographic pattern within the European cockle *C. edule*. Spatial differences of eight shell characteristics within samples from 18 sites were studied. However, biogeographic groups could not be discovered due to strong local variability in shell form parameters. Environmental conditions and/or co-existing species were suspected to shape the plasticity of cockle shells at each site. At sites where the sister species *C. glaucum* occurred together with *C. edule*, high variability of shell characters was found in both species. The lack of morphological character displacement in mixed populations confined reliable species identification to the DNA level.

Since parasites often strongly influence host morphology and its life history traits, the biogeography of the trematode assemblage was studied for *C. edule* as first and second intermediate host. At ten European locations ranging from Norway to Portugal, 30 cockle specimens per site were sampled to detect trematode diversity and abundance. Cockles from the Scandinavian sites shared dominant trematode species (*Himasthla elongata*, *Meiogymnophallus minutus* and *Renicola roscovita*) with the south-western European populations. However, two geographical groups could be revealed, based on the occurrence of rare species and changes in the abundances of common trematodes. The geographic distribution of suitable hosts for closing parasite life cycles is most likely responsible for the separation of a southern and a northern community. Thus, the scatter of shell forms was not reflected in the parasite assemblages, but a similarity to warm and cold temperate biogeographic provinces could be found.

The biogeographic groups in the parasite fauna raise the question whether the intermediate host may share the same pattern in terms of subpopulations. Hence, another hidden diversity was under survey comparing the common barcoding sequence (5' part of mitochondrial cytochrome c oxidase (COI)) for intraspecific variation of *C. edule* populations. Analyses revealed significant biogeographic clustering of cockle populations along the European shores. Among the 383 *C. edule* individuals studied, the occurrence of 79 sequence variants divided the distribution range in two large-scale geographic groups. These geographic clusters are similar to marine biogeographic provinces. The southwestern group includes all cockles from Morocco to the British Isles while one Scottish population showed particular differences. The northern group comprises the Wadden Sea, Shetland Islands as well as the Norwegian Sea. Within this northern group, Skagerrak/Kattegat populations showed both regional sequence characters indicating a secondary contact zone or a glacial refuge. The Arctic population near Murmansk deviates from all others but shows some genetic connection to Norwegian populations. In contrast to studies on other marine species, higher genetic diversity was found in populations at high latitudes than in the southern populations. Northern cockle populations displayed a complex network of genetic structure, and analyses indicate a surprisingly old demographic age.

This study revealed that European cockle populations exhibit a well structured pattern along the East Atlantic shore on two different levels, bivalve population genetics and the associated trematode assemblage. Morphological plasticity was not constant throughout the range, but varied without a clear large-scale geographical pattern. With this thesis study, I contribute to the knowledge of the large scale patterns of marine organisms endowed with pelagic larvae and corroborate the upcoming hypothesis that against expectation, coastal invertebrates are not panmictic on continental scale.

The extraordinary genetic pattern with indication for a northern refuge may provide a basis for further studies to clarify the influence of Pleistocene ice ages on coastal marine fauna and flora. Studies on physiological characteristics of cockles may reveal an adaptation of warm and cold temperate populations. Co-speciation of host and parasite with the possible development of subspecies may be discovered by a phylogeographic approach. A shift of biogeographic patterns is expected with global warming whereas local extinctions may reduce plasticity and diversity of coastal biota.

Biogeographische Muster der Meeresmuschel *Cerastoderma edule* entlang der europäischen Atlantikküste

ZUSAMMENFASSUNG

Die Herzmuschel *Cerastoderma edule* ist eine häufig anzutreffende Muschel der sandig bis schlackigen Gezeitenzone Europas. Da sie in hohen Individuendichten auftritt, spielt diese wirbellose Art eine Schlüsselrolle in den küstennahen Nahrungsnetzen der nordostatlantischen Küsten. Mit dem Ziel, die Variation der Populationsstruktur auf verschiedenen analytischen Ebenen zu beschreiben, habe ich Herzmuscheln von 19 Orten innerhalb des Verbreitungsgebietes untersucht. Die Proben aus Buchten der Barentssee bis zur Afrikanischen Atlantikküste wurden hinsichtlich ihrer geographischen Unterschiede in der Schalenmorphologie, dem Parasitenbefall und der Populationsgenetik bearbeitet.

Morphologische Merkmale wurden daraufhin geprüft, ob für die Herzmuschel *C. edule* ein mögliches geographisches Muster zu erkennen ist. Räumliche Unterschiede in acht Schalenmerkmalen wurden für 18 Probenahmestellen analysiert. Biogeographische Gruppen wurden jedoch nicht gefunden, da die Schalenparameter eine hohe lokale Variabilität zeigten. Lokale Umweltbedingungen und/oder der Einfluss anderer Arten sind wahrscheinlich der Grund für die Formenvielfalt der Herzmuschelschalen an den untersuchten Orten. Dort, wo die Schwesterart *C. glaucum* mit *C. edule* zusammen vorkam, wurde in beiden Arten eine hohe Variabilität der Schalenmerkmale gefunden. Aufgrund fehlender morphologischer Unterscheidungskriterien in gemischten Populationen ist eine verlässliche Artidentifizierung nur auf DNA-Ebene möglich.

Da Parasiten oft starken Einfluss auf die Morphologie und Biologie der Wirte haben, wurde nach einem biogeographischen Muster in der Zusammensetzung der Parasitengemeinschaft von *C. edule* als erster und zweiter Zwischenwirt gesucht. An zehn Orten zwischen Norwegen und Portugal habe ich jeweils 30 Herzmuscheln untersucht, um die Trematodenvielfalt in den Wirtstieren zu ermitteln. Die Herzmuscheln der skandinavischen Orte teilten einige dominante Trematodenarten (*Himasthla elongata*, *Meiogymnophallus minutus* and *Renicola roscovita*) mit den südwestlichen Populationen. Es konnten jedoch anhand der Befallsmuster zwei geographische Gruppen unterschieden

werden, die vorwiegend durch die Anwesenheit seltener Arten und der verschieden starken Präsenz der häufigen Arten geprägt wurden. Wahrscheinlich ist die geographische Verbreitung von weiteren Wirtsarten, die den Parasitenlebenskreislauf schließen, für die Bildung einer südlichen und einer nördlichen Gruppe verantwortlich. Somit wird das verstreute Muster in der Schalenmorphologie nicht in der Parasitenzusammensetzung wiedergefunden, aber eine Ähnlichkeit zu den biogeographischen Einteilungen in warm und kalt temperierte Zonen konnte erkannt werden.

Die biogeographische Gruppierung der Parasitenfauna lässt die Frage aufkommen, ob der Zwischenwirt das gleiche Muster in Form von Subpopulationen teilt. Somit wurde eine andere unsichtbare Diversität untersucht, indem die allgemeine Barcoding-Sequenz, das 5' Ende der mitochondrialen Cytochrome c oxidase I (COI), innerhalb der Herzschneckeart *C. edule* verglichen wurde. Die Auswertungen deckten eine signifikante geographische Strukturierung der Herzschnecke-Populationen entlang der europäischen Küsten auf. Innerhalb der 383 untersuchten *C. edule* Individuen wurden 79 Sequenzvarianten gefunden, anhand derer das Verbreitungsgebiet in zwei großskalige geographische Gruppen werden kann. Diese Gruppierungen ähneln den marinen biogeographischen Provinzen. Die südwestliche Gruppierung beinhaltet alle Herzschnecke-Populationen von Marokko bis zu den Britischen Inseln, wobei eine schottische Population auffällige Unterschiede zeigte. Die nördliche Gruppe besteht aus Populationen aus dem Wattenmeer, von den Shetland-Inseln sowie der Norwegischen See. Innerhalb des Gebiets dieser nördlichen Gruppierung, wiesen Populationen aus dem Skagerrak/Kattegat-Gebiet Sequenzeigenschaften sowohl der nördlichen als auch der südlichen Gruppe auf, was auf eine sekundäre Kontaktzone oder auf ein Rückzugsgebiet während der Eiszeit hinweist. Die arktische Population aus der Nähe von Murmansk unterschied sich von allen anderen, zeigte jedoch eine genetische Verbindung zu norwegischen Populationen. Im Gegensatz zu vielen anderen marinen Arten, wurde eine hohe genetische Diversität in den Populationen der hohen Breitengrade gefunden, und nicht in den südlichen Populationen. Die nördlichen Herzschnecke-Populationen zeigten dabei ein komplexes Netzwerk in ihrer genetischen Struktur, und die Analysen weisen auf ein überraschend hohes demographisches Alter hin.

Diese Untersuchung zeigt, dass Herzschnecke-Populationen entlang der Ostatlantikküste ein gut strukturiertes Muster auf zwei Ebenen aufweisen: für die Muschel-Populationsgenetik und die assoziierte Trematodenzusammensetzung. Die morphologische Plastizität dagegen

variierte ohne ein großräumiges Muster. Mit dieser Doktorarbeit trage ich zum Wissen über großskalige Muster in Meeresorganismen mit pelagischen Larven bei und unterstütze die aufkommende Hypothese, dass Wirbellose entlang kontinentaler Küsten nicht panmiktisch sind.

Das außergewöhnliche, genetische Muster mit dem Hinweis auf ein nördliches Refugium gibt Anlass zu weiteren Untersuchungen, die den Einfluss der Eiszeiten im Pleistozän auf die Küstenfauna und -flora aufklären sollen. Weiterführende Arbeiten zu physiologischen Eigenschaften der Herzmuscheln könnten warm oder kalt angepasste Populationen deutlich machen. Eine gleichzeitige Anpassung von Wirt und Parasit sowie die mögliche Entstehung von Unterarten könnte mit phylogeographischen Analysen aufgedeckt werden. Eine Verschiebung der biogeographischen Muster mit zunehmender globaler Erwärmung wird erwartet, wobei lokales Aussterben die Plastizität und Diversität der Küstenlebewesen verringern könnte.

GENERAL INTRODUCTION

For species and species communities, biogeographic patterns can be found on different levels (Bailey 1998, Olson et al. 2001, Spalding et al. 2007). Latitudinal gradients in species diversity are one of the most conspicuous phenomena in this distribution of organisms, and mainly have been shown for terrestrial habitats (reviewed by Rohde 1992, Willig et al. 2003). A focus was laid on explaining this spatial variation in community structure and species richness (Blanchard & Bourget 1999, Gray 2002, Ricklefs 2004, Gaston et al. 2008). Mainly climatic zonation seems to drive latitudinal variation of taxonomic richness (Currie et al. 2004, Hawkins et al. 2007, Kozak & Wiens 2007) but also shapes phenotypic variation. Bergmann's rule, for example, which refers to the increasing body size of endothermic organisms towards higher latitudes, is one of the most studied and controversial patterns. Recently, Diniz-Filho et al. (2007) emphasised that this pattern in mammal species can be explained mainly as a response to environmental conditions. In ectothermic organisms declining seasonal length and lower temperature towards higher latitudes have been shown to select for latitudinal variation in growth and development but growth efficiency may increase with latitudes (Lindgren & Laurila 2005). Latitudinal clines in genetic diversity have been reported in fauna and flora whereas genetic variation in these species decreased from south to north (Hewitt 2004, Kelly & Eernisse 2007).

Differentiation within species but also speciation is mainly caused by separation due to geographical barriers. On land, critical habitat characteristics can change drastically over short distances because of explicit barriers such as mountains, deserts, and water gaps. In other cases, the bunching of terrestrial range limits may derive from historical events such as glacial intrusion or land bridge submergence (Pielou 1979). In marine systems, however, it is more difficult to envision how persistent range boundaries become locally concentrated. Although substrate types vary spatially and rivers alter local salinity levels, a single, continuous, dispersal medium (the ocean) connects all available habitats, and environmental gradients within this medium are neither as striking nor as immutable as on land (Gaylord & Gaines 2000).

Marine species are generally characterized by large population sizes, high dispersal capacity of the pelagic larval stages, and wide biogeographical distribution. The lack of migrational barriers at sea seemed to guarantee a high connectivity between distant populations and precluded their allopatric subdivision (Palumbi 1992). Meanwhile, structures showing genetic discontinuities between conspecific populations appear to be related to the life history pattern and dispersal capability of marine species (e.g. Avise 1992). However, effective dispersal in the sea is still poorly understood (Hedgecock 1986, Gaylord & Gaines 2000). However, differentiation patterns have been shown to be influenced by habitat discontinuities and isolation by distance (Johnson & Black 1995), by patterns of estuarine circulation (e.g. Ayvazian et al. 1994) and oceanic currents (Shulman & Bermingham 1995, Rocha-Olivares & Vetter 1999) as well as by local adaptations (Powers et al. 1986).

The development of species complexes has most probably been enhanced by geographic separation during historical demographic events (Bleidorn et al. 2006, Remerie et al. 2006, Nikula et al. 2007). In particular, quaternary expansions and contractions of glacial ice sheets are thought to have played an important role in shaping the distribution of biodiversity among current populations in the north-temperate region (Rowe et al. 2004). During major glaciations the polar ice sheets spread considerably, and vegetation as well as coastal zones were shifted towards the equator (Hewitt 2000). Large volume of accumulated ice lowered sea levels by about 120 m, and thus changed the local existence of coastal ecosystems. Postglacial range expansion in suitable territories is assumed for populations at the northern limits of the refugial range resulting in loss of alleles and homozygosity because of these founding events (Hewitt 2000).

For present population structuring, Hanski (1999) stated that if all local populations have a substantial risk of local extinction, long-term survival is possible only at the metapopulation level. In coastal ecosystems, this risk may derive from the high variability of the environment, with gales disturbing the area, a long lasting ice-cover during cold winters in onshore areas of the north, or oxygen depletion below stratified waters in warm summers (Armonies 2001). This may give rise to metapopulation structures with genetic differentiation patterns on a rather large scale in coastal populations.

Biogeography and coastal ecology

Biogeographic regionalization as mapped classifications of patterns in biodiversity has long been an important tool in fields from evolutionary studies to conservation planning. For the world's coastal and shelf areas, recently a classification was reissued (Spalding et al. 2007) which may be of critical importance in supporting analyses of patterns in marine biodiversity (Fig.I.1). However, coastal taxa include several of the more prominent exceptions to the rule that species diversity increases towards the tropics and may deviate from these patterns (e.g. Gaines & Lubchenco 1982). Similarly, a number of the clearest counterexamples to observed patterns of equatorward reduction in north-south range breadth (Rapoport 1982, Stevens 1989) appear in coastal ocean species (Rohde et al. 1993, Roy et al. 1994, Stevens 1996).

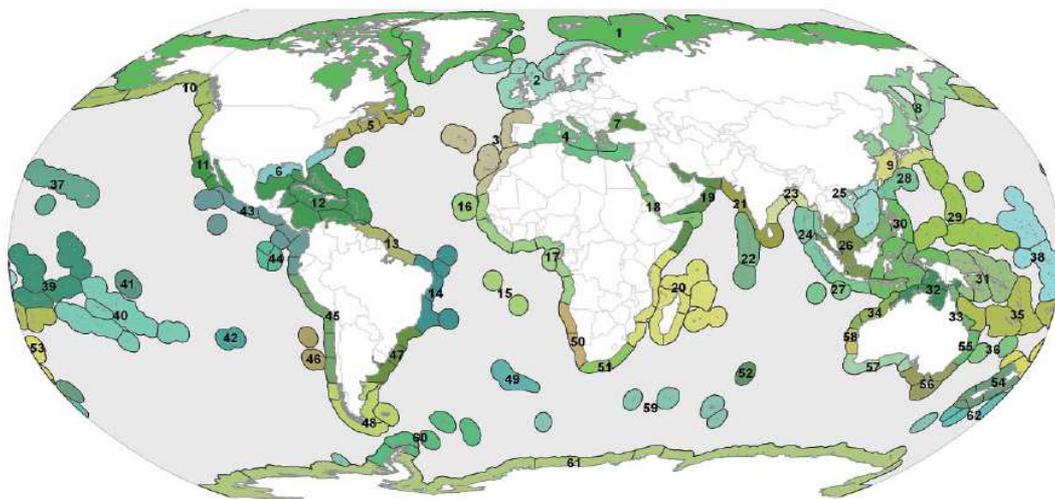


Figure I.1: Marine provinces with ecoregions outlined. European provinces: 1 – Arctic, 2 – Northern European Seas, 3 – Lusitanian, 4 – Mediterranean Sea (from Spalding et al. 2007).

Many benthic marine species, especially bivalves, achieve high rates of dispersal by means of planktonic larval stages. The presence of planktonic larvae has been long considered as a life-history strategy for the maintenance of the gene pool homogeneity of a species over its distribution area (Scheltema 1971, Buroker 1983, Palumbi 1996, Burton 1997). While some species show sufficiently high rates of gene flow to be almost panmictic, for other species the levels of gene flow are low enough to allow local selection, and genetic drift

may occur more or less independently in each deme (Slatkin 1981). Nonrandom larval dispersal may be responsible for phenotypic and genotypic clines due to a habitat gap or other dispersal barriers (Hare et al. 2005). Gaylord and Gaines (2000) suggested that the colliding areas of nearshore ocean currents have the potential to constrain a species' geographic range. Besides the rather continuous latitudinal changes of community composition, such currents might delimit the 'continental North Sea' benthos community in the English Channel, i.e., at its southern limit, and at the same time at its northern limit in the Skagerrak area (Armonies 2001).

Ecological gradients in population structure and differences in life history traits can already be observed, when warm sea water temperatures in low latitudes enhance e.g. larval development and growth (Thorson 1950, O'Connor et al. 2007). Temperature and salinity are probably the most influential factors regulating the duration of pelagic life stages in estuarine and brackish-water species (Thorson 1950, Kingston 1974). A variety of environmental factors are known to influence shell morphology and relative proportions of many bivalve species, such as latitude (Beukema & Meehan 1985), depth (Claxton et al. 1998), shore level (Franz 1993), tidal level (Dame 1972), currents (Fuiman et al. 1999), water turbulence (Hinch & Bailey 1988), wave exposure (Akester & Martel 2000), type of bottom (Claxton et al. 1998) and type of sediment (Newell & Hidu 1982). These abiotic factors influencing shell growth and shape are complemented by biotic factors including physiology and also enemies like predators and parasites (e.g. Thielges 2006a, Miura & Chiba 2007).

Parasitism as a biotic factor affecting host population dynamics has received little attention in latitudinal surveys of coastal ecosystems. However, Poulin & Mouritsen (2003) surveyed large-scale determinants of trematode infections for 54 intertidal gastropod species and pointed to an important role of local, small-scale factors, although shallow water parasites are known to generally respond to temperature (Marcogliese 2001). Because parasitic species do not just live at a locality per se, but also must reside within a host species, a further factor that may be important to consider in studies that examine the spatial variability of parasite prevalence is host genotype. For example, Lively (1989) and Grosholz (1994) demonstrated the role of genetics of molluscan hosts in influencing infection rates of trematodes.

The European cockle *Cerastoderma edule*

Along the East Atlantic coast, the European cockle *Cerastoderma edule* is an infaunal bivalve widespread in sandy bottoms. It displays high densities from the Barents Sea coast of the Kola Peninsula to West African lagoons. The cockle *C. edule* is largely confined to estuaries and sheltered bays (Baggerman 1953, Armonies 1992, 1994a, 1994b). Its broad range includes westerly mudflats of Iceland (Ingólfsson 1996) as well as the deeper subtidal at the entrance of the brackish Baltic Sea (Brock 1980). Recent records of southern occurrence prove extensive cockle beds in Moroccan lagoons (Bazairi et al. 2003) while southernmost range edge is reported for the coasts of Senegal (Hayward & Ryland 1995).

Cockles mature quickly and have a high fecundity (Honkoop & van der Meer 1998). They are relatively short-lived and have very variable recruitment and population size. Cockle larvae are exposed to coastal and tidal currents and drift normally about 30 days, before metamorphosing and settling to the seabed as postlarvae (Fig.I.2). A high degree of larval retention within a coastal system may be advantageous for these bivalves (self-recruitment), as it has been shown that populations tend to be unstable over time (Beukema 1978, Beukema & Dekker 2005). The duration of the larval period, and hence the overall mortality rate, depends on temperature (Fig.I.2) (Dare et al. 2004). Warm spring sea temperatures enhance growth and development, reduce mortality, and favour better recruitment (Widdows 1991).

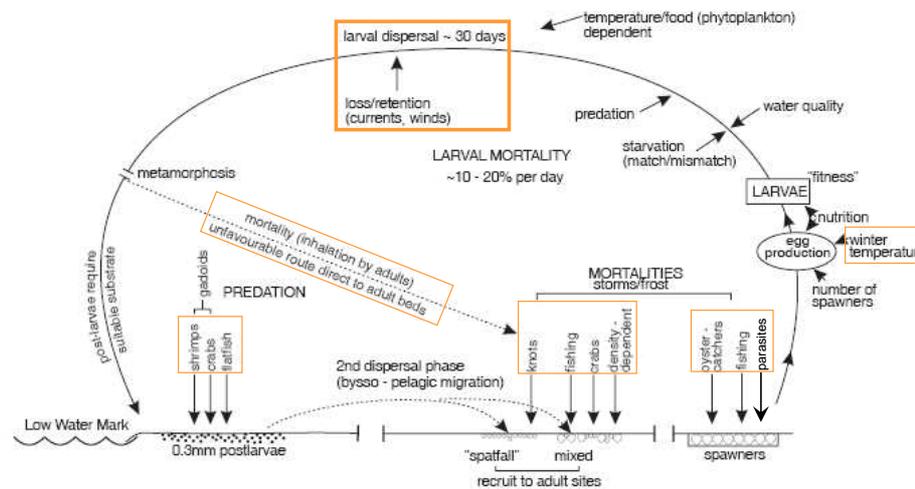


Figure I.2: Schematic diagram of cockle life-history. Factors structuring local population dynamics are highlighted (modified after Dare et al. 2004).

The survival of cockle spat recruiting onto established beds can be negatively affected by the density of older cockles as field data and experiments indicate (Fig.I.1) (Kristensen 1957, André & Rosenberg 1991, Bachelet et al. 1992, André et al. 1993, De Montaudouin & Bachelet 1996). In addition, spat mortality rates are presumed to be very high in the first weeks after settlement, when crustaceans are the main predators (Kuipers & Dapper 1981, Pihl & Rosenberg 1984, Sanchez-Salazar et al. 1987). Reduced or delayed crab predation pressure is generally considered to be an important factor contributing to high recruitment success of cockle and mussel following ‘ice winters’ in the Wadden Sea (Beukema 1991, Strasser & Günther 2001). Predator exclusion experiments in winter indicated furthermore that birds might locally eliminate juvenile cockles (Strasser 2002). Thus, cockles feature strongly in coastal foodwebs.

A large number of trematode and other parasites are known to use cockles as intermediate hosts (e.g. Carballal et al. 2001, De Montaudouin et al. 2000, Thieltges et al. 2006, De Montaudouin et al, in prep.). In many cases such infections are relatively harmless under normal environmental conditions (e.g. Wegeberg & Jensen 2003). However, some trematode species were implicated in mass mortality events of cockles (Jonsson & André 1992, Thieltges 2006b) (Fig.I.2).

Morphologically, the separation of the two sibling species *C. edule* and *C. glaucum* was a challenge for decades, as the lagoon cockle *C. glaucum* overlaps in habitat use with *C. edule* but generally lives further inshore (Høpner Petersen 1958, Rasmussen 1973, van Urk

1973). After physiological differences were shown (Lauckner 1972, Kingston 1974, Ansell et al. 1981), the species status became clearer with the use of biochemical methods (Jelnes et al. 1971, Brock 1978, Hummel et al. 1994, Machado & Costa 1994, André et al. 1999). Chromosome morphology has been described in an Atlantic population of *C. edule* (Fig.I.3) (Insua & Thiriôt-Quievreux 1992), and a chromosome complement of $2n=38$ has been reported in *C. edule* and *C. glaucum* (Thiriôt-Quievreux & Wolowicz 1996).

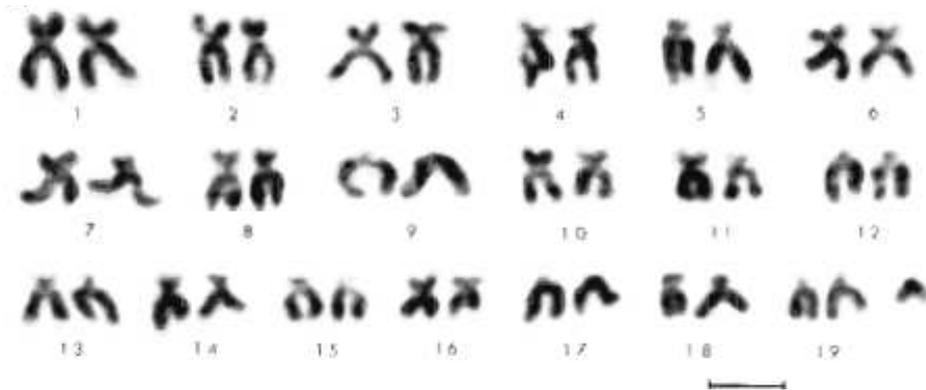


Figure I.3: Karyotype of *C. edule* chromosomes. In contrast to *C. glaucum* karyotype, no metacentric chromosomes were found in *C. edule*. Supernumerary chromosomes were only observed in *C. edule*. (graph from Insua & Thiriôt-Quievreux 1992)

While *C. glaucum* is thought to be structured into metapopulations distributed over insular habitats not regularly connected by dispersal (Reise 2003), the existence of large, coherent populations is suggested for the European cockle *C. edule*. This marine invertebrate seems to provide an interesting system to examine large-scale variation and biogeographic patterns in morphology, parasite assemblages and population genetics along the latitudinal gradient of the European shore.

Aim of the study

While the European cockle *C. edule* is reported from the Arctic Barents Sea to the subtropical Mauritanian coast, nothing is known about the structuring in morphological traits or population genetics along this large gradient. This large scale study focuses on varying expressions of biogeographic patterns by addressing three main questions:

- 1) How does the biogeographic distribution impact the morphology of the intertidal bivalve *C. edule*?
- 2) Does the trematode composition in this coastal bivalve reflect biogeographic provinces?
- 3) Is the East Atlantic coastline shaping the genetic diversity of the European cockle *C. edule* which is endowed with pelagic larvae?

Previous studies on growth parameters and local morphological descriptions indicate latitudinal differences within the East Atlantic range of the European cockle *C. edule*. In CHAPTER 1, a potential geographic pattern in morphology of this intertidal bivalve is investigated for spatial differences of eight shell characteristics. Samples from 18 sites representing the distribution range from Arctic to African coasts were studied. The degree of morphological similarity in sympatric populations at northern sites is investigated for *C. edule* and its sibling species *C. glaucum* using multivariate techniques.

Morphology and population dynamics of host species are often affected by parasites which use host resources sometimes resulting in destruction of host tissue. The cockle *C. edule* is utilized as first and second intermediate host by at least twelve known trematode species along its range from Portugal to Norway. With latitudinal sampling, I explore in CHAPTER 2 the question whether the parasite community composition in *C. edule* reflects biogeographic provinces or changes independently.

Biogeographic affiliation on DNA level was found for several marine species despite the assumption that planktonic larvae maintain gene flow between coastal populations. Additionally, a decline of genetic diversity towards high latitudes is expected following the “southern richness-northern purity” theory. In CHAPTER 3, population genetics of the European cockle *C. edule* has been analysed, as no information on this abundant species was available for its whole range. Cockles under survey originate from 19 sites, including sites near Casablanca at the Moroccan Atlantic coast to near Murmansk up to the Russian Barents Sea shore.

CHAPTER 1

Shell forms of the intertidal bivalve *Cerastoderma edule* L. from
Africa to the Arctic**Abstract**

Latitudinal patterns were revealed in the last decades for several life history traits as growth and physiological parameters. To unravel a potential geographic pattern in morphology of the intertidal bivalve *Cerastoderma edule*, I investigated geographic differences of eight shell characteristics along its distribution range. Samples from 18 sites were surveyed including cockle populations from Arctic to African coasts. The sister species *C. glaucum* was sampled at three localities where both species occurred together. The degree of morphological similarity was calculated using multivariate techniques.

Biogeographic proposed groups are not reflected in shell form but high site specific variability was apparent. The variability in shell form in *C. edule* was most likely due to environmental conditions and/or co-existing species and not due to genetic differentiation along the latitudinal gradient of cockle distribution. Contrary to previous studies, high variability of shell characters was detected in the European cockle *C. edule* compared to the lagoon cockle *C. glaucum* when found in sympatric populations. Species identification by morphometrics was shown to be most difficult in mixed populations where reliable species discrimination of these sibling species was only possible on DNA level.

Introduction

Biogeographic regions are mainly defined by species assemblages. At the European coast, Spalding et al. (2007) stretched the Lusitanian province from the West coast of Africa to the tip of Brittany, while Briggs (1995) included the Celtic Sea and set the western entry of the English Channel as provincial border. The adjacent northern region was named as Northern European Seas or Boreal region reaching to the Finnish-Russian border. This Boreal province includes among others the North Sea, Southern and Northern Norway as ecoregions defined by Spalding et al. (2007). The most northern European province was the Arctic spanning from the southern Barents Sea eastward and including Svalbard islands, Iceland and Greenland.

A variety of organisms showed differing phenotypic traits among conspecifics inhabiting different environments (Laudien et al. 2003, Pardo & Johnsons 2005, Irie 2006, Jarrett 2008). A survey on surf clams inhabiting two different temperature regimes showed that the population from the cold province were significantly flatter and less wedge-shaped than clams from the warm province (Laudien et al. 2003). Iglesias & Navarro (1990) assumed the existence of a European latitudinal trend in bivalve growth rates which increase with lowering latitude. This assumption supported early observations from Cole (1956), who showed that prolonged winter season resulted in reduced growth rates.

Coastal areas are highly variable habitats where organisms are exposed to abiotic factors like tidal movement of the water, wind as well as change in temperature and salinity. Soft-bottom species are supposed to cope with the threat of physical instability either by adaptation in morphology, burrowing behaviour or other escape strategies (Stanley 1988, Vermeij 1995). Particularly for bivalves, morphological shell characters are commonly used criteria for identification (Vermeij 1995). Suitable and reliable morphological criteria are still desired for identification purposes in field work (Machado & Costa 1994, Mariani et al 2002b). In early studies on cockles, the variability of shells has been interpreted variously as a consequence of environmental factors (Loppens 1923, Eisma 1965), or alternatively as an expression of the existence of hidden diversity (Chavan 1945). Many authors have thoroughly described physiological and ecological differences (Rygg 1970, Boyden 1972, Russell & Høpner Petersen 1973, Kingston 1974, Ivell 1979, Brock 1980) between the newly confirmed two sister species of cockles in Europe. One of these species,

C. edule, lives typically in the intertidal, and the other, *C. glaucum*, occurs mostly in lagoons and salt marsh creeks (Fig.1.1) of European coasts (Brock 1991, Hummel et al. 1994, Mariani et al. 2002a). A third morph (*C. lamarcki*) was proposed as subspecies which may have descended from *C. glaucum* in the Baltic Sea area (Brock & Christiansen 1989, Hummel et al. 1994). However, there is still some controversy about and uncertainty of the actual geographic distribution and local occurrence of these two species, especially in the Mediterranean Sea (e.g. Zenetos 1996, De Min & Vio 1997). However, at northern coasts variability of these sister species originating from similar localities is marginally investigated (Rasmussen 1973, Brock 1978).

There are several studies which dealt with the differentiation between the two sibling species *C. edule* and *C. glaucum* (e.g. in Portugal: Machado & Costa 1994). However, only a few surveys considered comparisons of geographically separated populations within the species (*C. glaucum* in Mediterranean Sea: Mariani et al. 2002b). Thus, information on morphological variation within these abundant coastal invertebrates is still limited to local studies.



Figure 1.1: Typical shells of both species of *Cerastoderma* genus. *C. edule* (left) with high number of quite flat ribs and *C. glaucum* (right) with a low rib number but pronounced rib structures.

To investigate geographical patterns in *C. edule* along its distribution range, I applied eight morphological characteristics of shells sampled from 18 sites of Arctic to African coasts. Sympatric populations with the sister species *C. glaucum*, which were found at three northern sites, were sampled to look for morphological adaptations comparing these two

sibling species. The following questions arise concerning morphological parameters differing with latitude between populations:

- (I) Are there any morphological differences between *C. edule* populations along the Atlantic distribution range?
- (II) If there are differences, do they correlate with biogeographic provinces?
- (III) Are there consistent differences in shell form between sympatric populations of the sibling species *C. edule* and *C. glaucum*?

Multivariate methods like discriminant function analyses were used considering size measurements and rib numbers of cockle shells.

Material and Methods

Cockle samples of the genus *Cerastoderma* were investigated from 18 intertidal sites of the eastern North Atlantic across biogeographic provinces (Tab.1.1, Fig.1.2).

Table 1.1: List of sampling localities for *Cerastoderma* species including geographic and sampling details. Sampling localities are indicated with sample code on the map in Fig.1.2.

Code	Species	Location	Country	n	Latitude	Longitude	Sampling date	samples provided by
MO	<i>C. edule</i>	Merja Zerga	Morocco	101	34°50' N	06°14' W	August 2005	H. Bazairi
AL	<i>C. edule</i>	Lagos	Portugal	51	37°07' N	08°37' W	September 2005	-
LN	<i>C. edule</i>	Lisbon	Portugal	50	38°43' N	09°00' W	September 2005	-
AR	<i>C. edule</i>	Arcachon	France	61	44°35' N	01°14' W	August 2005	X. de Montaudouin
RO	<i>C. edule</i>	Roscoff	France	44	48°72' N	03°97' W	June 2007	A. Wagner, K. Valentin
TH	<i>C. edule</i>	Southend	England	63	51°28' N	00°42' E	March 2007	D. Lackschewitz
DB	<i>C. edule</i>	Dublin	Ireland	50	53°19' N	06°12' W	August 2005	-
SO	<i>C. edule</i>	St. Andrews	Scotland	147	56°21' N	02°50' W	August 2006	J. Saunders
SL	<i>C. edule</i>	North Gluss	Scotland	138	60°28' N	01°21' W	September 20 07	J. Coyer, J. Olsen
LA	<i>C. edule</i>	Langoog	Germany	30	53°45' N	07°29' E	July 2007	K. Reise
SY	<i>C. edule</i>	Sylt	Germany	73	55°01' N	08°26' E	September 2005	-
LF	<i>C. edule</i>	Limfjord	Denmark	42	56°97' N	09°20' E	July 2007	K.T. Jensen
NM	<i>C. edule</i>	Norsminde	Denmark	36	56°02' N	10°25' E	July 2007	K.T. Jensen
KR	<i>C. edule</i>	Kristineberg	Sweden	50	58°14' N	11°26' E	July 2006	-
FL	<i>C. edule</i>	Arendal	Norway	49	58°26' N	08°48' E	August 2005	S. Mortensen
BN	<i>C. edule</i>	Bergen	Norway	50	60°23' N	05°20' E	August 2005	-
BO	<i>C. edule</i>	Bodoe	Norway	49	67°17' N	14°37' E	August 2005	K. Korsnes
DZ	<i>C. edule</i>	Murmansk	Russia	20	69°10' N	36°05' E	August 2007	E. Genelt-Yanovsky
SY	<i>C. glaucum</i>	Sylt	Germany	59	55°01' N	08°26' E	2004-2006	J. Fermer, S. Jacobsen
KR	<i>C. glaucum</i>	Kristineberg	Sweden	8	58°14' N	11°26' E	July 2006	-
NM	<i>C. glaucum</i>	Norsminde	Denmark	40	56°02' N	10°25' E	July 2007	K.T. Jensen

In the years 2005 to 2007, cockle individuals were sampled at low tide by hand or rake next to Lagos and Lisbon (Portugal), Dublin (Ireland), Sylt (Germany), Kristineberg (Sweden) and Bergen (Norway). In addition, samples from 12 other sites were kindly provided by colleagues (Tab.1.1). Samples of *C. glaucum* came from the Wadden Sea site Sylt (SY), and the Skagerrak/Kattegat region (KR, NM) (Fig.1.2).

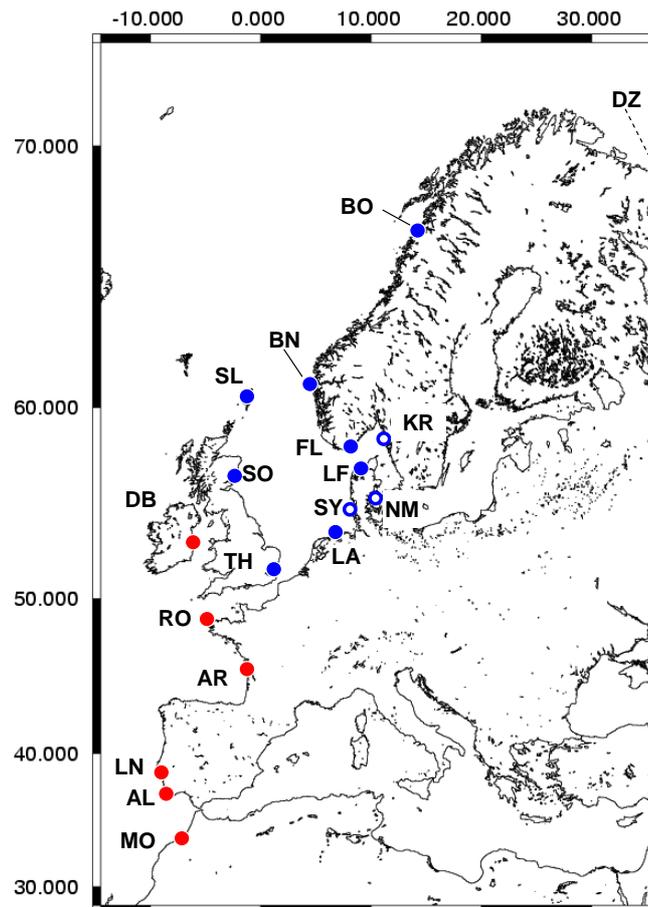


Figure 1.2: Map of sampling localities for *Cerastoderma* sp. Sample codes are labelled as in Tab.1.1. Rings indicate sampling of populations of the two species, *C. edule* and *C. glaucum*; blue circles show Boreal *C. edule* sites, red ones represent Lusitanian sites (after Briggs 1995).

Latitudinal survey on cockle morphology

Shells from 1104 individuals of *C. edule* plus 107 *C. glaucum* were measured examining a total of eight morphometric characters. Three shell dimensions – length (L), height (H) and width (W) – plus ligament length (LL) (Fig.1.3) were measured for all individuals to the nearest 0.01 mm using a digital measuring slide. Additionally, the number of ribs was

counted. For randomly chosen individuals of each population, rib width (RW), rib height (RH) and furrow width (FW) were scaled using the same digital calliper (Fig.1.3). The choice of morphological characters took those into account which were previously reported to be important for the differentiation of the two sister species *C. edule* and *C. glaucum* (e.g. in Rasmussen 1973, Brock 1978, Machado & Costa 1994). All data were length corrected to compare the results of the different sites. Additionally, curve index was derived of width and length data by using the following equation: $C=W/(2 \times L)$ (Brock 1991). Mean values plus information on minimum and maximum size were chosen to show the high variability in size parameters between the sites. The values in graphs are given as mean \pm standard deviation (SD) and standard error (SE).

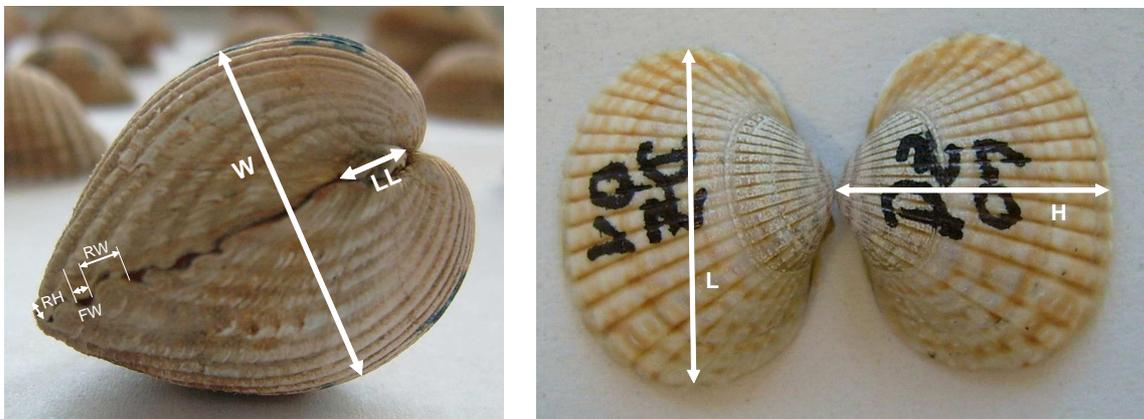


Figure 1.3: Measurement of shell characters. W: width, LL: ligament length, L: length, H: height; RH: rib height, RW: rib width, FW: furrow width.

Bivalve age was determined by counting growth rings. A growth curve was displayed by setting length versus number of growth rings counted for 140 individuals of *C. edule*, representing seven populations along the distribution range.

Inter-site comparisons

During size recording, size variability between samples could be recognised by eye. To avoid differentiation due to different age structures in the sampled populations, morphometric ratios relating size parameter to total length were chosen for direct comparison of sample sites and statistical analyses. Morphometrics ratios were calculated by relating all other metric measurements (H, W, LL, RH, RW, FW) to shell length (L).

Furthermore, rib numbers (RN) were used as an independent parameter. Statistical analyses and graphs were done by Statistica 6.0 (Statsoft, Hamburg, Germany).

To compare the different populations, arcsine-transformed data of morphometric ratios were used to carry out one-way analysis of variance (ANOVA) (cp. Reuschel & Schubart 2006). The post hoc Scheffe's test was used for statistical differentiation among sites.

To define the driving parameters within the morphological variables, a principal component analysis (PCA) was performed with log-transformed data. Discriminant function analyses (DFA) were used to display separation or overlay of populations by canonical analyses. Mahalanobis distances describe spatial proximity between population centroids defined by data of DFA analyses. The classification functions within DFA were then employed to assess how accurately individual shells had been assigned to the different populations.

Inter-specific comparisons

To reveal differences in variation of morphometric parameters between the sister species, *C. edule* and *C. glaucum*, populations co-occurring at the same location were analysed with arcsine-transformed data of morphometric ratios performing discriminant function analyses (DFA) as above described. Student t-Test and ANOVA were performed to compare populations among sites and between species.

Where morphological discrimination was not successful, sequence comparison of partial mitochondrial DNA was applied. A fragment of 582 bases of the cytochrome c oxidase I gene was analysed for seven randomly chosen cockles by Neighbor Joining method using the software BioEdit (Hall 1999). The resulting phylogram was displayed by TreeViewX, Version 0.5.0 (R.D.M. Page).

Results

Site-specific morphological differences were conspicuous when the age structure of sampled populations was not similar. Size and thickness of shells from Shetland Islands (SL) and Bodoe (BO) were in average much bigger than all the others (Tab.1.2), mainly due to old age of the sampled individuals. Smallest in shell size were cockles which originated from Murmansk (DZ) and St. Andrews (SO). These young cockles had thin and fragile shells and were probably in their first or second year (Fig.1.4).

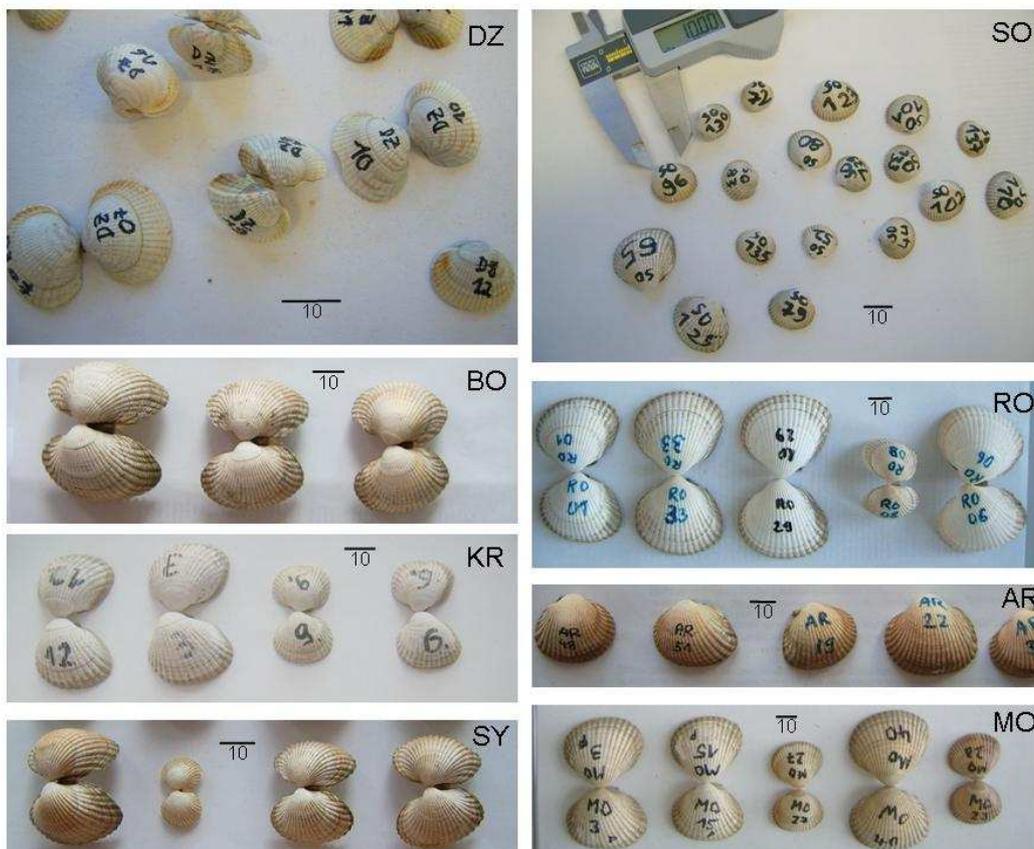


Figure 1.4: Representative specimens of *C. edule* from eight sample sites. Sample codes are used as in Tab.1.1. Note that there were different size scales! Scale bar: 10 mm.

Latitudinal gradients in morphological descriptors

Individuals of *C. edule* showed high variability of size and form between the 18 sites investigated along the latitudinal range (Tab.1.2). Strong variation in cockle length pointed at different age of the populations investigated.

Table 1.2: Morphometric data of the 18 *C. edule* samples. Mean value (Mean), minimum (Min), and maximum (Max) measurements are given for length (L), height (H), width (W), ligament length (LL), curve index (Curve) and rib number (RN) involving all individuals (N). Values for rib width (RW), rib height (RH), and furrow width (FW) were recorded for the subset (N_E).

site		N	L	H	W	LL	Curve	RN	N_E	RW	RH	FW
MO	Mean	101	29.0	25.9	19.9	6.1	0.343	24,71	50	1.5	1.3	1.0
	Min		18.6	16.4	12.1	2.7	0.297	22		1.0	0.9	0.6
	Max		36.9	32.0	25.9	9.2	0.422	28		2.1	1.8	1.6
AL	Mean	51	24.2	21.8	16.6	4.2	0.343	25,71	26	1.3	1.0	0.7
	Min		18.4	16.7	12.5	2.5	0.312	23		0.9	0.8	0.5
	Max		33.4	29.4	22.4	6.9	0.366	30		1.8	1.3	1.0
LN	Mean	50	26.4	24.7	18.3	5.5	0.348	21,76	26	1.5	1.2	1.0
	Min		18.3	17.3	12.2	3.0	0.307	18		1.2	0.9	0.8
	Max		31.8	29.5	22.0	8.4	0.389	24		1.8	1.9	1.3
AR	Mean	61	30.0	27.6	22.9	6.4	0.382	24,72	29	1.5	1.2	1.0
	Min		26.7	24.1	20.2	5.3	0.343	21		1.1	0.9	0.9
	Max		34.7	32.3	26.6	8.5	0.424	29		1.7	1.5	1.2
RO	Mean	44	30.6	28.6	21.8	6.5	0.357	24,66	22	1.6	1.5	1.4
	Min		19.1	18.5	14.4	3.1	0.336	22		1.2	1.2	1.0
	Max		40.3	37.8	28.2	10.9	0.377	27		2.4	1.9	1.9
TH	Mean	63	20.9	18.9	14.1	4.0	0.338	21,71	32	1.4	1.0	0.8
	Min		12.4	11.6	8.3	1.7	0.308	19		0.9	0.8	0.5
	Max		26.1	23.7	18.3	6.1	0.373	24		1.8	1.3	0.9
DB	Mean	50	32.1	30.0	24.4	7.9	0.308	23,28	25	1.9	1.4	0.9
	Min		20.1	19.0	14.2	2.9	0.338	21		1.4	1.1	0.8
	Max		39.5	35.9	30.6	11.1	0.422	26		2.5	1.8	1.3
SO	Mean	147	17.7	16.5	13.0	3.0	0.366	22,86	72	1.1	1.0	0.7
	Min		12.8	11.9	8.7	1.9	0.328	20		0.6	0.5	0.4
	Max		31.0	29.7	23.9	8.6	0.405	26		1.9	1.5	1.0
SL	Mean	138	42.8	39.7	31.7	10.0	0.371	25,25	71	2.6	1.9	1.4
	Min		32.3	30.2	23.1	4.5	0.331	22		1.6	1.1	0.8
	Max		53.8	49.6	38.4	15.1	0.423	29		3.5	2.6	3.1
LA	Mean	30	24.6	21.6	17.2	5.7	0.349	22,53	25	1.0	0.8	1.0
	Min		19.0	16.1	12.3	3.5	0.322	21		0.7	0.6	0.7
	Max		32.1	28.1	22.5	8.1	0.374	25		1.4	1.0	1.2
SY	Mean	73	32.5	29.1	22.5	7.9	0.343	22,67	48	1.8	1.0	1.0
	Min		15.5	13.9	9.4	2.3	0.305	20		0.8	0.4	0.5
	Max		44.2	39.5	32.7	13.2	0.393	26		3.1	2.1	1.8
LF	Mean	42	30.7	27.3	20.9	6.6	0.341	23,88	25	1.6	1.1	1.2
	Min		21.7	18.7	14.2	2.6	0.312	20		1.3	0.9	0.9
	Max		35.2	31.4	24.7	8.7	0.377	26		2.1	1.3	1.9
NM	Mean	36	32.8	30.0	23.0	6.9	0.352	22,56	17	1.8	1.5	1.2
	Min		16.9	15.9	12.7	2.1	0.316	19		1.1	1.2	0.9
	Max		44.8	39.7	30.2	12.7	0.408	26		2.5	1.9	1.4
KR	Mean	50	24.6	23.2	17.1	5.0	0.346	21,28	23	1.9	1.2	1.1
	Min		15.6	14.2	10.6	2.2	0.308	18		1.2	0.9	0.6
	Max		32.9	31.9	23.2	9.3	0.386	25		2.4	2.0	1.6
FL	Mean	49	30.6	28.2	21.4	6.6	0.349	23,24	24	2.0	1.4	1.1
	Min		19.9	18.5	13.2	2.6	0.315	20		1.3	1.1	0.8
	Max		50.0	45.9	35.6	17.6	0.394	26		2.9	2.1	1.7
BN	Mean	50	28.0	26.6	19.9	7.6	0.354	23,46	26	1.5	1.2	0.9
	Min		16.1	15.7	11.3	3.1	0.316	20		1.0	0.8	0.6
	Max		46.9	42.1	29.7	11.7	0.395	27		2.0	1.6	1.3
BO	Mean	49	40.5	35.7	28.4	9.0	0.350	25,49	25	2.1	1.8	1.3
	Min		31.7	28.0	20.4	5.2	0.306	23		1.3	1.2	1.0
	Max		49.0	44.2	35.1	12.6	0.386	29		2.6	2.6	1.6
DZ	Mean	20	15.4	12.7	9.2	1.7	0.298	26,05	20	0.7	0.6	0.5
	Min		13.0	10.5	7.2	1.1	0.268	22		0.5	0.5	0.4
	Max		20.1	16.5	12.9	2.6	0.325	29		1.0	0.8	0.7

Size values of the 18 different sites showed high correlation for length to width ($R^2=0.98$, Fig.1.5). Cockles with the most spherical shape came from Arcachon Bay (AR) and Dublin Bay (DB) for which the values deviated strongly from the linear regression line due to larger mean width (Fig.1.5).

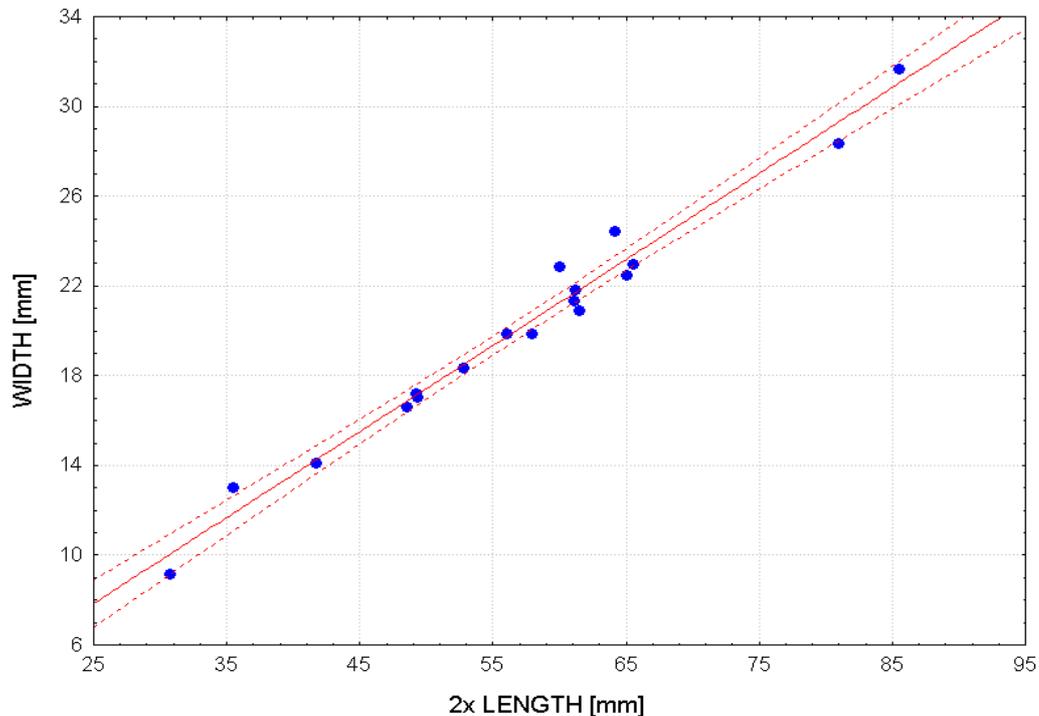


Figure 1.5: Curve index of *C. edule* as relation of width to double length. Equation of regression: $y=0.383*x-1.737$, $r=0.989$, $p<0.001$; mean curve indices represent the 18 sample sites. Regression line: solid; dotted lines indicate 95 % confidence interval.

Variation of *C. edule* shell size ratios with latitude showed no simple trend but a decrease towards the edges for height to length and ligament length to length (Fig.1.6A+B). Curve index represents a size ratio correcting for length-related variation among the populations and displays the globosity of the shell shape. It indicates low variation but reflects the tendency of lowering towards the distribution edges (Fig.1.6C). The high latitude population of Murmansk differed most from all other populations showing lowest values (Fig.1.6C). Most of the sample data on rib numbers ranged between 21 and 24 (11 sites, Tab.1.2). Mean rib numbers were similarly high for the northern populations (DZ, BO, SL: between 25 and 26; see Fig.1.6D) and showed no relation to size. Only the southern Portuguese site (AL) showed likewise high numbers of shell ribs (Fig.1.6D).

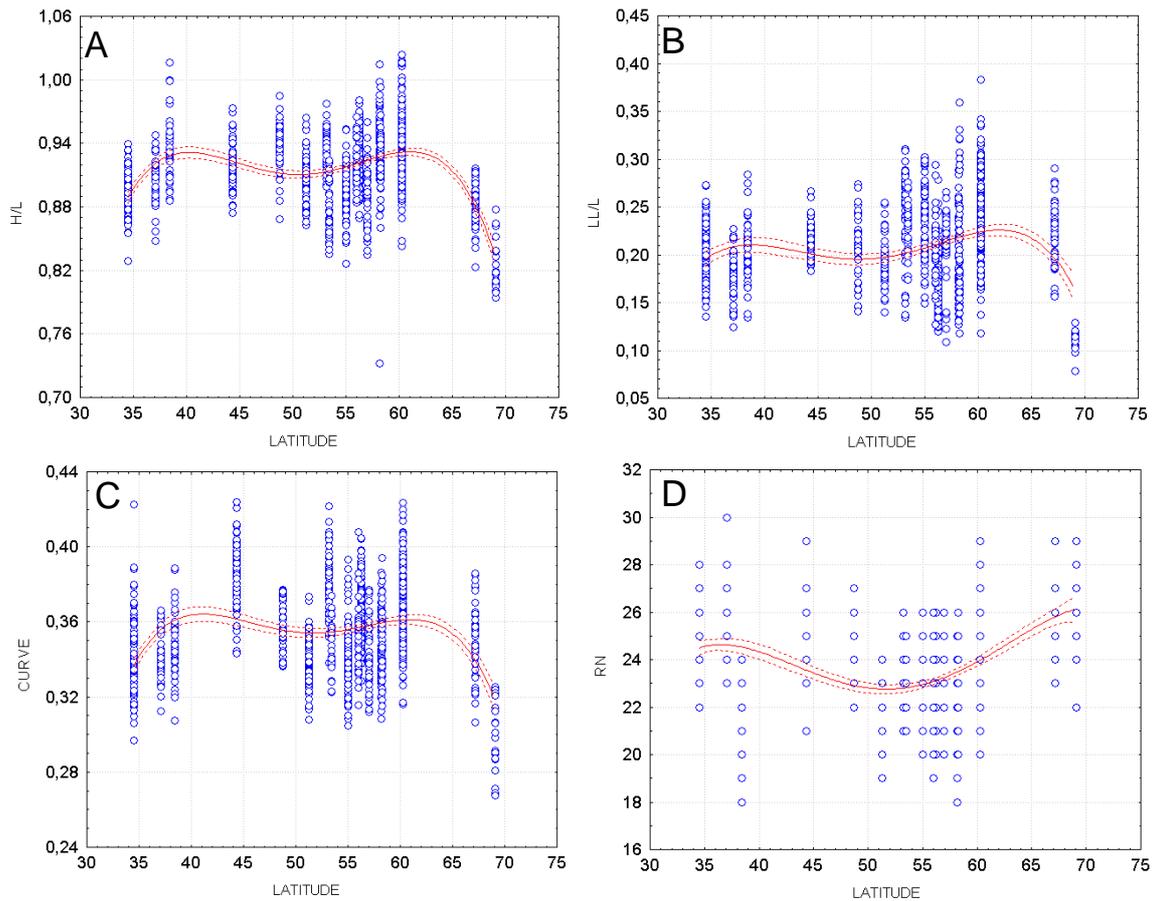


Figure 1.6: Latitudinal variation of shell parameters. A.-C. Variation with latitude showed no simple trend but a decrease towards the edges for all three shell size ratios, height to length (H/L), ligament length to length (LL/L) and curve (=W/2L). D. Rib number (RN) increased towards the edges. Lines indicate polynomial regression line with 95% confidence intervals.

The growth ring structure was more pronounced in northern cockles (DZ, BO, KR, SY) than in more southern populations (AR, MO) (see Fig.1.4). Samples of northern sites, coming from the North Sea and Norwegian Sea, were mainly composed of older individuals (one to nine years) than found in southern samples down to the African coast (one to five years).

An overlay of size-age relation with growth ring data pooled from seven cockle populations showed most variation in the mid-age data (two to five years, Fig.1.7). Following the regression equation, the estimated growth rate of *C. edule* varied between 0.055 and 0.041 mm/day in the first year and became reduced to 0.023 mm/day in the fourth year. A difference in growth rate between northern and southern sites could not be revealed with these data.

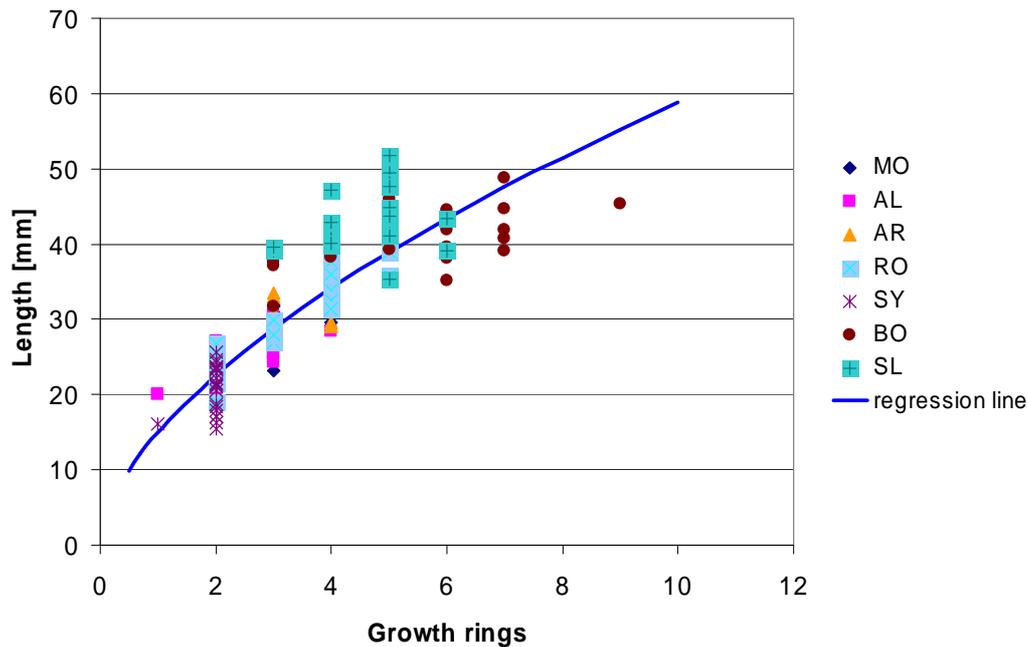


Figure 1.7: Size-age relation within the species *C. edule*. Data were pooled for seven different sites (each $N=20$) along the distribution range of European cockles. Regression equation: $y=14.998*x^{0.5933}$, $R^2=0.773$, $p<0.001$.

Inter-site comparisons of *C. edule*

For direct comparisons of morphological parameters, size ratios and rib numbers were transformed to consider changes in growth due to allometry. Only subsets of cockle individuals (N_E , Tab.1.2) per population were taken into account for calculations, since all eight parameters ought to be considered for the following statistical analyses.

Between sample sites, all morphometric ratio differences were highly significant (ANOVA: $p<0.001$), except for the relation of rib height to length (ANOVA: df 17, $F=1.537$, $p=0.077$). The post hoc Scheffe's test for log-transformed rib numbers showed no consistent pattern. Most similar were MO, AL, RO, SL, BO and DZ because of high rib numbers. Sites which were similar due to low rib numbers were LN, TH, LA, SY, NM and KR.

According to the principal component analysis, most important descriptive variables (component loads >0.7) were the size ratios of H/L for PC1 and LL/L for PC2. However,

all other parameters contributed to the distribution of the data set (Fig.1.8) with a component load of more than 0.5 and explaining together over 50 % of variance.

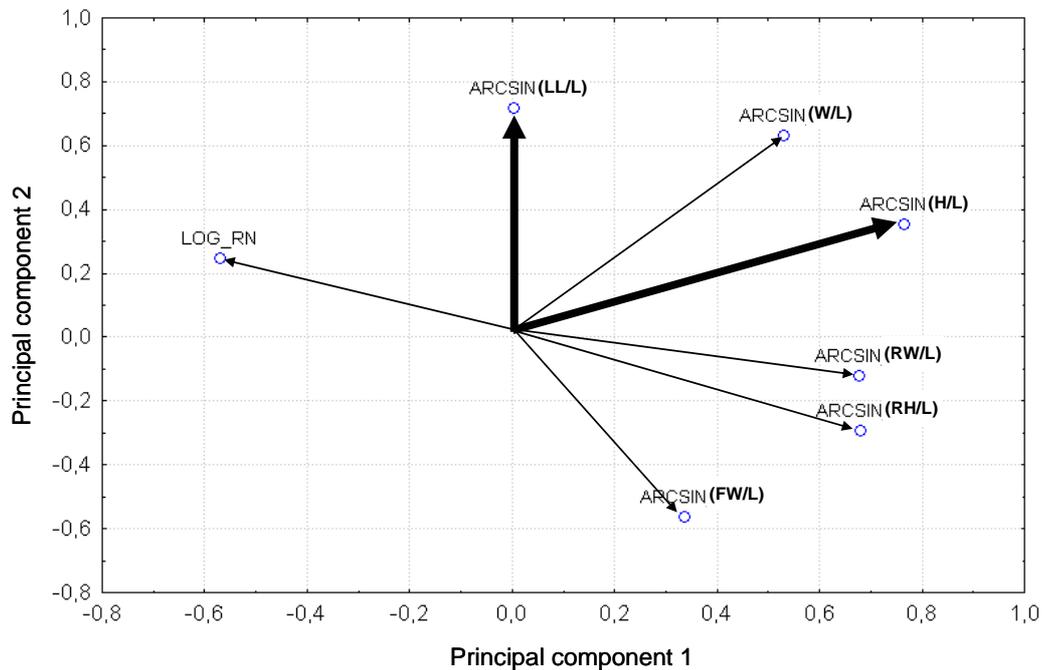


Figure 1.8: Principal component analysis of all morphological characters examined. Projection of variables on plane defined by first and second principal components.

In order to test the overall differentiation of the 18 populations, a discriminant function analysis was carried out using the six arcsine-transformed morphometric ratios plus log-transformed rib number. The data set was subjected to canonical analyses shown in Fig.1.9. The discrimination between the 18 groups was highly significant (Wilks' Lambda: 0.029, approx. $F(119.37)=22.056$, $p<0.0001$; 60.7 % correct classification) while affiliation of individuals to their population site (classification) was not always explicit.

The Arctic population of Murmansk was correctly classified with a likelihood of 100.0 %, the population of Bodoe and Bergen with a likelihood of 37.5 % and 72.0 %, respectively. The classification matrix showed that individuals belonging to the Skagerrak/Kattegat area (FL, KR, NM, LF) were only correctly classified in less than 50 % (Appendix 1.1) indicating high similarity of the data sets. In the Wadden Sea area, the population of Langeoog was correctly classified by 100.0 %, while individuals of the Sylt population were displayed scattered over the range and were classified with a likelihood of 64.6 %.

The southern sites of cockle distribution were correctly classified with 52.0 % to 79.2 %, showing highest specificity for SO and RO.

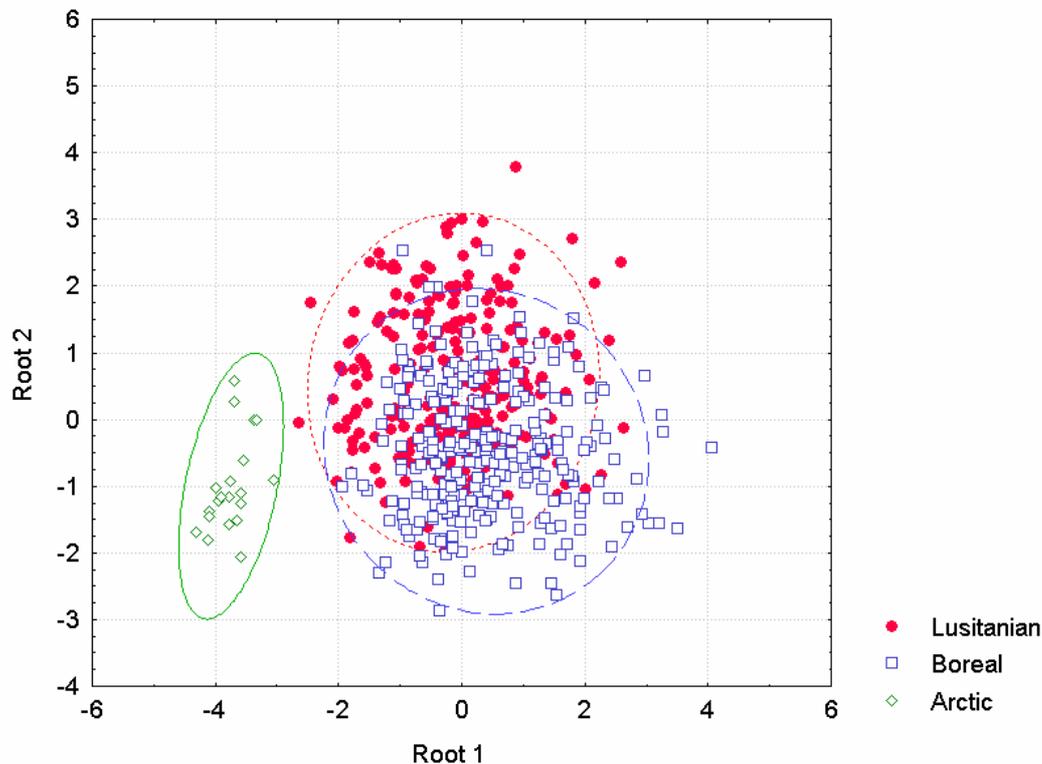
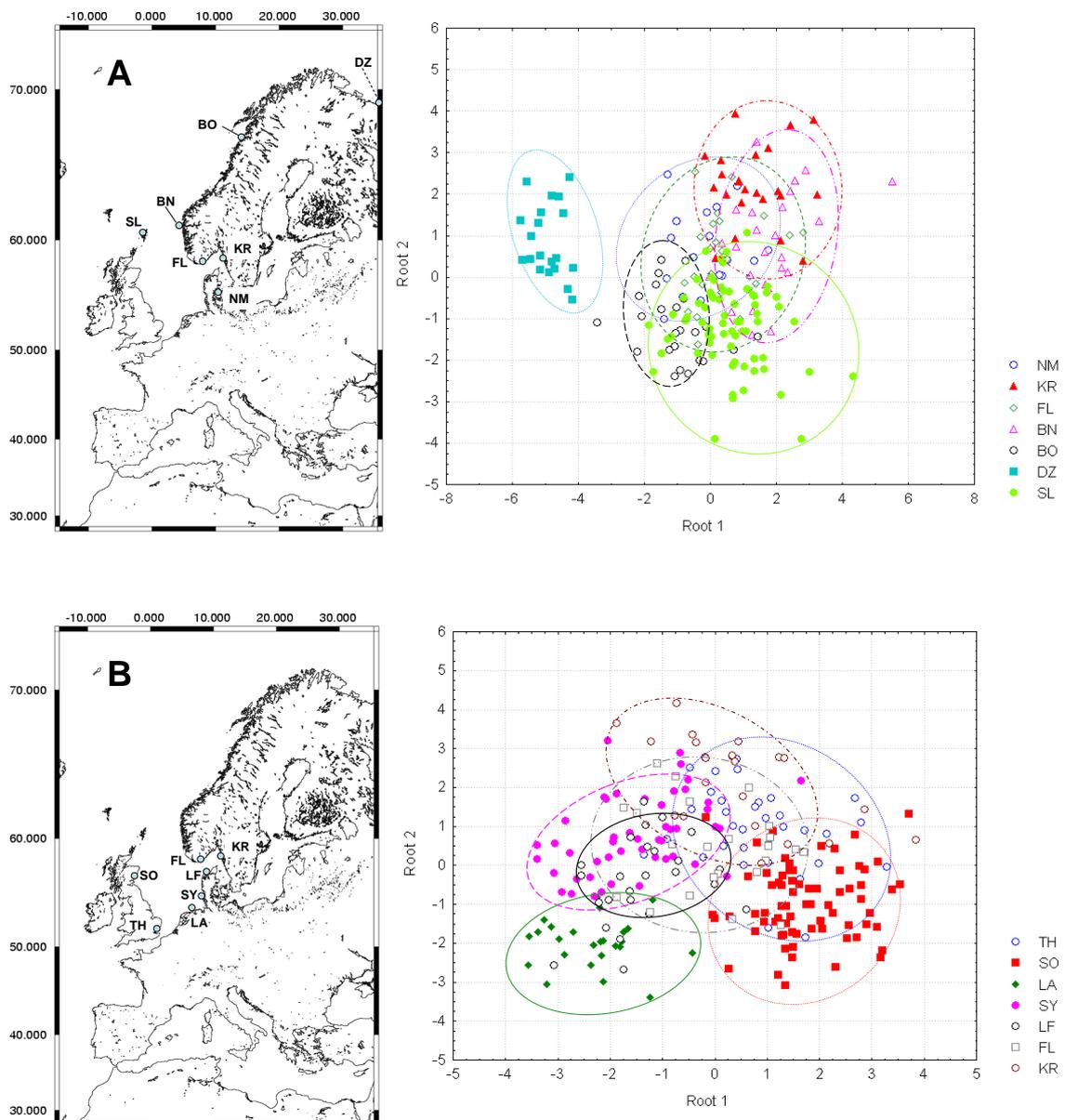


Figure 1.9: Similarity of morphological data pooled for all 18 sites. The plot shows first discriminant function (root 1) against the second (root 2) with canonical analysis depicting discrimination. Samples were grouped for illustration according to biogeographic provinces after Briggs (1995). Finer resolution is displayed in Fig.1.10.

Small scale differences were revealed when canonical analysis depicting discrimination was applied on regional population groups (Fig.1.10A-C). The group of most northern populations (Fig.1.10A) showed a strong outlier DZ with high Mahalanobis distances from all other populations (D^2 21.3-48.6), although arcsine-transformation was used to reduce the influence of allometry. Correct classification was about 70 % for all northern boreal population, with high values for DZ (100 %), SL (84.5 %) and KR (78.3 %). An overlay of BO and SL was shown by low distance for morphometric parameters (D^2 4.2), while FL seems to be centred between all sites (without DZ) with lowest correct classification (16.7 %) and low distances between centroids (D^2 1.0-5.8). The North Sea sites (Fig.1.10B) show a strong overlay of populations for LF, SY and FL within canonical ordination. The population of the Thames Estuary was nearest to Skagerrak/Kattegat populations, FL (D^2

4.3) and KR (D^2 4.7). Most distance showed here the Langeoog population (D^2 10.6-26.7) except for LF population (D^2 6.7). Within the southern sites (Fig.1.10C), 79.9 % correct classifications were found. The Mahalanobis distances (D^2) of the population of Morocco revealed short distances to the populations of AL (D^2 5.1), AR (D^2 6.7) and RO (D^2 7.9) followed by LN (D^2 9.0) and TH (D^2 9.6) and at last DB (D^2 15.1). The short distance of AR population to MO (D^2 6.7) and AL (D^2 6.9) is surprising and in contrast to a long distance to the geographically closer LN (D^2 14.0) and RO (D^2 13.3). A clear structure seems obvious when relating the closely situated populations of RO, TH and DB (D^2 >16.5).



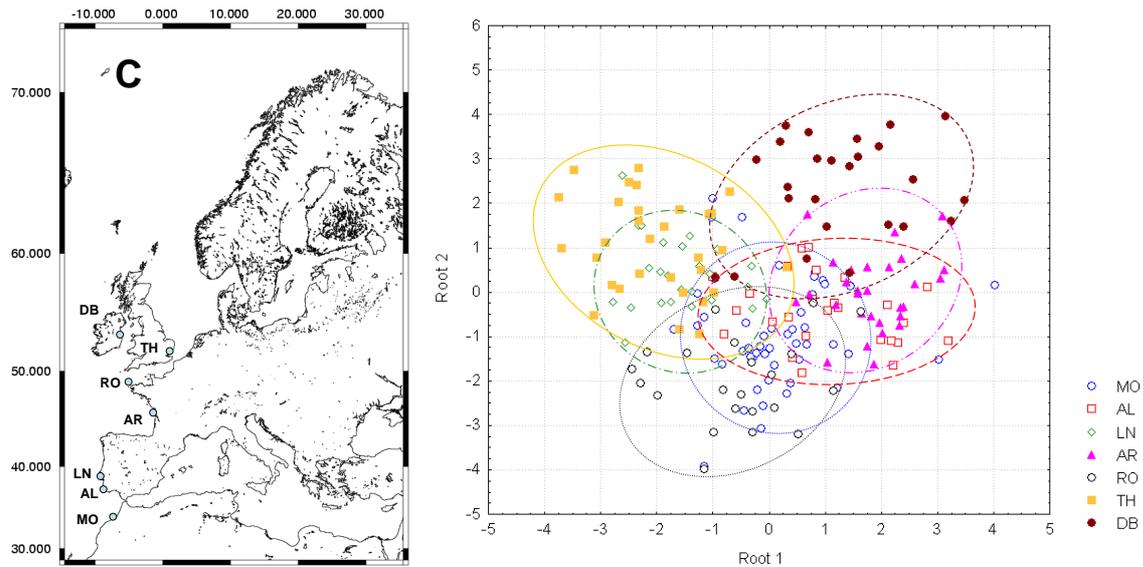


Figure 1.10: Regional resolution of morphometric clustering. Three coastal regions show internal variation by canonical analysis of morphological discrimination. **A.** Northern boreal group including Skagerrak/ Kattegat, Norwegian Sea and the Arctic site. **B.** Southern boreal group composed of sites situated at the North Sea coasts. **C.** Lusitanian group including populations from Morocco to the British Isles.

Comparison of *C. edule* and *C. glaucum* in sympatric occurrence

Morphological size parameters were compared between the species *C. edule* and *C. glaucum* at the three northern sites, Sylt (SY), Norsminde (NM) and Kristineberg (KR) (Tab.1.3). Differing patterns of shell morphology were found for cockle species co-occurring at two northern sites at the entrance of the Baltic Sea (KR, NM) and one in the Wadden Sea (SY) but also within species between sites (Tab.1.3, Fig.1.11).

Height to length ratio showed only significant differences between species for the Sylt site (ANOVA: Scheffe test, $p < 0.01$) while the *C. edule* sample was differing from all other samplings (Fig.1.11A). This size ratio was most similar for the two species at the Norsminde site (NM) (Fig.1.11A). However, differences could be shown for the ratio of ligament length to total length (Fig.1.11B) for *C. edule* and *C. glaucum* at all three sites (ANOVA: Scheffe test, $p < 0.05$). Curve index showed no differentiation by shell shape between the sister species at the Danish and the Swedish site (Fig.1.11C). Curve index was demonstrated to differ significantly between the two cockle species only for individuals of the Sylt sample (ANOVA: Scheffe test, $p < 0.01$, Fig.1.11C) which were sampled at different

habitats (tidal flat and saltmarsh creek). The high globosity value of Norsminde within *C. edule* species differed significantly from the other two sites (ANOVA: Scheffe test, $p < 0.01$, Fig.1.11C). Rib numbers did not differ significantly between species (ANOVA: Scheffe test, $p > 0.5$) but for *C. glaucum* significant differences could be detected between all three sites (ANOVA: Scheffe test, $p < 0.01$, Fig.1.11D).

Table 1.3: Morphological metrics for separation of cockle species at each site. Six morphometric ratios and rib number (RN) were tested with Student t-test. Strongest separation can be observed in the Sylt population.

site	species		N	L	H	W	LL	Curve	RN	N_E	RW	RH	FW
SY	G	Mean		28.6	26.4	20.2	3.4	0.354	21.35		1.6	1.0	1.0
		Min	26	20.4	19.2	15.2	1.7	0.324	19	25	1.1	0.7	0.6
		Max		33.4	30.0	24.3	6.9	0.389	24		2.6	1.9	1.4
	E	Mean		27.1	24.1	18.2	6.1	0.335	22.65		1.3	0.7	0.8
		Min	26	20.6	18.1	13.6	4.4	0.308	20	19	0.8	0.4	0.6
		Max		34.6	32.0	24.7	9.3	0.359	25		2.6	1.7	1.3
NM	G	Mean		25.9	24.2	18.6	3.4	0.359	21.04		1.8	1.3	1.1
		Min	25	20.5	19.0	14.8	2.3	0.341	17	17	1.5	0.9	0.8
		Max		32.2	30.6	24.5	5.4	0.389	24		2.2	1.5	1.5
	E	Mean		29.2	27.0	20.9	5.9	0.358	22.47		1.7	1.3	1.1
		Min	17	21.9	20.9	16.5	3.7	0.329	21	9	1.2	1.2	1.0
		Max		34.8	33.0	27.9	8.7	0.408	25		2.3	1.8	1.3
KR	G	Mean		27.8	26.6	19.7	4.6	0.355	20.13		1.8	1.5	1.3
		Min	8	23.5	22.0	17.0	3.2	0.334	19	8	1.3	1.1	1.0
		Max		33.5	31.8	24.2	7.0	0.382	21		2.3	1.9	1.6
	E	Mean		27.4	25.7	18.6	5.8	0.340	21.50		1.9	1.2	1.1
		Min	22	21.8	19.8	13.9	3.9	0.308	18	22	1.4	0.9	0.6
		Max		32.9	30.4	23.1	9.3	0.370	24		2.4	2.0	1.6

For further discrimination of the two sister cockle species, all shell descriptors were used (N_E , Tab.1.3). After transforming the data to correct for length variability between samples (length based ratios) and to reach variance homogeneity, best species determination was possible for the North Sea sample (SYG, SYE), where all parameters differed significantly (t-Test: $p < 0.05$) between *C. edule* and *C. glaucum* populations (Tab.1.4). Norsminde cockles (NMG, NME) showed differences in length based ratios of ligament length, rib width and rib number (t-Test: $p < 0.01$) but not in shape represented by curve index C (t-Test: $p > 0.05$) (Tab.1.4). At Kristineberg (KRG, KRE), shell characters showed small significant differences in size parameters like ligament length, rib height and furrow width (t-Test: $p < 0.05$) but also not for curve measurements ($p > 0.05$) (Tab.1.4).

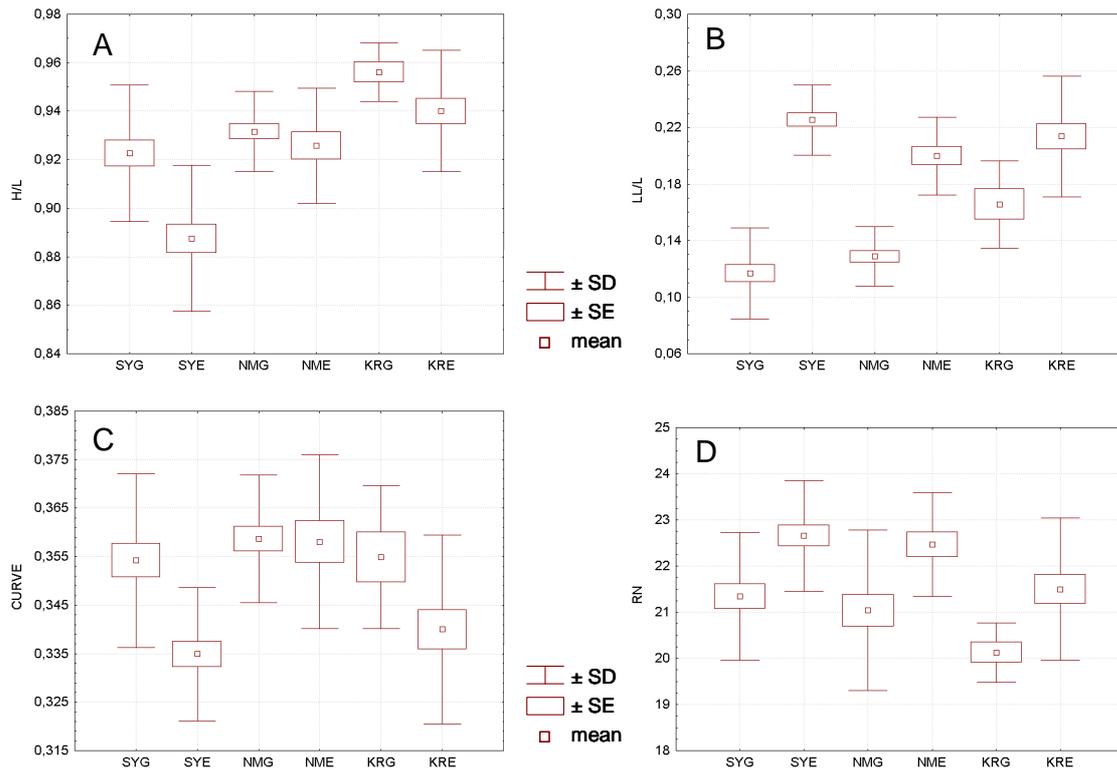


Figure 1.11: Shell parameters of sympatric populations in the north. **A.** Height to length (H/L) is most similar for the two species *Cerastoderma edule* (xxE) and *C. glaucum* (xxG) at Norsminde (NM). **B.** Ligament length to total length ratio (LL/L) showed smallest difference between species at Kristineberg (KR). **C.** Curve index (Curve) shows a large difference only for the two species from Sylt. **D.** Differentiation between species emerges from comparison of rib number (RN) at all sites. Number of cockles is shown in Tab.1.3.

Table 1.4: Morphological metrics for separation of cockle species at each site. Six morphometric ratios and rib number (RN) were tested with Student t-test. Strongest separation can be observed in the Sylt populations. For the sites at the Baltic Sea entrance, significant differences could be shown for the relation of ligament length to length (LL/L) and rib number (RN).

transformed characters	N		t value	df	p	N		t value	df	p	N		t value	df	p
	SYG	SYE				NMG	NME				KRG	KRE			
arcsine (H/L)	26	26	4.2179	50	0.0001	25	17	0.7792	40	0.4404	8	22	1.7441	28	0.0921
arcsine (W/L)	26	26	4.3482	50	0.0001	25	17	0.0783	40	0.9380	8	22	1.9493	28	0.0613
arcsine (LL/L)	26	26	-13.5429	50	0.0000	25	17	-9.5045	40	0.0000	8	22	-2.9052	28	0.0071
arcsine (RW/L)	25	19	2.1928	42	0.0339	17	9	3.5086	24	0.0018	8	22	-0.8483	28	0.4035
arcsine (RH/L)	25	19	2.8295	42	0.0071	17	9	0.3311	24	0.7435	8	22	2.6970	28	0.0117
arcsine (FW/L)	25	19	2.1130	42	0.0406	17	9	0.4579	24	0.6512	8	22	2.0931	28	0.0455
arcsine (C)	26	26	4.3554	50	0.0001	25	17	0.1235	40	0.9024	8	22	1.9577	28	0.0603
log (RN)	26	26	-3.6470	50	0.0006	25	17	-2.9650	40	0.0051	8	22	-2.3843	28	0.0241

A trend for separating *C. glaucum* and *C. edule* could further be detected using all arcsine-transformed size ratios and log-transformed rib number available from the subsets of cockles for discriminant function analyses (Fig.1.12). Here, the difference between all the six cockle groups is highly significant (Wilks' Lambda: 0.04307, approx. $F(35.372)=11.839$, $p<0.0001$). Both populations of Sylt cockles are correctly classified with a likelihood of 92.0 % for *C. glaucum* (N=25) and 78.9 % for *C. edule* (N=19). The populations of Norsminde show correct classification with a likelihood of 82.4 % for *C. glaucum* (N=17) and 66.7 % for *C. edule* (N=9). Lowest values are derived from Swedish samples (KRG: N=8, KRE: N=22) showing scattered distribution over all sites and species (Appendix 1.2).

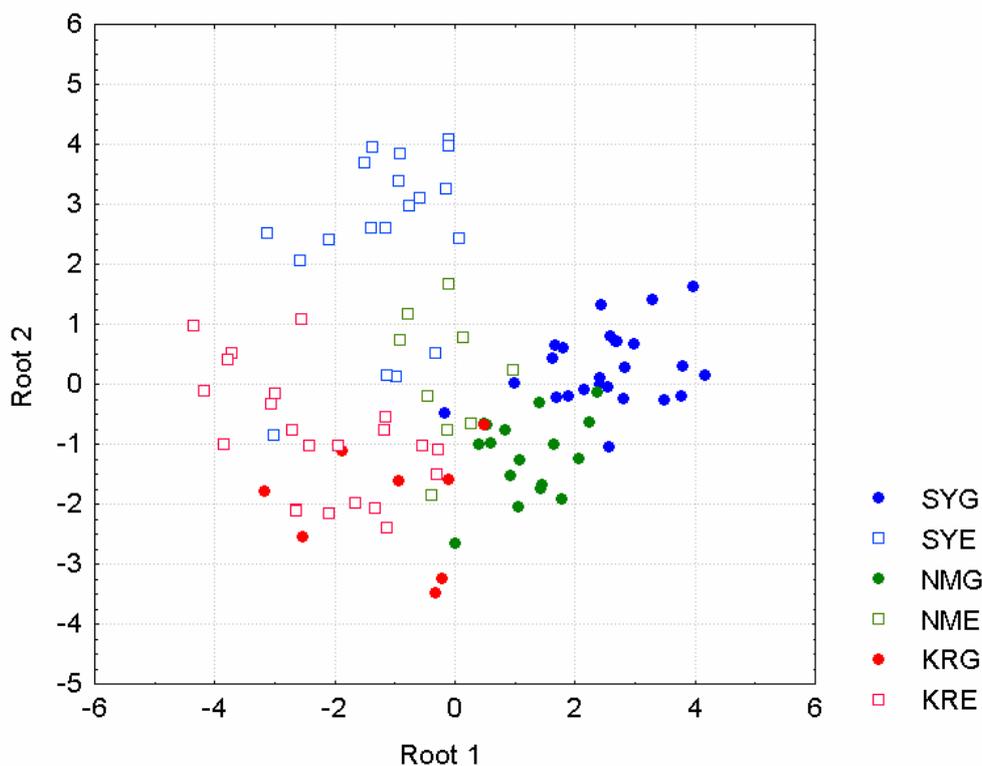


Figure 1.12: Weak species separation by morphometric data of all three sites. Morphometric ratios and rib number were used. First two letters indicate the site and the last one labels the species (G: *C. glaucum*; E: *C. edule*) (see Fig.1.11).

When data from *C. glaucum*-Kristineberg sample were excluded, the distinction of the two species became clearer (Fig.1.13). Only a small proportion of individuals were misclassified for *C. glaucum* (4.8 %) and *C. edule* (5.0 %) by discriminant function analysis.

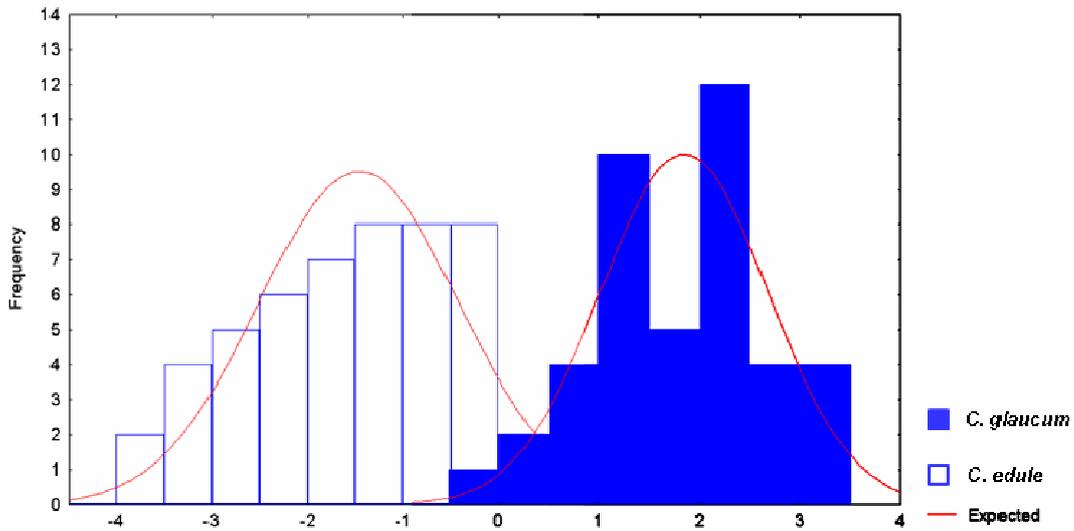


Figure 1.13: Separation of the two cockle species. Morphometric ratios and rib number contributed to differentiation between *C. edule* (N=50) and *C. glaucum* (N=42) along the first canonical axis of DFA.

Morphological siblings of Kristineberg bivalves could be clearly affiliated to each species by genetic sequence comparisons (Fig.1.14). Failed distinction at first glance (left: picture of valves of the two species) could be amended by sequence comparison (right: Neighbour Joining tree of partial mitochondrial DNA).



Figure 1.14: *C. edule* and *C. glaucum* sympatric at the Swedish site Kristineberg (KR). Left: picture of valves of the two species. Right: Neighbour Joining tree of partial mitochondrial DNA (COI) comparing the sampled bivalves.

Due to high morphological variability in both cockle species, a separation without reference specimen is often difficult to do. At Kristineberg, the ligament length was similar for both species, although *C. glaucum* has typically much smaller ligaments than *C. edule*. At the Danish site, *C. edule* individuals showed higher variability in globosity of shell form which may lead to a mix up of species.

When the data presented here were implemented in a relation of curve index to rib number (Fig.1.15) with some of the Mediterranean samples from Mariani et al. (2002b), no difference could be shown. High similarity could be found within *C. glaucum* for the samples from Sylt (SYG), Norsminde (NMG), Mazoma in western Greece (MAZ) and from the Ebro delta in eastern Spain (EBR). The Italian population of Chioggia (CHI) was different from all others due to low rib numbers as already pointed out by Mariani et al. (2002b). The population of Sabaudia in Italy (SAB) would group into a *C. edule* cluster due to low globosity and high rib numbers.

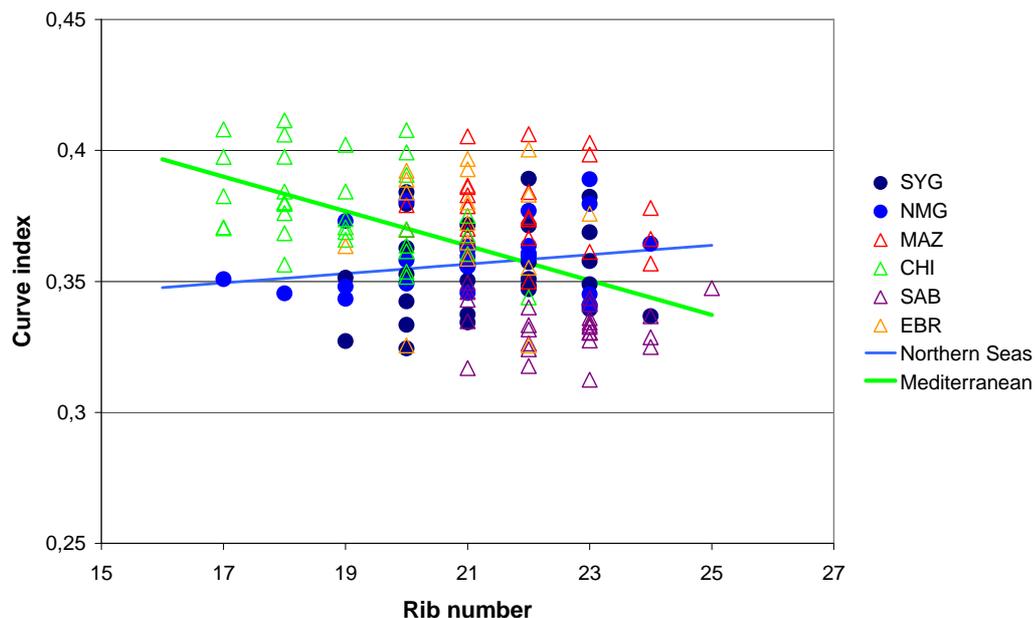


Figure 1.15: Site comparison for Lagoon cockles coming from six geographical sites. Mediterranean *C. glaucum* sites (MAZ, CHI, SAB, EBR; from Mariani et al. 2002b) were added to own data (SYG, NMG). In contrast to the Mediterranean samples (regression equation: $y = -0.0066x + 0.5022$, $R^2 = 0.248$, $p < 0.05$), the northern sites show no negative correlation ($y = 0.0018x + 0.3188$, $R^2 = 0.031$, $p < 0.05$).

Discussion

Within the European cockle species *C. edule*, morphological variation between the 18 populations was existent. However, a macro-scale pattern could not be inferred for regional groups. Most conspicuous was the separation of the Murmansk population which was attributed to high numbers of ribs and distinct flatness of cockle shells. Furthermore, the population of Langeoog situated in the Wadden Sea showed remarkable differences in comparison to the other North Sea sites. Similarity of cockle shells from spatially distant sites may be more under influence of locally differing factors like tidal range or intra- and inter-specific competition. This locality dependent pattern may also cause the high phenotypic plasticity for both cockle species, when *C. edule* is compared to its sister species *C. glaucum*. While *C. glaucum* showed variability mainly in ligament length, *C. edule* varied between the three sites investigated in rib number and shell form represented by the curve index.

Within species comparison (*C. edule*) along the European shores

Influence of short growth periods and cold temperatures may have shaped the cockle form at Murmansk site to be different than all other populations situated in temperate regions. However, negative allometric growth may also be responsible for the low curve index found in the young cockles which means that the animals increase length faster than other shell dimensions (cp. Mariani et al. 2002b). Another population which forms a morphometrically well separable group is found at Langeoog in the southern North Sea. However, no known regional difference could be assigned to this separation against the other North Sea sites. Populations from southern Scandinavia do not represent distinct groups but show a strong intra-similarity in morphological characteristics.

Over the latitudinal gradient, *C. edule* populations displayed similar curve indices with populations from Morocco and Portugal showing the same curve variability as English or Scandinavian samples. Most spherical shapes were found in Scottish and French populations which indicate a lower salinity at these sites (cp. Mariani et al. 2002b). As the Scottish population was next to the mouth of Eden estuary, a lower salinity environment

seems quite likely. In addition, type of sediment is suspected to shape cockle shells (Mars 1951, Rygg 1970, Barnes 1980).

Thus, a large-scale pattern related to temperature or regional seas was not found in European cockles, and a high degree of morphological differentiation could not be assigned to geographical locations as shown for Chinese clams (Kong et al. 2007). It is likely that local factors have a higher impact on the study than large scale factors (cp. Rasmussen 1973, Brock 1980, De Montaudouin 1996). Environmental influences affect growth plasticity and population dynamics which can be recognised in varying mean size (Jensen 1992a, De Montaudouin 1996). Already Høpner Petersen & Russell (1973) described *C. edule* with rib numbers of 19-29 and maximal length of 60 mm. This observation on rib number variability was recorded before, for example on a small scale study along the Dutch shoreline (Eisma 1965). He suggested that there is a positive relation of rib number with increasing salinity. However, causality has not been revealed yet.

In a local study at the French Atlantic coast, Montaudouin (1996) revealed immersion time due to tidal level as main factor for the large variation of phenotypes in cockle populations of Arcachon Bay. The growth curve of *C. edule* (Fig.1.7) suggested yearly shell extension of up to 20 mm for young cockles down to 8.4 mm for four year old individuals without clear geographical association. This range of values was recovered in a collection of growth rate data (Fig.3 in Iglesias & Navarro (1990)) where high numbers were assigned to low latitudes (Spanish coast) and low values to boreal sites (North Sea). They assumed the existence of a latitudinal trend, with growth rates increasing southwards. This conclusion is supported by early observations from Cole (1956), who showed that prolonged winter season resulted in reduced growth rates. However, a slow growing Spanish population showed similar values as boreal cockles (Iglesias & Navarro 1990). A reduction in feeding time by long emersion periods was discussed as a strong influence. This factor is reported as strongly limiting growth in different species of bivalves (Dehnel 1956, Griffiths 1981), including *C. edule* (Kreger 1940, Hancock 1967, Farrow 1971, Barnes 1973, Jones 1979, Jensen 1992a). In contrast, Cardoso et al. (2006) recorded higher growth rate in Dutch intertidal than subtidal habitats, while cockles of older age (>5 years) were not found in the subtidal. Jensen (1993) noted that population density through intraspecific competition is a growth influencing factor which can change expected growth

rates for a population. Thus, the variation of cockle shell morphology is dependent on multiple extrinsic factors which may overlay genetic differences.

Plasticity in organismal forms

The concept of phenotypic plasticity has recently become more important in evolutionary thinking (Pigliucci 2005). It can be defined as the production of multiple forms from a single genotype, depending on environmental conditions (Miner et al. 2005). Although several species show different phenotypic plasticity according to their environment (e.g. Todd et al. 2006, Brookes & Rochette 2007, Smith et al. 2007, Tsang et al. 2007), morphological criteria are still first choice descriptions to identify and distinguish species from each other.

Many species are very similar in form and living traits and have remained unrecognised until recently or are even still unknown. In last decades, progress in development of genetic markers enabled the detection of several cryptic or sibling species, sometimes with remarkable differences at the DNA level (De Vargas et al. 1999, Wilke & Pfenninger 2002, Kruse et al. 2003). For species that are seemingly widespread and abundant the probability is quite high to consist of cryptic species (e.g. Bleidorn et al. 2006, Remerie et al. 2006, Suatoni et al. 2006) which have low population sizes and may be highly endangered. The discovery of species that are morphologically indistinguishable also leads to the question why and how they came into existence (Sáez & Lozano 2005). However, it is proven that genetic diversity has important effects on communities and ecosystem processes (Weltzin et al. 2003, Gamfeldt et al. 2005, Crutsinger et al. 2008, Ehlers et al. 2008).

Environmental conditions and co-existing species are known to have an impact on morphology of organisms from different ecosystems. Although phenotypic plasticity is usually associated with sessile organisms such as plants (Bradshaw 1965) and corals (Todd et al. 2004) that cannot escape their environment once they have become established, plastic responses in motile organisms were recently reported (e.g. Imre et al. 2001, Moore et al. 2004). Studies on marine molluscs show that size differences and variability of shell

thickness can be related to space limitation and wave exposure at rocky intertidal zone (Conde-Padin et al. 2007).

Comparison of *C. edule* and *C. glaucum* of sympatric populations

Morphometric values of both cockle species vary with sites. Regional differences were found in *C. edule* for all parameters investigated between the three sites. When compared to *C. edule*, the lagoon cockle *C. glaucum* did not show higher variability in curve and rib numbers as stated before from English and Danish cockles (Russell & Høpner Petersen 1973). Noticeably, distinction by globosity and ligament length index was impossible when the species were taken from a mixed population at the Swedish site Kristineberg (Fig.1.14). Only sequence comparison revealed the sibling species occurring in a mixed population.

A strong negative correlation of the curve index versus rib numbers was revealed for *C. glaucum* in the Mediterranean Sea (Mariani et al. 2002b). The northern *C. glaucum* (SYG, NMG) of this study could not support this trend (Fig.1.15) which might be due to less geographical separation distance. High similarity could be found within *C. glaucum* for the samples from my own data and samples of Greece and Spain. Interestingly, a population genetic study on *C. glaucum* shows a connection of Wadden Sea populations on mitochondrial DNA basis with Mediterranean sites in the Gulf of Lyon and at Sardinia, but not with Greece (K. Tarnowska, pers. comm., Appendix 1.3).

Morphometric characters were found to be useless for identification purpose during a large scale study of mixed populations along the Portuguese coast, and qualitative characters as valve profile, type of calcareous scales and shell colour were suggested for experienced researchers (Machado & Costa 1994). These results on diminished separation conform the observations on shells in English estuarine populations of both cockle populations investigated by Boyden (1973) and contribute to the hypothesis on the steps which might have driven “the evolution of form” of the studied species during the process of adaptation to coastal tidal and confined habitats (cp. Mariani et al. 2002b). Following this concept, mixed cockle populations inhabiting the same environment adapted probably with same features leading to real morphological siblings. This is opposite to the concept of character

displacement where closely related species change morphological characters when they geographically overlap and compete for similar resources (Brown & Wilson 1956, Fenchel 1975, Marko 2005). There is no evidence in the literature for competition between the two cockle species, but displacement in spawning time was surveyed in mixed populations (Rygg 1970, Boyden 1971). In contrast, Gosling (1980) could show a convergence in allele frequency for *C. glaucum* in mixed intertidal populations which may occur also independent of the presence of *C. edule*. The most reliable separation could be shown for the Sylt populations where persistent Lagoon cockle populations are found in saltmarsh creeks. However, near the island of Sylt, intertidal populations of *C. glaucum* can be found at times in sea grass beds coexisting with its sister species *C. edule* (Reise 2003).

Implications for morphology and genetics of *Cerastoderma* genus

The study of functional shell morphology can provide very useful information about species' evolutionary ecology as it may indicate site characteristics within a species as proposed by Mariani et al. (2002b). They described the adaptive significance of cockle shell form in the Lagoon cockle, investigated mainly in the Mediterranean Sea. In general, adaptation to different salinity ranges and sediment characteristics was assumed for the two cockle species, *C. edule* and *C. glaucum* (Eisma 1965, Brock 1978, Mariani et al. 2002b). As these factors are strongly dependent on local conditions, a latitudinal pattern or biogeographic affiliation of morphological parameters becomes less possible.

In order to adapt to tidal shallow waters, *C. edule* may have evolved a more tapering, light and laterally compressed shell which facilitates burrowing during emersion time. According to genetic results, *C. glaucum* is believed to have originated from a form similar to *C. edule* during the partial or total separation events between the Atlantic Ocean and the Mediterranean Sea, which is traced back to the Messinian salinity crisis (Brock 1991, Nikula & Väinölä 2003). Temperature and salinity stress might have re-shaped the newly adapted individuals of *C. glaucum* towards a decrease of rib numbers and a consequent more spherical appearance (Mariani et al. 2002b). Site preference of *C. glaucum* for lagoons or salt marsh creeks is thus connected to life trait as this cockle has no efficient burial capacity and no possibility to reduce the effect of current dislodging (cp. Gaspar et al. 2002). If this evolutionary process is assumed to be plausible, such morphological

features may turn out to be neutral, or even advantageous, for *C. glaucum* (Mariani et al. 2002b).

This study shows that intra-specific variation in the morphology of cockles is common without a clear pattern describing latitudinal groups. Instead, the variability may be related to a different spectrum of predators, temperature, salinity regimes and tidal influence at the collection sites. Plastic responses to heterogeneous environmental conditions seem to be one of the most common phenomena characterizing the world (Pigliucci 2005). High shell form variability of bivalves of intertidal areas is most likely found in environments, where marine organisms are known to be exposed to naturally changing stress factors. Therefore, environmental conditions as well as co-existing species have a strong impact on phenotypes of organisms. As shown also for phenotypic diversity in crabs (Brian et al. 2006, Reuschel & Schubart 2007), differences in abiotic factors like salinity and/or sediment and not genetic differentiation are hold responsible for shaping different plasticity in *Cerastoderma* shell forms along the latitudinal gradient of cockle distribution.

CHAPTER 2

Cockle parasites across biogeographic provinces

Abstract

The European cockle *Cerastoderma edule* is an abundant coastal bivalve hosting at least twelve trematode species along its distribution range. As a species, this bivalve host crosses the Lusitanian and Boreal biogeographic provinces. I here explore the question whether the parasite community composition in *C. edule* (1) is uniform throughout the East Atlantic coasts, (2) reflects biogeographic provinces, or (3) shows geographic structuring independently from province boundaries.

From Norway to Portugal, ten cockle populations were examined for parasite composition. Although Boreal and Lusitanian cockle populations share dominant trematode species (*Himasthla elongata*, *Meiogymnophallus minutus* and *Renicola roscovita*), a northern and a southern cluster based on prevalence and abundance level can be recognized. However, the change in parasite assemblage was found within the Wadden Sea instead of the English Channel where a biogeographic province boundary has been located. No general decline in trematode diversity towards high latitudes was found, but a peak of species richness occurred in the North Sea region.

Introduction

Species diversity showing latitudinal gradients is one of the most conspicuous phenomena in organismal patterns (reviewed by Rohde 1992). In marine habitats, the decline of diversity with rising latitude is still under debate (Clarke 1992, Clarke & Crame 1997, Attrill et al. 2001, Rex et al. 2005). Several studies could not confirm a clear trend (Kendall & Aschan 1993, Rex et al. 1993, Brey et al. 1994). Rex et al. (2005) summarized that latitudinal gradients in coastal mollusc diversity show poleward declines in diversity, but patterns are highly asymmetrical between hemispheres, and irregular both within and among regions.

For the European coasts, marine biogeographic provinces and ecoregions were defined by Briggs (1995) and Spalding et al. (2007). The Lusitanian province includes the Atlantic east coast from Portugal to the tip of Brittany at least. Sometimes the Celtic Sea is included and the western entry of the English Channel is set as the provincial boarder (Briggs 1995). The northern adjacent region is named Northern European Seas or Boreal region reaching to the Norwegian-Russian border. This Boreal province includes among others the North Sea, Southern and Northern Norway as ecoregions (Spalding et al. 2007).

Latitudinal gradients in hidden communities in its sense of non-visible fauna came quite recently into research focus, although e.g. parasites form a large proportion of all known organisms (Windsor 1998, Bush et al. 2001). Changes in the relative success of populations caused by the evolution of biotic interactions, perhaps particularly those between parasites or pathogens and their hosts, are expressed as variations in geographical and habitat distributions (Poulin 1999, Ricklefs 2004). Varying abiotic factors are assumed to be responsible for the spatial pattern of parasites in fish and snails (Rohde & Heap 1998, Poulin & Mouritsen 2003). These parasite communities are mainly comprised of monogenean and digenean trematode species. Digenean trematodes are often found in invertebrates of intertidal ecosystems. Their life cycle includes a snail as first intermediate host, a second intermediate crustacean or mollusc host, and a bird or fish as final host (Cheng 1967, Galaktionov & Dobrovolskij 2003). Sexual reproduction takes place inside the gastrointestinal tract of the vertebrate host, and trematode eggs are passed out into the external environment e.g. with the faeces of the bird. Mainly molluscs subsequently get infected either by ingesting a trematode egg while feeding non-selectively or when they are

penetrated by miracidia hatched from trematode eggs. Using molluscs as first intermediate host, the parasite stresses host individuals by the production of thousands of cercariae, often resulting in tissue destruction of gonads or digestive glands. Second intermediate hosts are often actively infected by swimming or drifting cercariae emerging from first intermediate hosts. The impact of trematodes on intermediate host ecology and community structure is well documented in some intertidal ecosystems (Sousa 1991, Thomas et al. 1998, Mouritsen & Poulin 2002, Miura & Chiba 2007). These parasites can have strong influence on host populations causing castration and even local extinctions (Briers 2003, Galaktionov & Dobrovolskij 2003, Fredensborg et al. 2005). However, growth alteration or shell deformations have also been recognised to be caused by trematode infestations (Feirrer et al. 2005, Thieltges 2006a, Miura & Chiba 2007).

Structuring in parasite communities and within species may be caused by three main factors: climate, host occurrence and host specificity (Combes 2001, Rohde 2002). Latitudinal gradients in abiotic factors like temperature may impact transmission success of free-living trematode stages (Galaktionov & Bustnes 1995, Mouritsen 2002, Thieltges & Rick 2006). Secondly, regionally absent host species influence the presence of trematode species e.g. at coastal sites (De Montaudouin et al. 2000, Skirnisson et al. 2004, Thieltges & Reise 2007, Gam et al. in press). In contrast, birds as final hosts may reduce parasite assemblage structures in intermediate hosts along the shore due to regular admixture by migration along the intercontinental flyways (Fig.2.1).

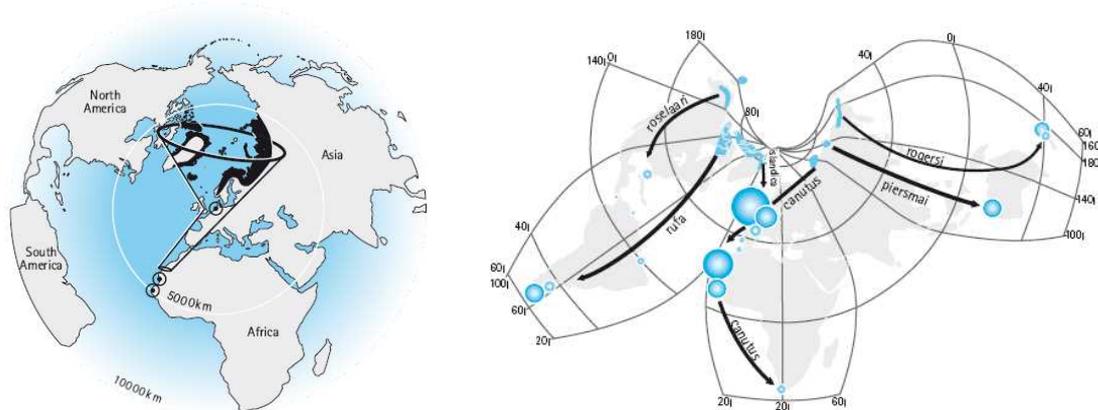


Figure 2.1: Central position of the Wadden Sea for migrating birds. Left: Flyways from Arctic breeding grounds (shown in black) to the African coast with assembling area in the Wadden Sea. Right: Worldwide connectedness: the flyways of the six identified subspecies of Red Knots *Calidris canutus* which is a common shellfish eating shorebird. (graphs from: Piersma 2007)

And thirdly, parasite species richness may be higher in host species with more genetic diversity than in those with less (cp. Pariselle 1996). Up to now, mainly relation of diversity in fish parasites to their host was under survey which underlined host specificity (Jousson et al. 2000, Desdevises et al. 2002). Host specificity of parasites is defined as the extent to which a parasite taxon is restricted in the number of host species used at a given stage of life cycle (Poulin 1998). This includes different host species, but also host races should be considered. Digeneans are thought to have coevolved most likely with their highly specific molluscan host (Pearson 1972, Adamson & Caira 1994). However, little knowledge is available about the influence of host's genetics on the probability and structure of parasite infections in invertebrates. To better understand parasite occurrence and phylogeography, it is important to integrate geography and biogeographic information from potential hosts (Nieberding et al. 2008).

The abundant European cockle *C. edule* is an important link in trematode life cycles serving as first and second intermediate host (Lauckner 1971, De Montaudouin et al. in prep). It provides a habitat for about 15 known trematode species along its distribution range (Lauckner 1971, De Montaudouin et al. 2000, Carballal et al. 2001, Russell-Pinto et al. 2006, Thieltges & Reise 2006, Gam et al. in press, De Montaudouin et al. in prep). These digenean trematodes can be considered as the most important metazoan parasites of molluscs (Lauckner 1983). In addition to fish trematodes, a considerable number of digeneans maturing in the intestine, gall bladder or renal tubes of shore birds utilize the cockle as second intermediate host. Larval echinostomatids, renicolids and psilostomatids encyst in various tissues, while gymnophallids occur free within the tissues or the extrapallial space of the molluscan host. (Lauckner 1983). Reports are rare on metazoan caused infections in European cockles towards the range margin (Jonsson & André 1992, Gam et al. in press), although studied since decades. An impact of this variety of parasitic symbionts on host genetic traits (or vice versa) may be expected for this host-parasite relationship.

This survey of the intermediate host *C. edule* along the European shore includes ten sites covering trematode communities from Norway to Portugal. Asking whether parasite composition reflects biogeographic divisions, four consecutive hypotheses are put forward:

- (1) The parasite community is uniform due to highly mobile final hosts
- (2) The parasite community reflects a separation congruent to biogeographic provinces (Lusitanian/Boreal along the European North Atlantic)
- (3) The parasite community is divided into geographic groups independent of biogeographic provinces.

These hypotheses are tested with respect to prevalence and abundance of trematode species using the cockle as first or second intermediate host by statistical comparative methods including the discriminant function analysis.

Material and Methods

Sampling

A total of 300 individuals of *C. edule* from ten sites were collected at intertidal coasts between the years 2005 and 2007 (Tab.2.1).

Table 2.1: Sample site information of cockles investigated.

Code	Location	Country	Latitude	Longitude	Sampling date
AL	Lagos	Portugal	37° 07' N	08° 37' W	September 2005
LN	Lisbon	Portugal	38° 43' N	09° 00' W	September 2005
TH	Southend	England	51° 28' N	00° 42' E	March 2007
DB	Dublin	Ireland	53° 19' N	06° 12' W	August 2005
LA	Langeoog	Germany	53° 45' N	07° 29' E	July 2007
SY	Sylt	Germany	55° 01' N	08° 26' E	September 2005
KR	Kristineberg	Sweden	58° 14' N	11° 26' E	July 2006
FL	Arendal	Norway	58° 26' N	08° 48' E	August 2005
BN	Bergen	Norway	60° 23' N	05° 20' E	August 2005
BO	Bodø	Norway	67° 17' N	14° 37' E	August 2005

Random sampling was done at low tide by hand in the intertidal zone next to Lagos and Lisbon (Portugal), Dublin (Ireland), Langeoog and Sylt (Germany), Kristineberg (Sweden)

and Bergen (Norway) (Fig.2.2). In addition, cockles from the area of Bodø and Arendal (Norway) and in the Thames estuary (England) next to Southend were fished at high tide in the lower intertidal zone by boat (Fig.2.2).

Sample sites were chosen to be as similar as possible but showed several differences concerning biotic, abiotic and human impacts. Mid eulittoral level of sheltered bays was mostly successful for cockle sampling and pictures of some sample sites are shown in Appendix 2.1. At Kristineberg, the cockle population was sampled in a small bay of the Gullmar fjord which was not exposed to regular tidal movements. Both Portuguese sites were strongly affected by clam harvesting people whose preference for larger individuals may have caused the small size of cockles in my samples.

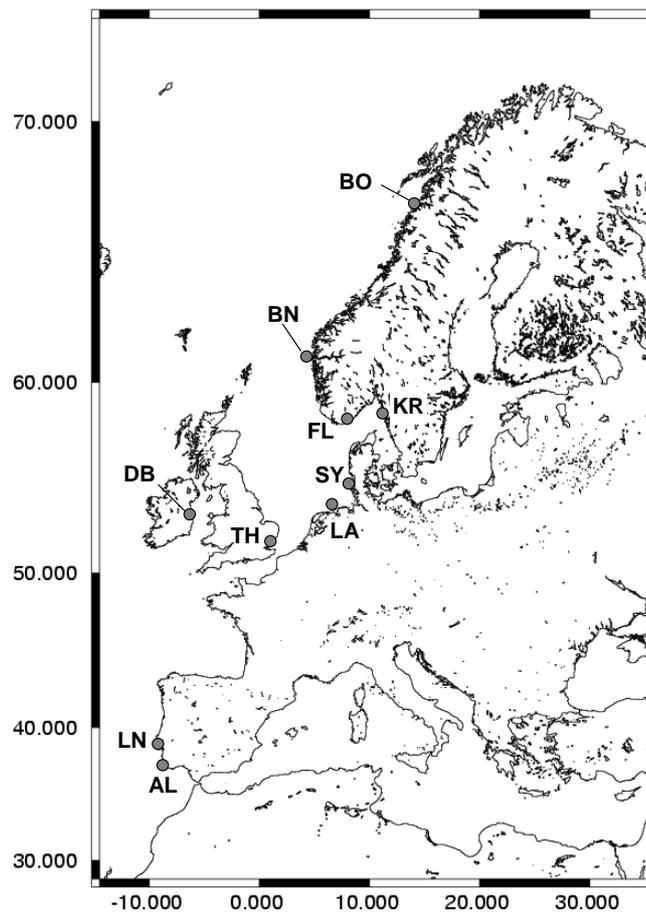


Figure 2.2: Distribution of samples sites at the European coast. Code for sampling sites and exact location are shown in Tab.2.1.

Southend cockles (TH) and the Lisbon population (LN) were sampled at estuarine sites next to the Thames and the Tagus, respectively (Fig.2.2). Sediment characteristics of all

the sites varied mainly between mud and coarse sand. But individuals of the population at the island of Askøy next to Bergen were found to lie on muddy sand between cobbles and pebbles. Rest areas for birds or nature conservation areas were next to all sample sites, and mud flats were used as feeding grounds for migrating shore birds.

Sample processing

Cockles were brought to the laboratory alive. For each specimen, shell length was measured with a vernier calliper to the nearest mm (Tab.2). All cockles investigated were at minimum one year old. Variability in cockle size distribution of samples was tested with one way ANOVA after taking the logarithm to the base of 10 of the length data (log-transformation). Length data for northern and southern European populations were tested by non-metrical Mann-Whitney U-test.

Table 2.2: Size of cockles investigated. Code referenced in Tab.2.1. N: number of host individuals. SD: standard deviation. Min: minimum, Max: maximum.

Code	N	Mean length (\pm SD) [mm]	Min length [mm]	Max length [mm]
AL	30	22.1 \pm 3.5	14.2	31.6
LN	30	25.5 \pm 2.5	17.4	30.0
TH	30	20.7 \pm 1.5	12.2	25.7
DB	30	30.5 \pm 6.0	16.3	37.0
LA	30	24.7 \pm 3.1	19.0	32.0
SY	30	33.6 \pm 5.3	15.5	41.2
KR	30	22.6 \pm 4.0	15.5	32.5
FL	30	29.4 \pm 6.3	17.3	41.2
BN	30	27.4 \pm 4.8	19.4	40.7
BO	30	40.4 \pm 5.4	32.4	53.2

Soft parts of the cockle were removed and squeezed between two glass plates to investigate the tissue for trematode infection. Species identification based on Werding (1969), Lauckner (1971, 1983), Desclaux et al. (2006) and Russell-Pinto et al. (2006). Examples of trematodes are shown in Fig.2.3. More information on cockle infecting trematodes can be found in Appendix 2.5. Metacercarial stages of trematodes were counted under a dissection

microscope. For species represented by sporocysts and rediae, only presence/absence was recorded because numbers were too high for routine assessment.

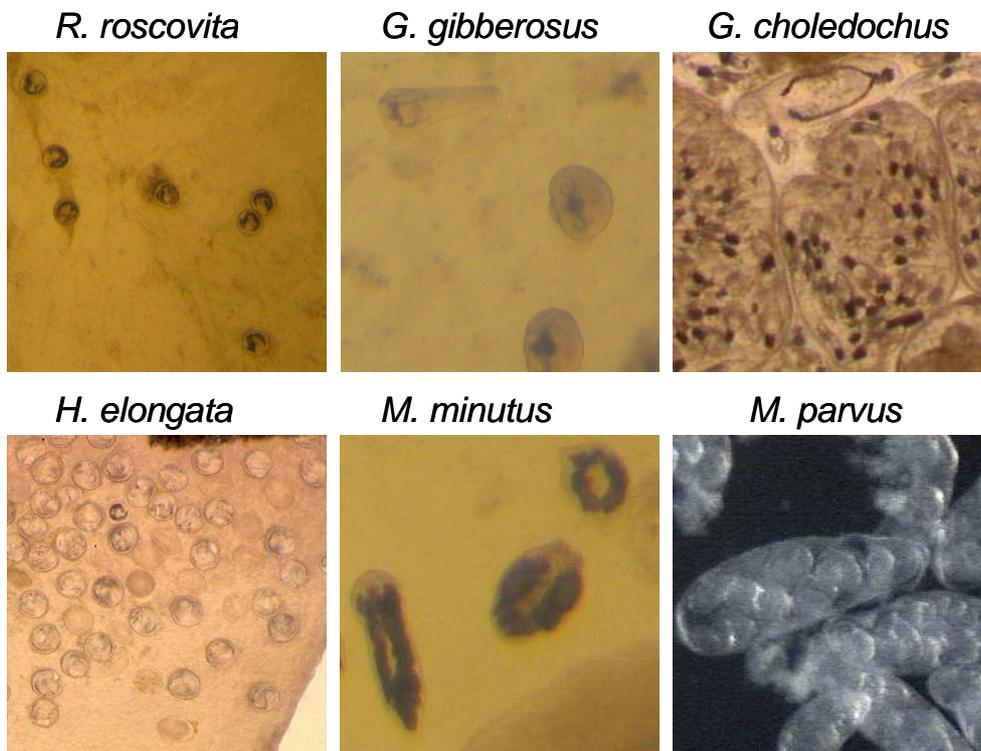


Figure 2.3: Examples of trematodes infecting the cockle *Cerastoderma edule*. *Renicola roscovita* and *Himasthla elongata* show encapsulated metacercariae (left column). *Gymnophallus gibberosus* and *Meiogymnophallus minutus* are found freely moving in tissue cavity (middle column). *Gymnophallus choledochus* and *Monorchis parvus* develop sporocysts with cercariae and/or metacercariae inside (right column).

Statistical analysis

Separate analyses for prevalence (all infective trematode species) and abundance (only metacercariae) were conducted to include trematode species utilizing the cockle in their first intermediate infective stage. Parasite prevalence is defined as percentage of infected host individuals per sample. Abundance of parasite individuals which is used in all analyses is given as numbers of metacercariae per host.

Parasite assemblage clustering based on prevalence data was analysed using the Bray-Curtis similarity index (Bray & Curtis 1957). This is provided in the Plymouth Routines in

Multivariate Ecological Research (PRIMER) package (Clarke & Gorley 2001). An analysis of the similarity matrix (using the non-parametric routine ANOSIM, Clarke & Green 1988) was conducted to test for significant differences in parasite composition among regional groups. ANOSIM test produces a statistic (R-statistic) that lies in the range (-1 to +1). Values of R=1 are obtained only when all replicates within groups are more similar to each other than any replicates from different groups (Clarke & Warwick 2001).

Metacercarial abundance were calculated as mean values with standard deviation or are shown as median with minimum and maximum value. Parasite abundance was used for discriminant function analysis (DFA) in Statistica 6.0 (StatSoft, Hamburg, Germany) which was displayed by canonical graphs. These analyses of metacercarial abundance should indicate separation or overlay of populations. Mahalanobis distances were calculated to describe spatial proximity between population centroids defined by data of DFA analyses. The classification functions within DFA were employed to assess how accurately individual parasite communities had been assigned to each population.

Results

In tissues and cavities of the 300 individuals of *C. edule*, I found twelve species of parasitic trematode stages and three other metazoan parasitic taxa. The parasitic crustacean *Mytilicola* sp. was found in the gut of cockles from Lisbon and Langeoog with 20 % and 3 % infected cockles per site, respectively. A common metazoan in cockle cavities was the turbellarian *Paravortex* sp. which was often found in cockles from Portugal to the Wadden Sea (with up to 43 % prevalence), but was absent further north. Nematodes were found in low numbers freely in tissue cavities at all locations investigated. More detailed data overview for non-trematode parasites is given in Appendix 2.2. However, the following analyses are confined only to the trematode community.

Trematode species and their prevalence pattern

The species number was mainly comprised of digenean trematodes using the cockle as second intermediate host. The highest numbers found per sample peaked in the North Sea

with eight trematode species (Tab.2.3). Within one specimen at Langeoog site, the maximum number of seven trematode species was recorded. The trematode species composition in the north was dominated by *R. roscovita* and *H. elongata*. Southern sites comprised of *H. interrupta* and *M. minutus* in high prevalences. Low diversity was found towards the edge of range within this study, with three species in the northernmost population and four species in the southernmost population (Tab.2.3).

Three out of twelve trematode species, namely *Bucephalus (Labratrema) minimus*, *Monorchis parvus* and *Gymnophallus choledochus*, used the cockle *C. edule* as first intermediate host for clonal reproduction. This group was found at six sites and the level of prevalence was rather high ($\leq 20\%$, Tab.2.3) considering that infected host specimens are functionally dead for the cockle population. The bifurcate trematode *B. minimus* was only found in the Dublin Bay cockles. The monorchiid *M. parvus* was recorded at the Skagerrak sites, Kristineberg and Arendal. The gymnophallid *G. choledochus* infected cockles near Dublin, Langeoog, Sylt and Bergen (Tab.2.3).

Nine species used *C. edule* as second intermediate host in the metacercarial stage, often with a prevalence of 100 % population infection (Tab.2.3). The echinostomatid *H. elongata* occurred all over the range infecting always more than 50 % of sampled cockles. With prevalence values from 10 % to 100 %, the variation of this infection parameter for *R. roscovita* was higher than in *H. elongata* which results in 52 % averaged prevalence (Tab.2.3). Both of these trematodes use *Littorina* snails as first intermediate host. In southern populations, *M. minutus* which uses the bivalve *Scrobicularia plana* as first intermediate host, showed dominance with up to 100 % infections and even in the northernmost record, at Bergen, a high percentage of cockles was infected (Tab.2.3). A southerly dominated pattern could also be shown for *H. interrupta*, but in this case, an infection was also recorded for the Bergen population as the northernmost occurrence. The absence in cockles from the Skagerrak populations may indicate a low density of the first intermediate host, *Hydrobia* sp., at these sites. This would be supported by lowest prevalence records of *Psilostomum brevicolle* at these sites which needs also *Hydrobia* snails and birds to complete the life cycle. Prevalence of *P. brevicolle* peaked in the Dublin Bay population with 73.3 % infested bivalves (Tab.2.3). An irregular occurrence showed *G. gibberosus* infecting cockles from Portugal to Norway but with prevalence values varying between 3.3 % and 90 % (Tab.2.3). The presence of species like *H. continua*,

Curtuteria arguinae and *Meiogymnophallus fossarum* were recorded only at one or two sites. The latter two species showed at the single site high infections while *H. continua* showed lowest average prevalence of all trematode species (Tab.2.3).

Table 2.3: Overview of prevalence data for each trematode species investigated. Code as referenced in Table 2.1. N_{TS}: Trematode species number; Sites: Sum of occurrence; Av Prev: Average prevalence.

Code	1st intermediate			Prevalence									Prevalence of all species [%]	N _{TS}
	<i>Gymnophallus choledochus</i>	<i>Monorchis parvus</i>	<i>Bucephalus minimus</i>	2nd intermediate host										
Final host	Bird	Fish	Fish	<i>Himasthia elongata</i>	<i>Meiogymnophallus minutus</i>	<i>Renicola roscovita</i>	<i>Himasthia interrupta</i>	<i>Gymnophallus gibberosus</i>	<i>Psilostomum brevicolle</i>	<i>Curtuteria arguinae</i>	<i>Meiogymnophallus fossarum</i>	<i>Himasthia continua</i>		
BO				60.0		40.0			16.7				70	3
BN	10.0			100.0	93.3	100.0	36.7	3.3					100	6
FL		20.0		100.0	46.7	100.0			3.3				100	5
KR		3.3		100.0	53.3	96.7		46.7	6.7			3.3	100	7
SY	6.7			100.0		100.0	30.0	6.7	13.3				100	6
LA	13.3			53.3	100.0	16.7	93.3	86.7	46.7			20.0	100	8
DB	6.7		10.0	90.0	100.0	53.3	86.7		73.3				100	7
TH				63.3	100.0	10.0	76.7		10.0				100	5
LN				63.3	83.3		53.3	90.0			93.3		97	5
AL				86.7	86.7		100.0			100.0			100	4
Sites	4	2	1	10	8	8	7	5	7	1	1	2		
Av Prev	4	2	1	82	66	52	48	23	17	10	9	2		

By cluster analysis of a Bray-Curtis similarity matrix, a pattern for a northern and a southern group could be defined based on prevalence data of the twelve parasite species recorded (Fig.2.4). The belonging of the Wadden Sea site, Langeoog, to the southern group became clear when ANOSIM values were compared. The global R was small (Global R: 0.373, p=0.043) with geographically assumed affiliation to the northern group. With the new position of the Langeoog population in the southern group (Global R: 0.772, p=0.008), the grouping was much stronger.

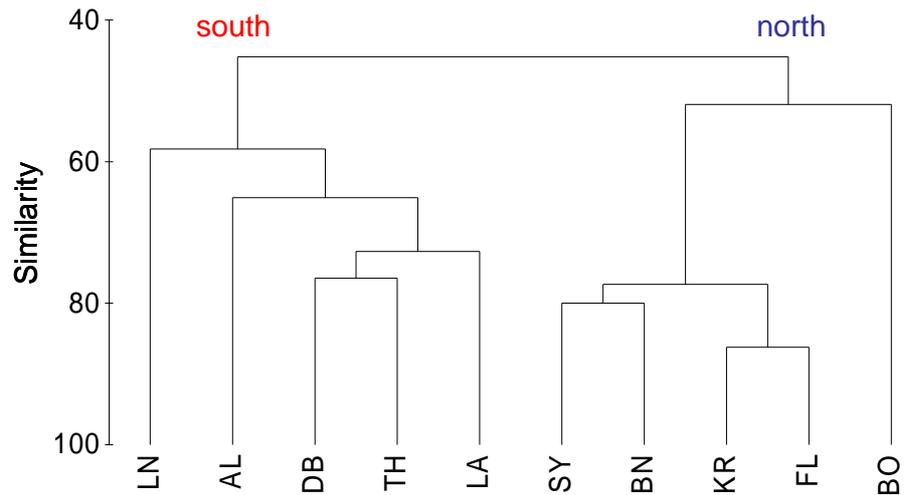


Figure 2.4: Cluster analysis for prevalence data. A separation of groups was generated, where the North Sea site Langeoog strongly clustered in the southern group. Analysis based on Bray-Curtis similarity matrix of none-transformed prevalence data.

After presence/absence transformation, prevalence data produced a different pattern which is more in line with the biogeographic provinces (Fig.2.5). This grouping may be assigned to the southern Lusitanian province versus the northern Boreal region, as the Portuguese sites showed strong separation from the other sites (ANOSIM: Global R: 0.759, $p=0.022$).

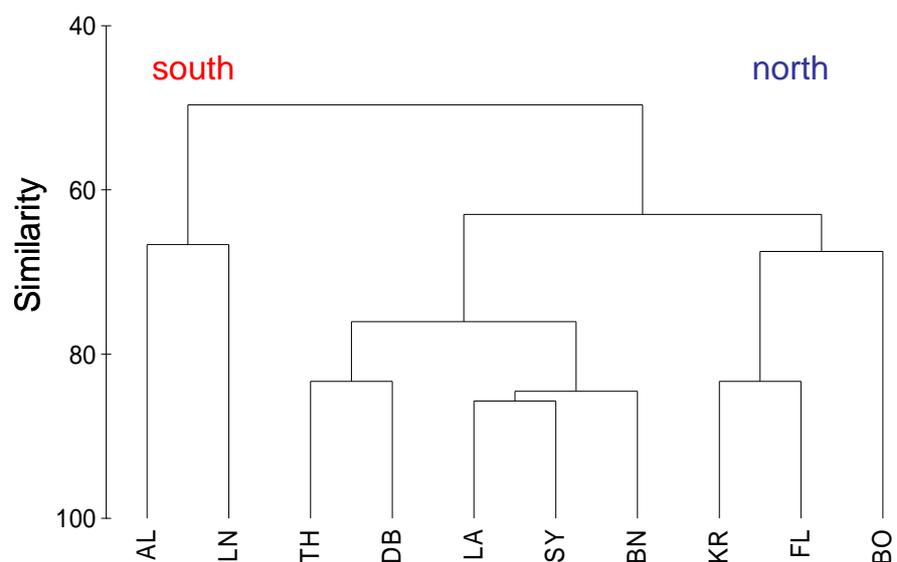


Figure 2.5: Cluster analysis for presence/absence data of all trematode species. A separation of groups was generated, where the British Isles are incorporated in the northern group. Analysis based on Bray-Curtis similarity matrix.

Patterns of metacercariae in cockles

Cockle size distribution was uneven when site groups were compared. Length was significantly different between sites (one-factorial-ANOVA of log-transformed length: $F=43.46$, $df=9$, $p<0.001$; Appendix 2.3). Northern samples (LA-BO) were composed of bigger cockles than the samples taken at the southern sites (LN-TH) (Mann-Whitney U-test, $p<0.001$). However, when metacercarial abundance in *C. edule* samples was tested for relation to size, no significant correlations were obtained. A correlation of trematode load (metacercariae) with shell length could be shown only for the intensity values (mean abundance per infected host individuals) of *M. minutus*: $R^2=0.25$, $p<0.05$ (Appendix 2.4).

Maximum overall infestation was detected within one individual in the Arendal population (FL) which was hosting 2189 metacercariae. This was mainly due to the contribution of the highest amount of metacercariae found for one trematode species (1347 of *R. roscovita*) (Fig.2.6). Similarly high maximum numbers were found in *M. minutus* in Dublin Bay (1200) and in Bergen for *H. elongata* (765).

The most abundant species along the European shoreline was the metacercariae *M. minutus* (Fig.2.6), which is not encysted in cockle tissue. With mean abundance over all sites of no less than 50 metacercariae per cockle, *H. elongata* and *R. roscovita* infected the cockle populations. Heaviest parasite burden was found at the northern sites (Fig.2.6). *H. interrupta* and *G. gibberosus* could be found with quite high abundance values for a few sites. The number of *P. brevicolle* metacercariae in the visceral mass was typically low in the samples where it was recorded. Individual trematode species showed different patterns of abundance along the European cockle distribution (Fig.2.6). At the northernmost site Bodø, few trematode species and low abundances were observed (Fig.2.6, Tab.2.3). The maximum number of the three species found in a single Bodoie cockle individual was six metacercariae. Disregarding this deviant site, for *R. roscovita* and *H. elongata*, an increasing abundance could be recorded towards the north (Fig.2.6). The more common *M. minutus* infection peaked in the Dublin Bay population, but there were also heavy infections found at the northern sites, Kristineberg and Bergen (Fig.2.6). For *H. interrupta*, a peak of infection was found in a Langeoog cockle with 192 larval trematodes, preferentially distributed in mantle tissue. The abundance of these metacercariae at more southerly sites was lower, but showed a maximum of 66 metacercariae in a cockle of the

Algarve site (Fig.2.6). As *H. interrupta*, also *P. brevicolle* uses *Hydrobia sp.* as first intermediate host. The highest abundance was found in Dublin Bay cockles with a decreasing trend towards the north (Fig.2.6). *G. gibberosus* occurred quite patchy, showing the highest abundance at Langeoog. The single site records of *C. arguinae* and *M. fossarum* showed high maximum abundances (Fig.2.6). *H. continua* showed very low abundances with a maximum of 6 metacercariae per individual cockle.

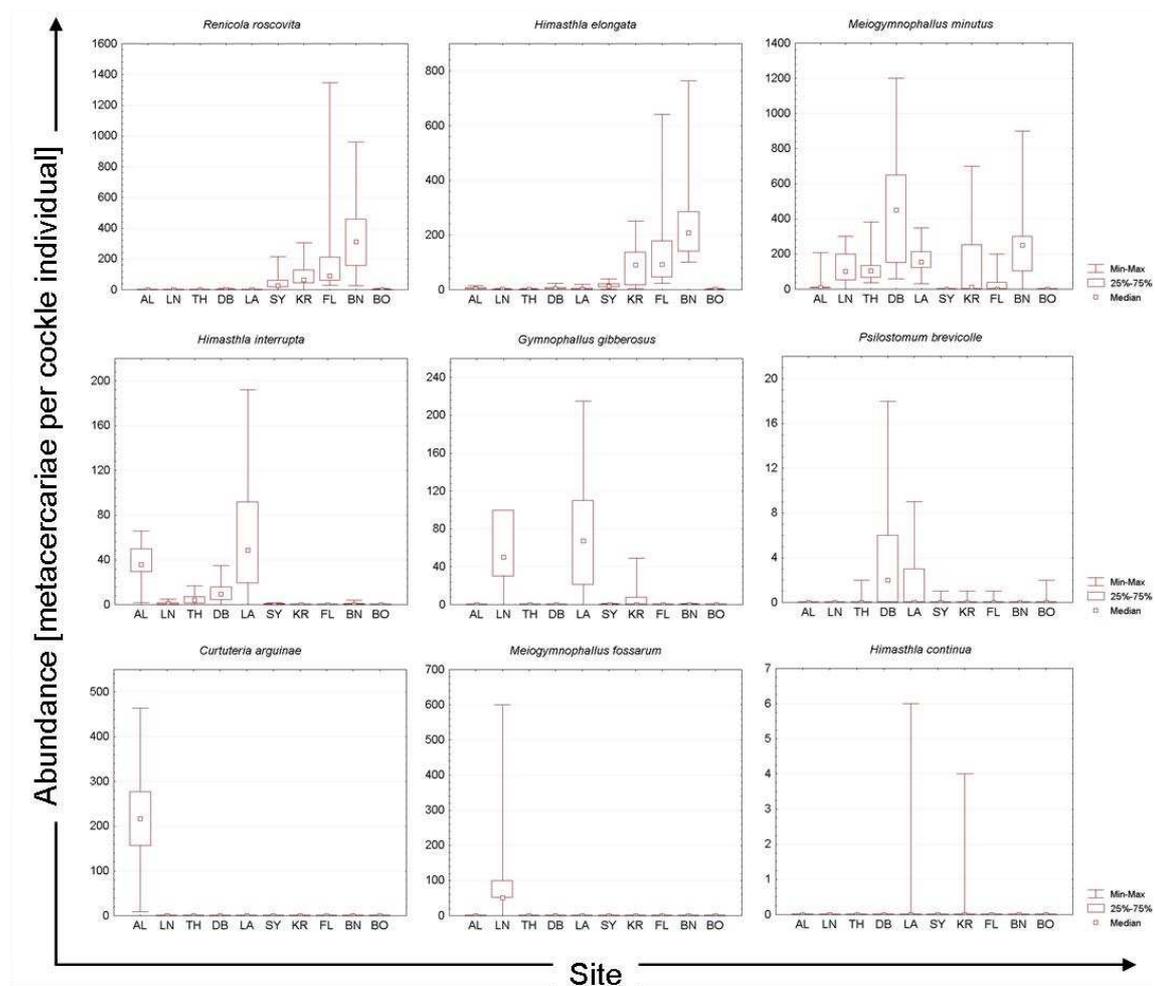


Figure 2.6: Abundance of metacercarial infections. Note different scales on y-axis! An increase in trematode abundance along the latitudinal site distribution was recorded for *R. roscovita* and *H. elongata*. A slight decrease can be seen for *P. brevicolle* from the Irish site northwards. The other abundance data were more scattered along the European shore.

When metacercarial assemblages based on abundances were tested by DFA for affiliation to northern and southern groups, the result supports grouping displayed in the prevalence cluster (Fig.2.4) rather than that based on presence/absence data (Fig.2.5). Several options were tested with the result that best classification (99.3%, Fig.2.7) was possible with

Langeoog (LA) and Southend (TH) populations grouped in a southern group. The separation between these two groups was mainly based on the contribution of more than 0.5 for *M. minutus*, *R. roscovita*, *H. interrupta* and *M. fossarum* to the canonical variable Root 1 (Eigenvalue: 3.684) (Fig.2.7). Mahalanobis distance (D^2) changed from 15.4 to 24.2 after a shift of the Langeoog population from the northern to the southern group indicating a reduced mixing of the groups.

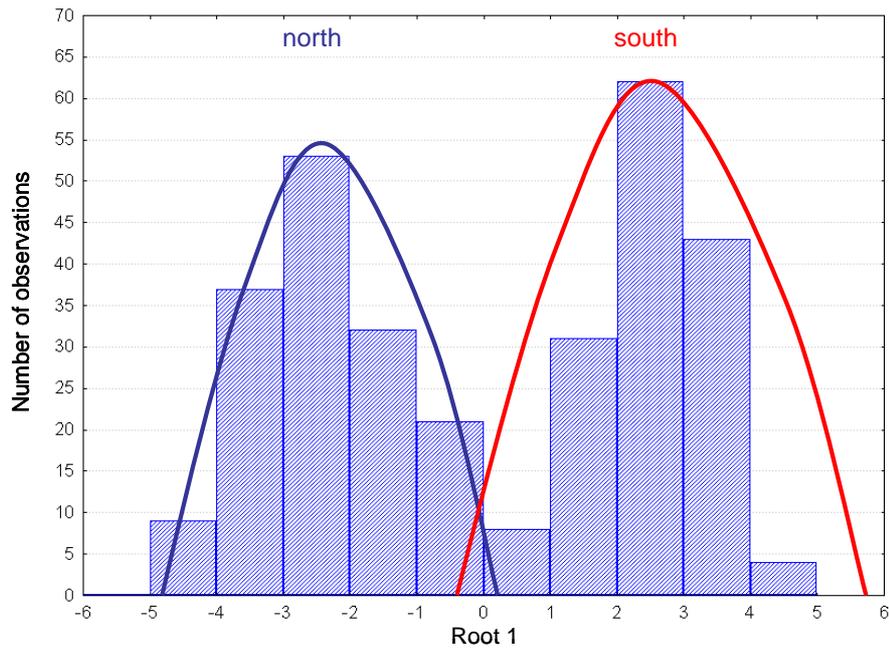


Figure 2.7: Separation of trematode communities (all metacercarial abundances) for provincial groups. Data were log-transformed for discriminant function analysis. Separation was most explicit along Root 1, when LA and TH are included into southern group (Wilks' Lambda: 0.14201, approx. $F(9.290)=194.68$, $p<0.0001$; correct classification: 99.3 %).

To display the data in two-dimensional canonical analyses, sites were grouped for DFA not as large-scale groups but on regional characteristics, except the Wadden Sea populations Langeoog and Sylt (Wilks' Lambda: 0.00216, approx. $F(45.1282)=83.884$, $p<0.0001$) (Fig.2.8). The Langeoog population clustered in the British Isles group which comprises Dublin Bay and Southend. The Sylt site was grouped with the Norwegian Sea and Skagerrak regions (Fig.2.8A+B). The populations of Lagos and Lisbon showed strong distinction from all others as group "Iberian" due to the influence of the rare species *C. arguinae* and *M. fossarum* (Root 1: 59 %) (Fig.2.8A). The trend for northern and southern group affiliations is given by the abundant species, *R. roscovita* and *M. minutus*, contributing to Root 2 (31 %) (Fig.2.8A). *G. gibberosus* and *H. interrupta* abundance,

represented mainly in Root 3 (9 %), did not clarify the overall parasite community structure for provinces but pointed out the special trematode composition in the Langeoog cockle population (Fig.2.8B).

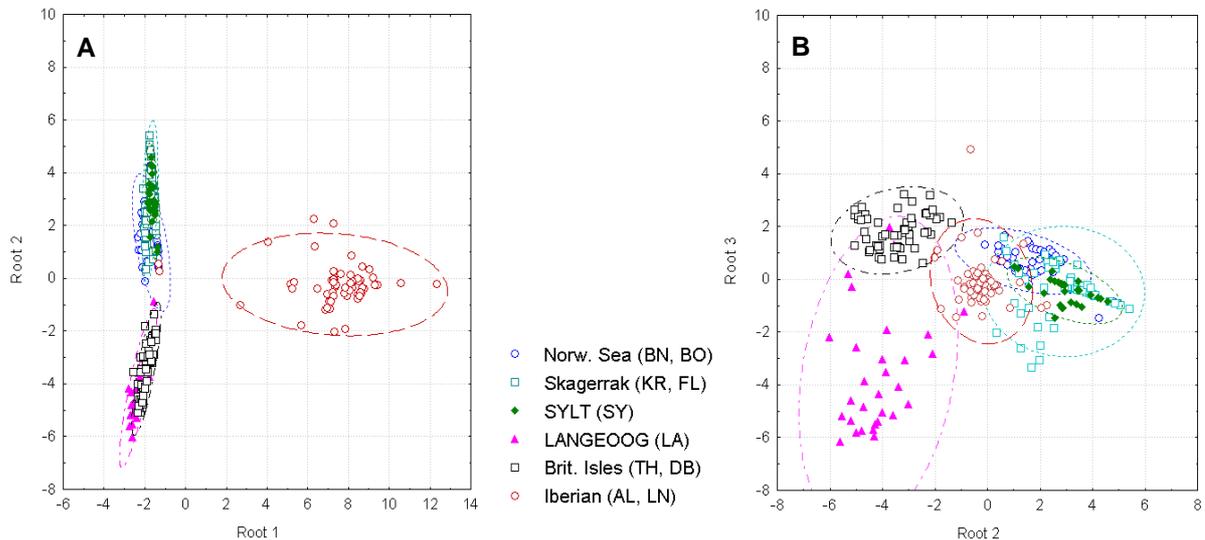


Figure 2.8: Grouping of metacercarial abundance (including nine trematode species). Small scale groupings show significant differences within high regional resolution. DFA of log-transformed data is displayed along canonical roots. **A.** Strong influence of the rare species on Root 1 separates the Iberian populations from the other five groups. Along Root 2, Langeoog is positioned in British Isles group, while Sylt is found with the Norwegian and Swedish sites. **B.** More common trematodes displayed by Root 2 vs. Root 3 show an overlay of northern and southern sites while Langeoog attains a separate position.

Discussion

Parasite assemblages in the cockle *C. edule* are not uniform in distribution, but show a geographic separation at the European coastline. Regionally dominating trematode species were the reason of a northern and southern site cluster. Here, high prevalences of *R. roscovita* and *H. elongata* metacercariae determined the northern group which was in line with the rising abundance towards the north for these two species. The southern group comprises several rare local species but is dominated by the abundance of *M. minutus*. The preferential range of these trematode species may be affected by extrinsic factors like environmental conditions or co-occurring species to cause these patterns. Trematodes using the cockle as first intermediate host showed low influence on the cluster generation.

However, intrinsic factors, represented by co-evolutionary processes between host and parasite, may play a role for generating regionally distinct groups of the host species.

Biogeographic divisions and small scale structuring of parasites

The biogeographic border of the Northeast Atlantic as proposed by Briggs (1995) and Spalding et al. (2007) lies to the west of the English Channel separating the Lusitanian and the Boreal province. While presence/absence data may reflect this biogeographic pattern, the southern parasite group analysed with trematode prevalence and abundances in the cockle *C. edule* incorporates the south-eastern English site Southend as well as the island of Langeoog in the North Sea (see Fig.2.4+2.8). The latter population is situated in the East Frisian Wadden Sea and may be more interesting, since cockles showed a peculiar pattern also in morphological analyses (CHAPTER 1).

Although a qualitatively good comparison of parasite communities is provided here due to consistency of examination procedure at each site, the revealed pattern may be biased because of several methodological problems implicated by this kind of large scale study. Thus, this survey makes no claim to show complete parasite diversity by the small sample size of 30 cockles at each site. The lack of parasites using the cockle as first intermediate host at the southern sites can probably be assigned to the low numbers of hosts investigated. However, more extensive regional studies at several European and African sites (De Montaudouin et al. 2000, Thieltges et al. 2006, Gam et al. in press) have revealed similar trematode compositions as detected in this study, and a large scale structured trematode occurrence in this defined number of randomly sampled cockles could be shown. The intermediate position in the trematode life cycle (Tab.2.4) seems not to be important for creating the large-scale community pattern which was observed in the cockle *C. edule*. Thus, the trematode species using the cockle as first intermediate host does not contribute much to the final cluster. Besides, all three species, *M. parvus*, *B. minimus* and *G. choledochus*, were recently recorded in Moroccan cockles (Gam et al. in press) extending the known range of occurrence farther to the south. These records indicate that the final hosts (Tab.2.4) are distributed from Africa to the North Sea. Changing climate may alter expansion potential by hosts and parasites by producing ecological perturbations, which cause geographical and phenological shifts, and alteration in the dynamics of

parasite transmission (Mouritsen et al. 2005, Brooks & Hoberg 2007). Recently, range expansion of a cockle parasite was demonstrated after a migratory fish host occurred more often in the North Sea and introduced *B. minimus* into the Wadden Sea (Thieltges et al. 2007).

Table 2.4: Host species of cockle trematodes. There are mainly molluscs and birds involved in these trematode's life cycles.

Trematode species	1st intermediate host	2nd intermediate host	final host
Echinostomatidae			
<i>Himasthla elongata</i>	<i>Littorina</i>	Bivalves	Birds
<i>Himasthla cotinua</i>	<i>Hydrobia</i>	Bivalves	Birds
<i>Himasthla interrupta</i>	<i>Hydrobia</i>	Bivalves	Birds
<i>Curtuteria arguinae</i>	unknown	<i>Cerastoderma</i>	unknown
Psilostomatidae			
<i>Psilostomum brevicolle</i>	<i>Hydrobia</i>	Bivalves	Birds
Renicolidae			
<i>Renicola roscovita</i>	<i>Littorina</i>	Bivalves	Birds
Gymnophallidae			
<i>Gymnophallus choledochus</i>	<i>Cerastoderma</i>	<i>Cerastoderma</i>	Birds
<i>Gymnophallus gibberosus</i>	<i>Macoma</i>	Bivalves	Birds
<i>Meiogymnophallus minutus</i>	<i>Scrobicularia</i>	Bivalves	Birds
<i>Meiogymnophallus fossarum</i>	<i>Scrobicularia</i>	Bivalves	Birds
Monorchidae			
<i>Monorchis parvus</i>	<i>Cerastoderma</i>	<i>Cerastoderma</i>	Fish
Bucephalidae			
<i>Bucephalus minimus</i>	<i>Cerastoderma</i>	Fish	Fish

Although there was a significant difference between average size of northern and southern cockles in this study, the span of size ranges for cockles investigated should be representative for trematode community characteristics of the sample sites. Metacercarial trematode infestations usually occur when cockle length is at least 15 mm (De Montaudouin et al., in prep). For the development of sporocysts and rediae, fertile hosts are chosen to use gonadal tissue reserves for multiplication. Fertility of cockles is expected at the age of one year and about 15 mm shell length depending on the environment, and young cockles reach larger size at the southern sites than e.g. in the cold-temperate climate of the North Sea region (Cole 1956, Iglesias & Navarro 1990, De Montaudouin et al. in prep).

Most of the samples were taken in late summer (Tab.2.1) when trematode activity is promoted by warm temperatures. Although the Southend sample was taken in spring, it shows no strong differences in composition and abundance to the Dublin Bay sample and clusters well into the British Isles group on all levels analysed. However, low temperature is known to reduce new trematode infections due to longer development time within the first intermediate host and modest mobility of infecting cercariae (Galaktionov & Bustnes 1995, Mouritsen 2002, Thieltges & Rick 2006). Thus, cold water seems to facilitate autonomic life-cycles as found in microphallids in North Norway (Galaktionov & Bustnes 1995). This could be a possible reason for low or no infection with metacercarial stages in cockles of the northern Norwegian and Arctic seas as found at more distinct the Bodø site within the northern group. At the Russian Barents Sea coast, trematode composition in cockles are expected to be similar to Norwegian assemblages as there are records of infected first intermediate hosts, *Littorina sp.* and *Hydrobia ulvae* (Chubrik 1966, Galaktionov & Bustnes 1999), although there are no published surveys of trematodes in European cockles at their northernmost range (except this study).

In a large scale approach, Poulin & Morand (1999) figured out that mainly distance between sample sites has reasonable impact on variation of parasite communities between fish populations of lakes. As the coastal sites which cockles inhabit are not restricted in connectivity like lakes, other factors may show more influence on their parasite composition. The distribution range of all hosts involved in trematode life cycle (Tab.2.4) is likely to be responsible for patterns in trematode communities on a large scale (Latham & Poulin 2003, Hechinger & Lafferty 2005, Fredensborg et al. 2006, Thieltges 2007). Significant differences of trematode prevalence and intensity in cockles within the Wadden Sea area were explained by differing first intermediate host density between local sampling sites (Thieltges 2007). But regional differences between parasite communities and species richness were investigated more extensively for final hosts, mainly vertebrates like fish or birds. For example, differences in trematode occurrence were found in *Hydrobia ventrosa* in relation to habitat use of waterfowl in Icelandic salt marsh ponds (Skirnisson et al. 2004). However, the proof of this relation failed in a more extensive bird study in the Baltic Sea area (Kube et al. 2002). For parasite species diversity in fish hosts, latitudinal gradients were found (Rohde & Heap 1998), but different trends could be detected for ectoparasites and endoparasites. Rohde & Heap (1998) stated that parasites living in

vertebrate tissue or cavities show no or less latitudinal change in species diversity or abundance than parasitic organisms exposed to environment. A similar result was found in terrestrial host-parasite relationships where only parasitoid assemblages in exposed hosts showed geographically and climate-driven variation on a global scale (Hawkins 1990). These regional differences recognised in infection pattern qualified parasites as biological markers to distinguish host populations of marine fish and study their migration (Rohde 2002).

Small scale heterogeneity of parasite assemblages within locations may change results on large scale pattern. Variability of parasite species occurrence and their abundance around the island of Sylt was shown to be high with significant differences between intertidal habitat structures (Thieltges 2007, Thieltges & Reise 2007). Host condition and density were found to be additional regulative factors for spatial parasite heterogeneity (Mouritsen et al. 2003). Finally, the cockle itself may structure parasite heterogeneity in infection levels at a small scale due to its body size variation (Wegeberg et al. 1999, De Montaudouin et al. 2005). Thus, the large scale pattern found needs to include more site specific studies with larger sample size to be verified.

Coevolution and host specificity in trematodes

Small scaling factors like host size distribution, physical condition or population density are important factors causing spatial parasite heterogeneity (Wegeberg et al. 1999, Mouritsen et al. 2003, De Montaudouin et al. 2005, Thieltges & Reise 2007). These characteristics are possibly results of an interplay between cockle and trematode. Fredensborg & Poulin (2006) focussed on the host life traits and showed that the snail *Zeacumantus subcarinatus* adapts to trematodes by reaching maturity early. Thus, the snail population maximizes its chance of reproduction before experiencing a high prevalence of castrating infection by trematodes.

The intimate interaction of host-parasite relationship is mainly influenced by the trematode specificity. The species range which can be infected by trematodes gets broader with down-stream hosts (Galaktionov & Dobrovolskij 2003). This means that host specificity is narrowest for first intermediate host and getting less stringent with second intermediate and

for definitive hosts. Recent findings suggest that the degree of congruence depends mainly on host position in the parasite life cycle and on the nature of transmission dynamics between host and parasite (Nieberding et al. 2004, Criscione et al. 2005). As cockles are affected by their trematodes at two different levels, a consistency between host and parasite phylogeography is not expected for all parasite taxa. *C. edule* serves as first and second intermediate host, and closest relation would be expected for the small number of cercariae producing species, *M. parvus*, *B. minimus* and *G. choledochus*. Especially, *G. choledochus* was shown to be strictly host-specific at this level, but displayed less specificity for its second intermediate host (Loos-Frank 1969). However, if a misidentification of cockle species could be excluded, *M. parvus* and *B. minimus* were also recorded from the sister species *C. glaucum* at first host level (Boyden 1969, Maillard 1976). For meiogymnophallid parasites, both cockle species, *C. edule* L. and *C. glaucum* (Poiret), serve as second intermediate hosts. While the metacercariae of *M. strigatus* and *M. minutus* can be found only in *C. edule*, the sibling trematode species *M. fossarum* could be detected in both cockle hosts (Russell-Pinto 1990, Bowers et al. 1996).

Members of the parasite population may be adapted to local available host species, and thus be more host specific than the species as a whole. This may lead to ecological, morphological and also genetic differentiation within the parasite species (Galaktionov et al. 2004). Cryptic speciation could be shown for several fish parasites (Pariselle 1996, Jousson et al. 2000, Desdevises et al. 2002). However, coevolution between host and parasite is difficult to demonstrate. Reconstruction of phylogeny patterns of this close relationship became very useful (Brooks 1988, Jousson et al. 2000, Paterson & Banks 2001). Poulin (1998) briefly reviewed the progress in host-parasite coevolution regarding Fahrenholz's Rule which assumes perfect congruence of host and parasite phylogeny. Testing this prediction for terrestrial rodents and their ectoparasites, Hafner & Nadler (1988) concluded that the speciation of hosts and parasites was roughly contemporaneous and probably causally related. However, simple comparisons of phylogenies may lead to incongruence of cophylogenetic history because of the complex interplay of cospeciation with other intrinsic factors (Paterson & Banks 2001, Desdevises et al. 2002). First hints on potential reciprocal coevolution between host resistance and parasite infectivity and/or virulence were demonstrated in the *Schistosoma* complex (Webster et al. 2004). However, the effect of co-differentiation on parasite diversification is often confounded by

underlying geographic patterns of host distribution and host ecology (Nieberding et al. 2008).

Conclusion and Outlook

All these factors influence the occurrence of parasite species at different host levels and on different spatial scales. For the cockle, *C. edule*, it seems most likely that migratory birds and, as a counterpart, the presence of snails used by trematodes as first intermediate host are mainly shaping the parasite assemblages along the European shores in relation to defined biogeographic provinces (Fig.2.5). The connectivity of parasite populations and possibly the finding of southern trematode dominance in parts of the Wadden Sea seem to be strongly related to migration routes (e.g. in Fig.2.1) and population dynamics of birds as final hosts. These have been noticed to change in the last decades (Bart et al. 2007, Reineking & Südbeck 2007) which may influence trematode occurrence and distribution by lower local bird densities and following reduced transmission. However, species-specific characters of trematodes and cockles have to be kept in mind to evaluate patterns concerning the close host-parasite relationship. Host specificity and co-evolution in the complex host-parasite relationship are not yet well understood. It seems possible that single trematode species may have potentially adapted to the cockle serving as first intermediate host. A phylogenetic approach may test, for example, the close relationship of *G. choledochus* to its cockle host, and a congruent evolutionary development may be revealed (Jousson et al. 2000). Phylogeographic studies on the widely occurring *H. elongata* or the abundant *M. minutus* could be promising to understand the transmission and infection success in cockles. A genetically based survey on common trematode species including cross-infection experiments may clarify the connection to the cockle as invertebrate host. Further studies may reveal physiological or behavioural co-adaptation of the cockle host and its specific parasites. Adaptation to or against trematode infection expressed in changes of host life history traits might be related to host genetics pattern.

Aside from the interplay of factors which generate species clusters, global warming is expected to influence the parasite host relationship. Promoting the proliferation of the infective stages in coastal ecosystems may increase lethal effects of trematodes using molluscs as first intermediate host (Poulin 2006). Additionally, the high cercarial output

may result in more regular extinctions of the second intermediate host (Jensen & Mouritsen 1992, Mouritsen et al. 2005). Thus, a change in food web structures affecting the whole species community of the ecosystem may be enhanced.

CHAPTER 3

Genetic diversity in high latitudes –
An intertidal bivalve contradicts a common pattern

Abstract

Despite planktonic larva which is assumed to maintain gene flow between coastal populations, biogeographic differentiation at different scales was found for several marine species on DNA level. Here, the European cockle species, *Cerastoderma edule*, often dominating intertidal and inshore subtidal sediment fauna in biomass, has been analysed for its genetic structure over the entire distribution range. Analyses of mitochondrial DNA revealed surprisingly high numbers of haplotypes as well as a genetic discontinuity east of the English Channel within the East Atlantic coastal range. However, the pattern included strange outliers and a peculiar inversion in the southern Scandinavian region.

Cockles were sampled at 19 sites, ranging from near Casablanca at the Moroccan Atlantic coast to near Murmansk at the Russian Barents Sea shore. 79 sequence variants were detected among 383 individuals with 15 haplotypes shared between two or more sites. Analysis of molecular variance (AMOVA) shows that 43.6 % of total genetic variability is explained by differences between populations grouped as “south-western” and “northern”. Highest haplotype richness was found in the North Sea and the Norwegian Sea. This contrasts other marine biogeographic studies which show a loss of diversity at high latitudes. The influence of the Pleistocene glaciations and application of sudden population expansion models are discussed in respect to a potential Boreal origin of present genetic patterns in *C. edule*.

Introduction

Compelling evidence for the presence of cryptic species in the oceans suggests that speciation in the marine realm is more frequent than expected (Palumbi 1996, De Vargas et al. 1999, Wilke & Pfenninger 2002, Kruse et al. 2003, Remerie et al. 2006). Marine species are often characterized by large population sizes, high potential for dispersal, and in some cases wide geographic distribution. These factors, coupled with the continuity of the marine habitat, tend to oppose isolation and divergence of populations, thus preventing cladogenesis through allopatry (Palumbi 1992). The presence of planktonic larvae has been considered as a life-history strategy for successful dispersal in marine benthic species. Thus, the maintenance of the gene pool homogeneity of a species over its distribution area is expected producing panmictic populations (Scheltema 1971, Buroker 1983, Palumbi 1996, Burton 1997). However, levels of gene flow may be low enough to allow natural selection that genetic drift occurs independently in each subpopulation (Slatkin 1981). As the occurrence of closely related species in sympatry is rather common, this emphasizes the fact that mechanisms of speciation in marine organisms are still poorly understood (Knowlton 1993, Miya and Nishida 1997).

A growing number of genetic surveys have found evidence of restricted dispersal in marine organisms with planktonic larvae (e.g. Johannesson 1992, Barber et al. 2002, Kojima et al. 2000, Taylor & Hellberg 2003, Viard et al. 2006, Luttikhuisen et al. 2008). Long distance movements of larvae may be impeded by the existence of hydrological or ecological barriers, such as currents, temperature or salinity, whereas self-recruiting populations may favour local differentiation (David et al. 1997). Genetic drift also occurs, when high fecundities are associated with high variance in reproductive success (Hedgecock 1994, Palumbi 1994, Gosling 2003). Low sea surface temperatures in temperate regions were hypothesized to contribute to longer larval duration and greater connectivity among lecithotrophic species (Kelly & Eernisse 2007). However, instead of high variability at the DNA level with very low genetic differentiation among populations, factors such as historical fragmentation, restriction to gene flow due to marine barriers, demographic instability and local selection may depart population structures from these expectations as shown by studies on several bivalves (Koehn et al. 1980, Karl & Avise 1992, Quesada et al. 1995, David & Jarne 1997, Luttikhuisen et al. 2003a).

Patterns of genetic variation and species diversity in boreal and temperate regions often show clear signatures of range compression and extension due to glacial cycles in the last two million years (Pamilo & Savolainen 1999, Hewitt 2004, Schmitt 2007). During the Last Glacial Maximum (18,000 years ago), coastal fauna along the European coast was presumably pushed as far south as the south of Britain and France or even the Iberian Peninsula (Dawson 1992). To estimate the influence of ice ages on species history, a detailed analysis of nucleotide variation and phylogeography of DNA sequences may be applied (Posada & Crandall 2001, Hey & Machado 2003).

Recent studies using the mitochondrial gene cytochrome c oxidase I (COI) in marine crabs and polychaetes have revealed population subdivision in European species (e.g. Roman & Palumbi 2004, Jolly et al. 2005). The results imply that genetic exchange is restricted for these populations within the eastern Atlantic coast, defining a genetic discontinuity between south-western and northern Europe next to the English Channel. This border roughly aligns with biogeographic regions proposed by Briggs (1995). He defined for the northeastern Atlantic a warm-temperate Lusitanian province, extending from the Cape Verde Islands off the coast of West Africa northward to the southern part of the English Channel, and including the Mediterranean. A cold-temperate Eastern Atlantic Boreal region extends from the western entrance of the English Channel with the British Isles to the Kola Fjord near the Russian border, and includes the southern coast of Iceland and the Faroes.

Along the north-eastern Atlantic coast, the European (or Edible) cockle *C. edule* (L.) is found frequently in soft-bottom intertidal and at shallow subtidal coasts. It shows a distribution from West Africa to the Barents Sea, reaching into marginal seas like the Baltic and the Mediterranean (Hayward & Ryland 1995). Since it often dominates biomass, this filter-feeding bivalve plays a key role in coastal food webs of the Northeast Atlantic and is also commercially fished in some countries (Rasmussen 1973, Beukema 1978, Sutherland 1982, Jensen 1992b, MacKenzie et al. 1997, Beukema & Dekker 2005). Planktonic larvae of *C. edule* were observed to settle after a up to four weeks drift in the water column. Thus, populations with low genetic differentiation are expected in the Atlantic range according to findings of other invertebrate species (e.g. Luttkhuizen et al. 2003b, 2008).

The genus *Cerastoderma* has been under genetic study since the 1970s because of uncertainties in morphological species recognition (Brock 1978, Brock 1987, Machado & Costa 1994). In the fossil record the genus dates back to the Oligocene (Keen 1969). The divergence of *C. edule* and its closest living relative, the Lagoon cockle, *C. glaucum*, has generally been attributed to the isolation of the Atlantic basins and the Mediterranean in the Late Miocene-early Pliocene times (Rygg 1970, Brock and Christiansen 1989, Hummel et al. 1994). Mainly the species complex, *C. glaucum* with the possible subspecies *C. lamarcki*, has been in focus over the last decades because of the patchy distribution in lagoons and limited larval drift (Brock 1987, Brock & Wolowicz 1994, Mariani et al. 2002a, Nikula & Väinölä 2003). Most genetic studies on this genus have mainly been aimed at identifying diagnostic genetic markers for distinguishing the two cockle species (Jelnes et al. 1971, Brock 1991, Hummel et al. 1994, Machado & Costa 1994, Freire et al. 2005) rather than analysing the differences among populations within the species (Beaumont & Pether 1996, Nikula & Väinölä 2003).

By using mtDNA sequences, I test the applicability of commonly assumed population structure and historical biogeography models on the coastal bivalve *C. edule*. For the analyses of samples from 19 North East Atlantic localities, including range margins like Baltic and Barents Sea, I hypothesize:

- (I) Cockle populations in southern regions have higher genetic diversity than those in the North of Europe because of a Pleistocene ice age retreat, and
- (II) No or low genetic intraspecific differentiation occurs throughout the distribution range because of high larval connectivity between the populations.

Material and Methods

Samples

A total of 383 specimens of *C. edule* from 19 European and one African population were sampled between 2005 and 2007 (Fig.3.1).

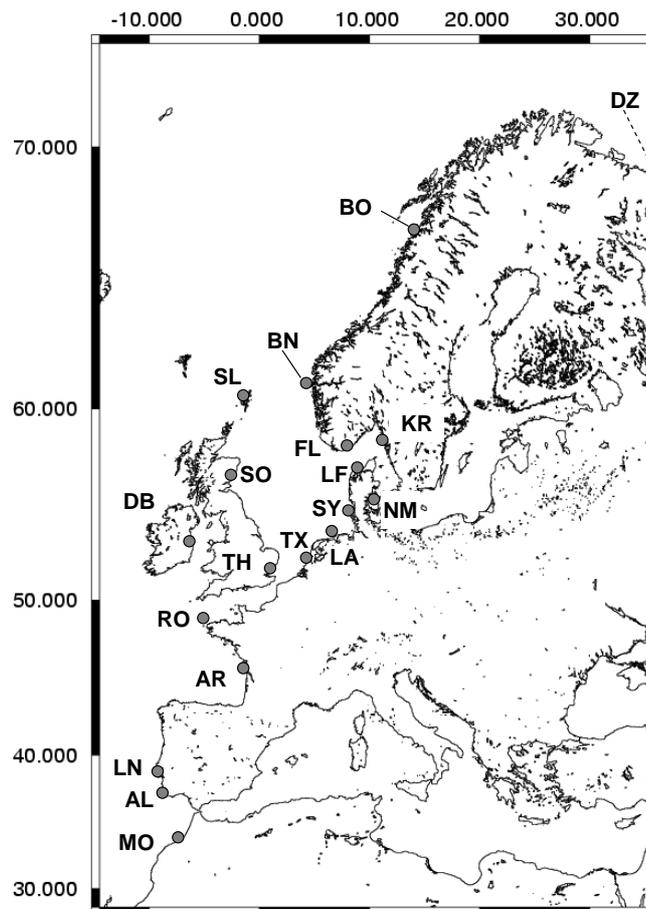


Figure 3.1: Sample localities for *C. edule*. Sample codes correspond to those in Tab.3.1.

Sampling was done at low tide by hand and rake next to Lagos and Lisbon (Portugal), Dublin (Ireland), Sylt (Germany), Kristineberg (Sweden) and Bergen (Norway). In addition, samples from 13 other sites were kindly provided to me by colleagues (Tab.3.1). Soft tissue or whole bivalves were preserved in 96-100 % ethanol until further processing. Randomly sampled individuals showed different ages (1-6 years), with size ranging from 13.7 to 52.5 mm.

Table 3.1: Site information for *C. edule* sampling. Localities are indicated on the map in Fig.3.1.

Code	geographic region	Location	Country	Latitude	Longitude	Sampling date	samples provided by
MO	North eastern Atlantic	Merja Zerga	Morocco	34°50' N	06°14' W	August 2005	H. Bazairi
AL	North eastern Atlantic	Lagos	Portugal	37°07' N	08°37' W	September 2005	-
LN	North eastern Atlantic	Lisbon	Portugal	38°43' N	09°00' W	September 2005	-
AR	North eastern Atlantic	Arcachon	France	44°35' N	01°14' W	August 2005	X. de Montaudouin
RO	North eastern Atlantic	Roscoff	France	48°72' N	03°9'7" W	June 2007	A. Wagner, K. Valentin
TH	English Channel	Southend	England	51°28' N	00°42' E	March 2007	D. Lackschewitz
DB	Irish Sea	Dublin	Ireland	53°19' N	06°12' W	August 2005	-
SO	North Sea	St. Andrews	Scotland	56°21' N	02°50' W	August 2006	J. Saunders
SL	North Sea	North Gluss	Scotland	60°28' N	01°21' W	September 2007	J. Coyer, J. Olsen
TX	North Sea	Texel	The Netherlands	53°13' N	04°94' E	August 2004	H. Andresen
LA	North Sea	Langeoog	Germany	53°45' N	07°29' E	July 2007	K. Reise
SY	North Sea	Sylt	Germany	55°01' N	08°26' E	September 2005	-
LF	North Sea	Limfjord	Denmark	56°97' N	09°20' E	July 2007	K.T. Jensen
NM	Kattegat/ Baltic	Norsminde	Denmark	56°02' N	10°25' E	July 2007	K.T. Jensen
KR	Skagerrak	Kristineberg	Sweden	58°14' N	11°26' E	July 2006	-
FL	Skagerrak	Arendal	Norway	58°26' N	08°48' E	August 2005	S. Mortensen
BN	Norwegian Sea	Bergen	Norway	60°23' N	05°20' E	August 2005	-
BO	Norwegian Sea	Bodoe	Norway	67°17' N	14°37' E	August 2005	K. Korsnes
DZ	Barents Sea	Murmansk	Russia	69°10' N	36°05' E	August 2007	E. Genelt-Yanovsky

DNA extraction and PCR

Total DNA was extracted from adductor muscle or foot tissue by following the protocol of the QIAGEN® DNeasy Blood and Tissue Kit (Qiagen; Hilden, Germany). DNA was used to amplify a fragment of about 700 base pairs (bp) of the mitochondrial cytochrome oxidase I (COI) gene (Fig.3.2).

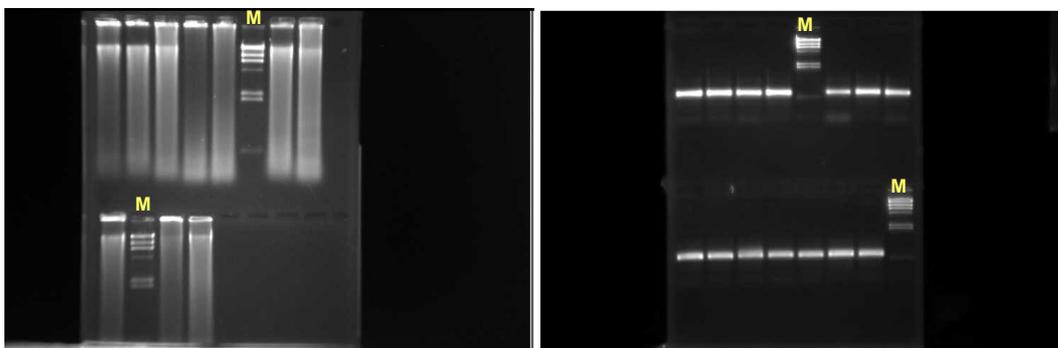


Figure 3.2: DNA extraction and PCR product agarose gel imaging. Left: Typical gel of DNA after extraction with DNeasy Kit. Right: Purified COI PCR products of ~700 bp length. Lane M shows the size reference marker λ /HindIII (125 to 23,130 bp, Fermentas; St. Leon-Rot, Germany).

Polymerase chain reaction (PCR) amplification was performed according to standard methods on a Biometra® Cycler (Biometra; Göttingen, Germany). First, published primers (LCO1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'; Folmer et al. 1994) were used. Because of weak amplifications, a new forward primer (COI5Fc: 5'-CAT AAA GAT ATT GGG ACT TTA TAC-3') was designed after the results of the first reactions.

PCR was carried out in 50 µL reaction volume with 2 µL template DNA (~150 ng), 0.2 mM of each dNTP, 0.6 pM of each primer, 1.5 mM of MgCl₂, and 0.5 U Taq polymerase (Qiagen; Hilden, Germany) in the buffer supplied by the manufacturer. The PCR conditions were as follows: after first denaturation at 94 °C for 2 min followed 40 cycles with denaturation at 94 °C for 60 s, annealing at 50 °C for 60 s, and extension at 72 °C for 60 s. Amplification results were confirmed by 1% TBE agarose gel electrophoresis. The fragments were purified using QIAquick purification kit (Qiagen; Hilden, Germany). Direct sequencing of PCR products was done in one direction by MWG Biotech (Ebersberg, Germany). Randomly chosen singleton haplotypes were verified by repeating DNA extraction, PCR and additional reverse sequencing.

Sequence alignment and population genetic analyses

Sequence characterization and genetic diversity

Quality of sequencing results was manually checked with CHROMAS Lite 2.01, and sequences were aligned with CLUSTAL W (Thompson et al. 1994) as implemented in BIOEDIT (Hall 1999).

Haplotype and nucleotide diversity analyses were performed with the software package DnaSP 4.10 (Rozas et al. 2003). With this software, number of nucleotide substitutions and aminoacid changes were analysed based on invertebrate mtDNA code (flatworm). A linear regression of nucleotide diversity with latitude was carried out in Statistica 6.0 (Statsoft, Hamburg, Germany).

Phylogenetic relationships

To uncover the relation of COI sequences within the species *C. edule* and in comparison to its sister species *C. glaucum*, maximum likelihood (ML) analyses were done for a 529 bp fragment in PhyML 3.0 (Guindon & Gascuel 2003), after removing identical haplotypes from the dataset. For the lagoon cockle *C. glaucum*, a Genbank sequence was used [AY226938]. Since mtDNA shows usually pronounced differences in transition and transversion rates (Nei & Kumar 2000), distances between haplotypes were computed using the models K80 (equal base frequency; Kimura 1980) and HKY85 (unequal base frequency; Hasegawa et al. 1985). Nonparametric bootstrapping was applied for the ML analyses using 100 pseudoreplicates.

To reveal the connection between COI haplotypes based on nucleotide differences, phylogenetic relationships were calculated in a minimum spanning network using parsimony in the computer program TCS v1.18 (Clement et al. 2000).

Geographical population structure

To estimate the degree and statistical significance (employing 500 permutations) of genetic subdivision between sampled populations and grouped samples, the AMOVA program (analysis of molecular variance) was used (Excoffier et al. 1992, Excoffier 1995), as implemented in the program Arlequin (Version 3.1; Excoffier et al. 2005). This approach performs a standard analysis of variance (ANOVA), partitioning the genetic variability in different hierarchical levels from the individual to the population level (for details see Excoffier et al. 2005).

The significance of pairwise F_{ST} (Hudson et al. 1992) was calculated in Arlequin (Version 3.1; Excoffier et al. 2005). In addition, for the detection of group structure (cp. Quinteiro et al. 2007), a graphic representation of Kimura's 2-parameter (K2P) pairwise genetic distance (Kimura 1980) between population samples was obtained on the basis of non-metric multidimensional scaling (MDS) of similarities (Clarke & Green 1988). The Euclidian distance index was used as provided in the Plymouth Routines in Multivariate Ecological Research (PRIMER®) package (Clarke & Gorley 2001) without transforming genetic distance data. An analysis of the similarity matrix (using the non-parametric routine ANOSIM, Clarke & Green 1988) was conducted to support the defined regional

groups statistically. The detailed structure was displayed by a hierarchical cluster analysis of the K2P data in PRIMER®.

Two methods were employed in order to detect the “isolation by distance” (IBD) model (Wright 1943, Slatkin 1993). To assess correlation between matrices of the pairwise genetic divergence and geographic distance matrices, a Mantel test (1000 permutations) was performed in Arlequin (Version 3.1; Excoffier et al. 2005), which calculated the probability that two matrices will be independent from one another. Geographic distances between populations were calculated in kilometers as shortest along-shoreline and across-water distance using ArcGIS 9.2 (ESRI; Kranzberg, Germany). To test for a relation of genetic diversity to the latitudinal gradient, a linear regression was applied in Statistica 6.0 (StatSoft; Hamburg, Germany).

Population demographic history

Neutrality tests and mismatch analyses were performed with the software package DnaSP 4.10 (Rozas et al. 2003). Additionally, demographic parameters under a sudden expansion model were calculated in Arlequin (Version 3.1; Excoffier et al. 2005) after Schneider & Excoffier (1999) with the distance method using the variable sites of all haplotypes defined.

To estimate the time frame of population expansion, generation time was assumed to be one year. Based on general estimates of mtDNA variation for other invertebrates (DeSalle et al. 1987, Knowlton & Weigt 1998), a molecular clock of 1.5–2.0 % sequence divergence per million years was used in the equation: $t = \tau/2\mu k$; with τ : moment estimator of time to expansion; μ : mutation rate per nucleotide and year; k : length of sequence investigated (Rogers & Harpending 1992).

Additionally, the time frame of intraspecific divergence was evaluated through comparison of the interspecific divergence between *C. glaucum* and *C. edule*. Divergence time is tentatively placed at the date of Atlantic-Mediterranean isolation about 6 million years ago (Hummel et al. 1994, Nikula & Väinölä 2003), which is linked with the Messinian Salinity Crisis in the late Miocene (Krijgsman et al. 1999).

Results

Analyses of mitochondrial DNA sequences of the European cockle *C. edule* revealed a distinct population genetic pattern in spite of low nucleotide variation. Along the distribution range, high genetic diversity was found at northern European sites. Northern haplotypes were found to be derived from sequence types of the southern cluster by phylogenetic analyses using the sister species *C. glaucum* as outgroup. Biogeographic separation could be defined on a large scale similar to marine provinces with a boundary next to English Channel. However, the Kattegat/Skagerrak-region showed an admixture of northern and southern haplotypes which influenced demographic analyses.

Sequence characterization and genetic diversity in *C. edule*

The aligned data set of COI sequences consisted of 582 nucleotide positions, with 78 (13 %) variable positions of which 23 (4 %) were parsimony informative. The 79 COI haplotypes identified displayed between 0.17 % and 2.23 % nucleotide difference related to the 582 base pair sequence (Appendix 3.1). Nucleotide substitution was predominantly found at the 3rd position (69.2 %). However, at 1st and 2nd codon position considerable variation could be detected with 24.4 % and 6.4 %, respectively. Of the 78 polymorphic sites, 12 were replacement mutations, placed mainly at the end of the 194 amino acid sequence (Appendix 3.2). Two replacements within one amino acid sequence occurred in singleton haplotypes belonging to Roscoff and Langeoog populations. Single replacement mutations were observed in three singletons of the Southend site (TH), in one of Dublin (DB), Langeoog (LA), Limfjord (LF), the Swedish site (KR), and in two singletons of Murmansk site (DZ). Single replacement of amino acid was also detected in the 29 individuals with haplotype “so1” which occurred mainly at the St. Andrews (SO), but was also found at the Norwegian sites Bergen (BN) and Bodo (BO) (Tab.3.2). Noticeably, replacement mutations were absent in the most southern region (MO, AL, LN).

Of the 79 COI haplotypes identified in the data set (Appendix 3.1), 15 haplotypes occurred at least at two different localities (=shared haplotypes, Tab.3.2), while nine were local and 55 singletons (see Appendix 3.4). The distribution of haplotypes in the populations investigated revealed two common haplotypes (“ATL”: 28.5 %; “NSea”: 20.4 % frequency

occurrence in total sample size), which can be found at ten or more localities (Tab.3.2). The “south-western” group which unites the south-western European sites from Portugal to Great Britain and the African site showed “ATL” as dominant haplotype with 66.7 %. Within a northern group, including all sampling sites north of the English Channel except the British Isles, “NSea” was the characteristic and most common haplotype (35.0 %).

Table 3.2: Frequency of shared haplotypes in *Cerastoderma edule* populations. N_{SH}: Number of individuals with haplotypes shared between minimum two sites. ATL-bo1: haplotypes. Haplotypes with an asterisk are restricted to populations in south-western Europe (red coloured: south-western) or northern Europe (blue coloured: northern).

site	N _{SH}	ATL	atl1*	atl2	atl3	atl4*	so1	Nsea*	ns1	ns2*	ns4*	ns5	ns6*	ns7*	nm1*	bo1*
MO	20	16	1	-	2	1	-	-	-	-	-	-	-	-	-	-
AL	19	16	-	1	1	1	-	-	-	-	-	-	-	-	-	-
LN	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AR	12	10	-	-	2	-	-	-	-	-	-	-	-	-	-	-
RO	15	12	-	-	2	-	-	-	1	-	-	-	-	-	-	-
TH	14	11	-	2	-	-	-	-	1	-	-	-	-	-	-	-
DB	17	16	1	-	-	-	-	-	-	-	-	-	-	-	-	-
SO	28	1	-	-	-	-	26	-	-	-	-	1	-	-	-	-
SL	18	1	-	-	-	-	-	10	1	1	-	-	-	-	-	5
FL	18	5	-	2	-	-	-	5	-	-	2	3	-	-	1	-
KR	19	12	-	-	-	-	-	1	1	-	2	2	1	-	-	-
NM	18	2	-	-	-	-	-	4	2	-	6	3	-	-	1	-
LF	15	-	-	-	1	-	-	9	1	-	-	3	1	-	-	-
SY	10	-	-	-	-	-	-	6	-	1	-	-	2	1	-	-
LA	18	-	-	-	-	-	-	15	3	-	-	-	-	-	-	-
TX	18	-	-	-	-	-	-	15	1	-	-	1	-	1	-	-
BN	16	-	-	-	-	-	1	10	2	1	2	-	-	-	-	-
BO	12	-	-	-	-	-	2	3	-	2	1	-	-	-	-	4
DZ	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13
all	307	109	2	5	8	2	29	78	13	5	13	13	4	2	2	22

Comparing the occurrence of haplotypes shared between sites, a greater number of private haplotypes were found exclusively in south-western or northern cockle populations than expected by chance (Chi-Square=5.6, d.f.=1, p<0.05). This was probably due to the fact that seven haplotypes were found only at northern sites. Noticeably, the number of individuals per private haplotype was also higher in the north, as only two individuals exhibited the southern haplotypes “atl1” and “atl4”, while e.g. all 78 individuals of the “NSea” haplotype appeared only in northern populations (Tab.3.2).

In general, haplotype and nucleotide diversity were highly different between populations and high in value for the whole dataset (Tab.3.3). Highest diversity indices were found at the North Norwegian site Bodo (BO; Tab.3.3: in bold). Highest numbers of polymorphic

sites and most unique haplotypes, which were not recorded from any other site, were detected in the northern Wadden Sea (SY, Tab.3.3: in bold).

On average, haplotype and nucleotide diversity was observed to be lower in the southern (MO to AR) than in northern edge populations (SL to DZ). At Roscoff (RO) and Southend (TH) higher values emerged than expected in regional comparisons. Relatively low index values were found in the Wadden Sea populations Texel (TX) and Langeoog (LA). Number of total haplotypes was low in three populations, Lisbon (LN), Arcachon (AR) and St. Andrews (SO). However, at Lisbon and Arcachon sites only 10 and 13 individuals were investigated, respectively. Lowest genetic diversity was found in St. Andrews (SO, Tab.3.3: highlighted in grey). This Scottish sample showed no unique haplotype.

Table 3.3: Genetic diversity indices are shown for cockle sequences. N: number of cockles investigated; H_t : number of total haplotypes; H_u : number of unique haplotypes at the site; h : haplotype diversity; π : nucleotide diversity; k : average number of nucleotide differences; standard deviation (SD) in parentheses. Highest values are indicated by bold numbers in frames, while lowest values are greyish highlighted.

site	N	H_t	H_u	Nr polymorphic sites	h (SD)	π (SD)	k
MO	21	5	1	4	0.424 (0.131)	0.00105 (0.00037)	0.60952
AL	21	6	2	6	0.429 (0.134)	0.00113 (0.00043)	0.65714
LN	10	3	2	5	0.511 (0.164)	0.00225 (0.00089)	1.31111
AR	13	3	1	4	0.410 (0.154)	0.00128 (0.00067)	0.74359
RO	23	10	7	14	0.731 (0.099)	0.00264 (0.00075)	1.53360
TH	21	9	6	11	0.729 (0.102)	0.00263 (0.00064)	1.53333
DB	23	8	6	9	0.526 (0.126)	0.00135 (0.00046)	0.78261
SO	28	3	0	3	0.140 (0.087)	0.00048 (0.00034)	0.28042
SL	20	6	1	7	0.705 (0.086)	0.00336 (0.00070)	1.95263
FL	20	8	2	13	0.868 (0.044)	0.00524 (0.00063)	3.04737
KR	20	7	1	8	0.642 (0.118)	0.00352 (0.00093)	2.04737
NM	20	8	2	11	0.863 (0.049)	0.00553 (0.00053)	3.21579
LF	19	9	4	8	0.772 (0.094)	0.00350 (0.00058)	2.03509
SY	19	12	8	17	0.901 (0.059)	0.00428 (0.00074)	2.49123
LA	21	5	3	10	0.486 (0.124)	0.00191 (0.00074)	1.11429
TX	20	6	2	6	0.447 (0.137)	0.00176 (0.00066)	1.02632
BN	22	8	3	14	0.771 (0.080)	0.00402 (0.00082)	2.33766
BO	22	11	6	15	0.931 (0.028)	0.00646 (0.00072)	3.75758
DZ	20	8	7	13	0.589 (0.130)	0.00223 (0.00083)	1.30000
ALL	383	79	64	78	0.866 (0.012)	0.00511 (0.00017)	2.96606

Between latitude and nucleotide diversity π , regression analysis of cockle sequence data reveals significantly positive correlation ($R^2=0.22$, $p=0.040$; Fig.3.3). Peculiar is the high variability of nucleotide diversity at the sites in the mid-latitudes, between $53^{\circ}00'N$ and

58°99'N, which spans from 0.00048 (SO) to 0.00553 (NM) (Tab.3.3). For haplotype diversity h , the regression only approaches significance ($R^2=0.28$, $p=0.024$), after the outlier St. Andrews (SO) was excluded from the analysis (not shown). This Scottish site shows a much lower degree of haplotype diversity than expected for its latitude.

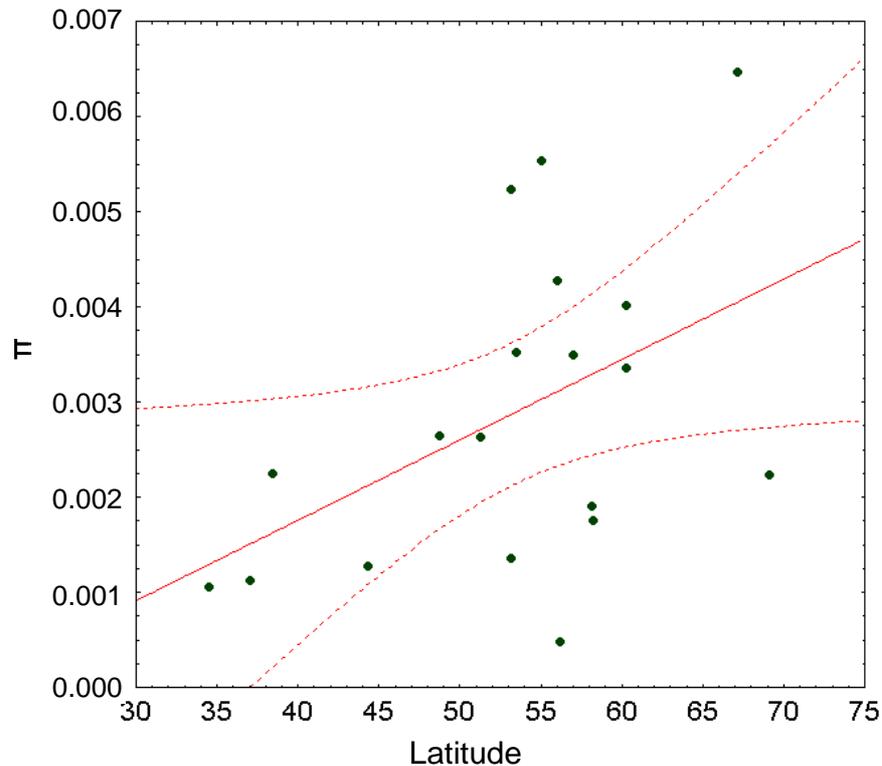


Figure 3.3: Linear regression of nucleotide diversity π against latitude (30°00'N to 75°00'N). Regression equation: $y=0.00008*x-0.0016$, $r=0.473$, $p<0.05$, line: solid; dotted lines indicate 95% confidence interval.

Phylogenetic relationships

The 79 haplotypes identified (Appendix 3.1) were analysed for phylogenetic relationships. When the *C. edule* haplotype set was rooted by an outgroup sequence of *C. glaucum*, the Maximum Likelihood (ML) tree displayed shallow divergence for *C. edule* haplotypes. The sister species showed 18% nucleotide difference between the aligned 529bp COI fragments (Appendix 3.3). As the singleton haplotype “ln2” (from Lisbon site) was closest related to *C. glaucum* (Fig.3.4), a basal position was proposed for the within species tree based on the outgroup rooting.

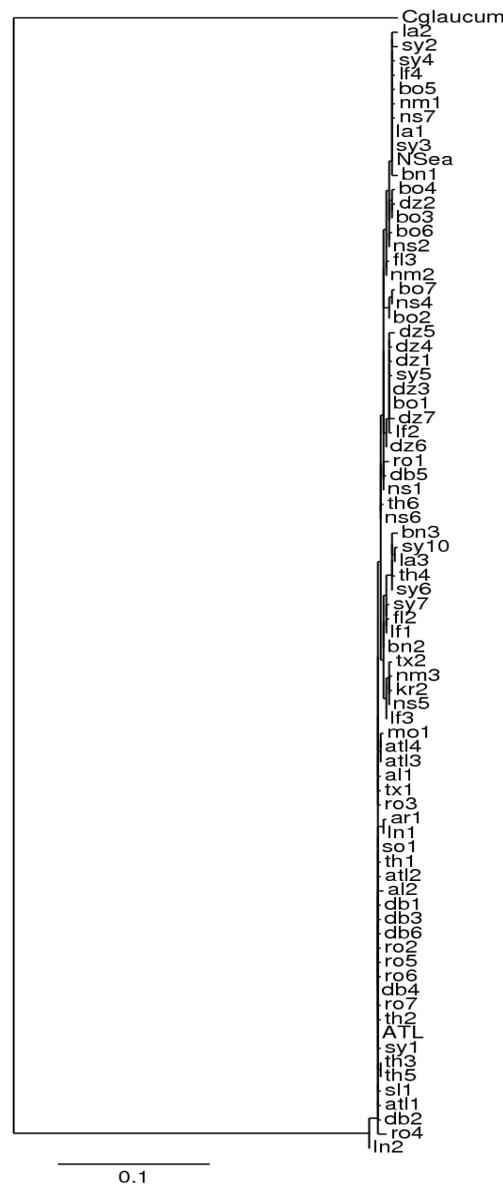


Figure 3.4: Phylogenetic analyses of the 79 *C. edule* haplotypes compared to *C. glaucum*. The outgroup comparison using a 529 bp fragment of COI sequence shows a shallow divergence within *C. edule* haplotypes with a basal placement of the “ln2” and “ATL”.

A comprehensive view of the genetic relationships among *C. edule* haplotypes is given in the ML tree in Fig.3.5, and a separation of a northern and south-western clade is apparent. In more detail, the biogeographical grouping of south-western sites (south-western: MO, AL, LN, AR, RO, TH, DB, SO) versus northern locations (northern: SL, TX, LA, SY, LF, NM, KR, FL, BN, BO, DZ) could be derived (Fig.3.5). However, the characteristic haplotype “ATL” in Atlantic populations differed only in a few nucleotide changes from “NSea” representing northern seas (Fig.3.5+3.6). Noticeable was the closely connected

position of the predominant haplotype at St. Andrews “so1” to the south-western “ATL” haplotype (Fig.3.5).

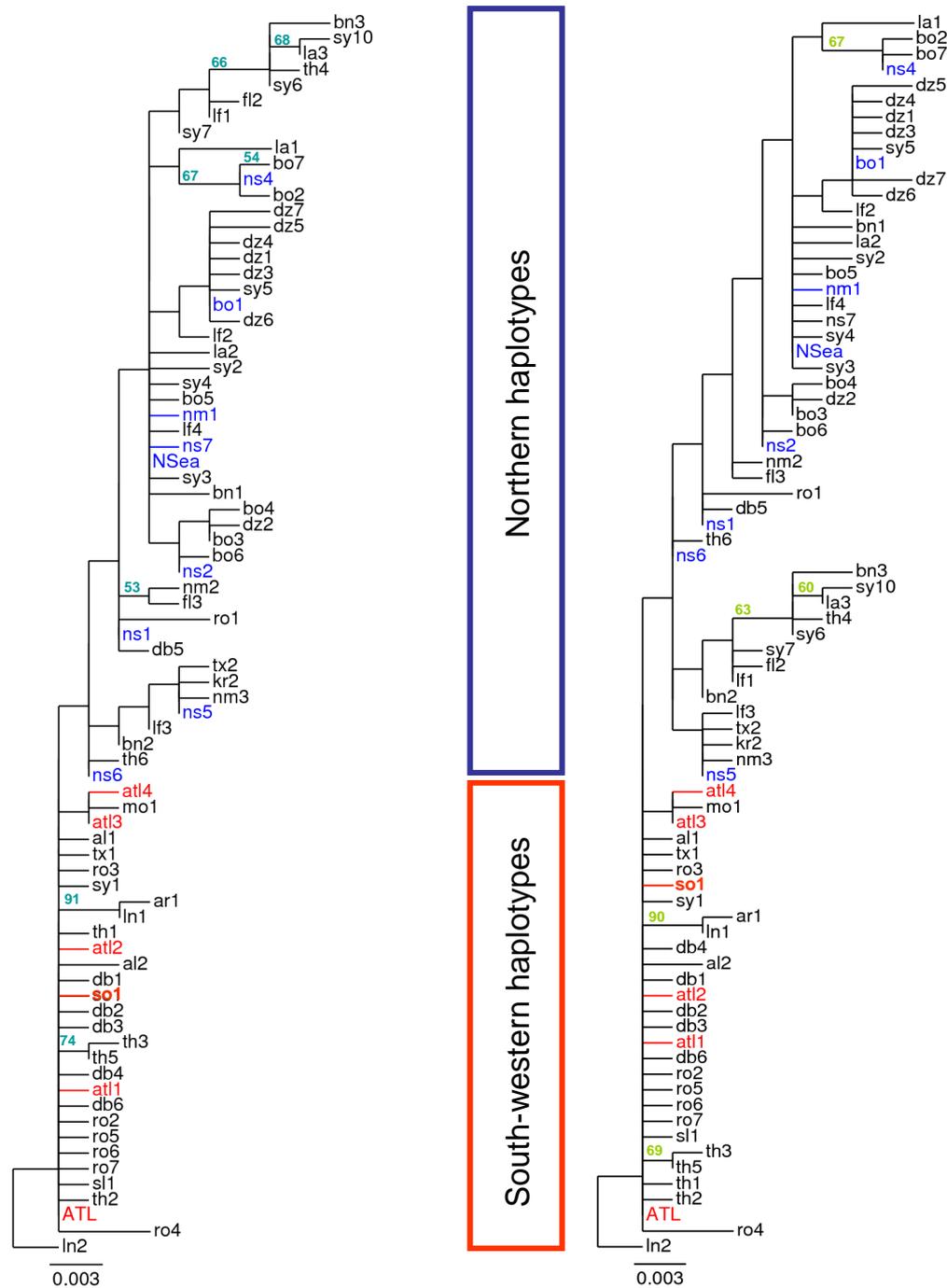


Figure 3.5: Phylogenetic analyses of all *C. edule* haplotypes. Left: ML tree (HKY85). Right: ML tree (K80). Both trees are rooted with “ln2” and display a separation of south-western and northern lineages (only bootstrap values >50 are shown). All haplotypes occurring at more than one site are coloured.

Further insights into the population genetic structure of *C. edule* are provided by the application of a parsimony network (Fig.3.6) which demonstrates the differences between the observed haplotype clusters. The possible mutation steps between haplotypes seem more complex for northern haplotypes than for the south-western ones (Fig.3.6).

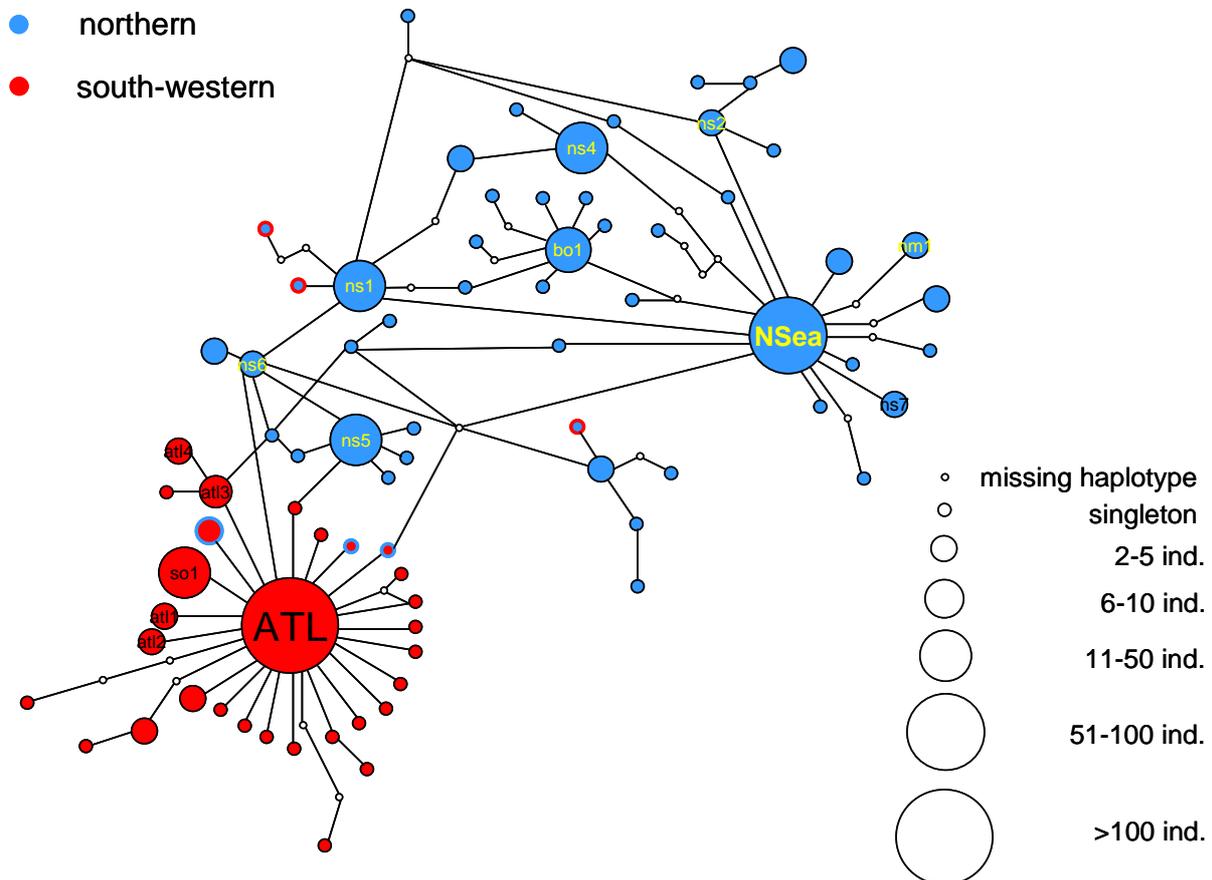


Figure 3.6: Minimum Spanning Network (TCS) for the 79 COI haplotypes of *C. edule*. Haplotypes shared between two or more sites are labelled (cp. Tab.3.2). One line means one nucleotide change. Colours correspond to ML tree. Ringed circles indicate haplotypes placed in the net divergent from geographic origin.

Northern haplotypes show several well connected mini-clusters centering the “NSea” and “bo1”-haplotype which might indicate a developing sub-structure of northern populations. The similarity of haplotypes in the south-western populations correspond to shallow divergence among occurring haplotypes with most sequences differing only by a single base pair from the dominant haplotype “ATL” (Fig.3.6). Here, “ATL” occupies a central position in the parsimony network forming a star-like pattern for the south-western haplotypes (Fig.3.6). The three steps in nucleotide change separating the main haplotypes,

“ATL” and “NSea”, mean a difference of only 0.51 %. Three singleton haplotypes of cockles located in the North Sea populations Texel, Sylt and North Gluss are included in the south-western phylogenetic pattern (blue ringed circles, Fig.3.6). On the other side, single haplotypes of Roscoff and Southend populations probably derived from northern haplotypes (red ringed circles, Fig.3.6).

Geographical mapping of haplotype frequencies shows the dominance of “ATL” (red coloured) and the simultaneous absence of “NSea” (blue coloured) along the southern European coast and the African site (Fig.3.7).

In the south-western sites, a few northern haplotypes were detected in single individuals in Roscoff (RO), Southend (TH) and St. Andrews (SO) (Tab.3.2). The composition of haplotypes in Scotland (SO, St. Andrews) with its lowest diversity (Tab.3.3) and dominance of the “so1”-haplotype (rose coloured) is outstanding. In North Sea, Norwegian Sea and Arctic, cockle populations were characterized by several shared northern haplotypes, while, interestingly, a mix of south-western and northern haplotypes was observed for the Skagerrak populations, KR and FL (Fig.3.7). The “ATL”-haplotype was additionally found in a few northern individuals at North Gluss (SL) and Norsminde (NM). The “NSea”-haplotype dominated the Wadden Sea region, most notably at Texel (TX) and Langeoog (LA), while it was not at all recorded at the Barents Sea site (DZ). Here, the population seemed strongly connected to northern Norway, as the dominating haplotype “bo1” was found in Bodo (BO) and at the Shetland Islands (SL) with a smaller share (Fig.3.7).

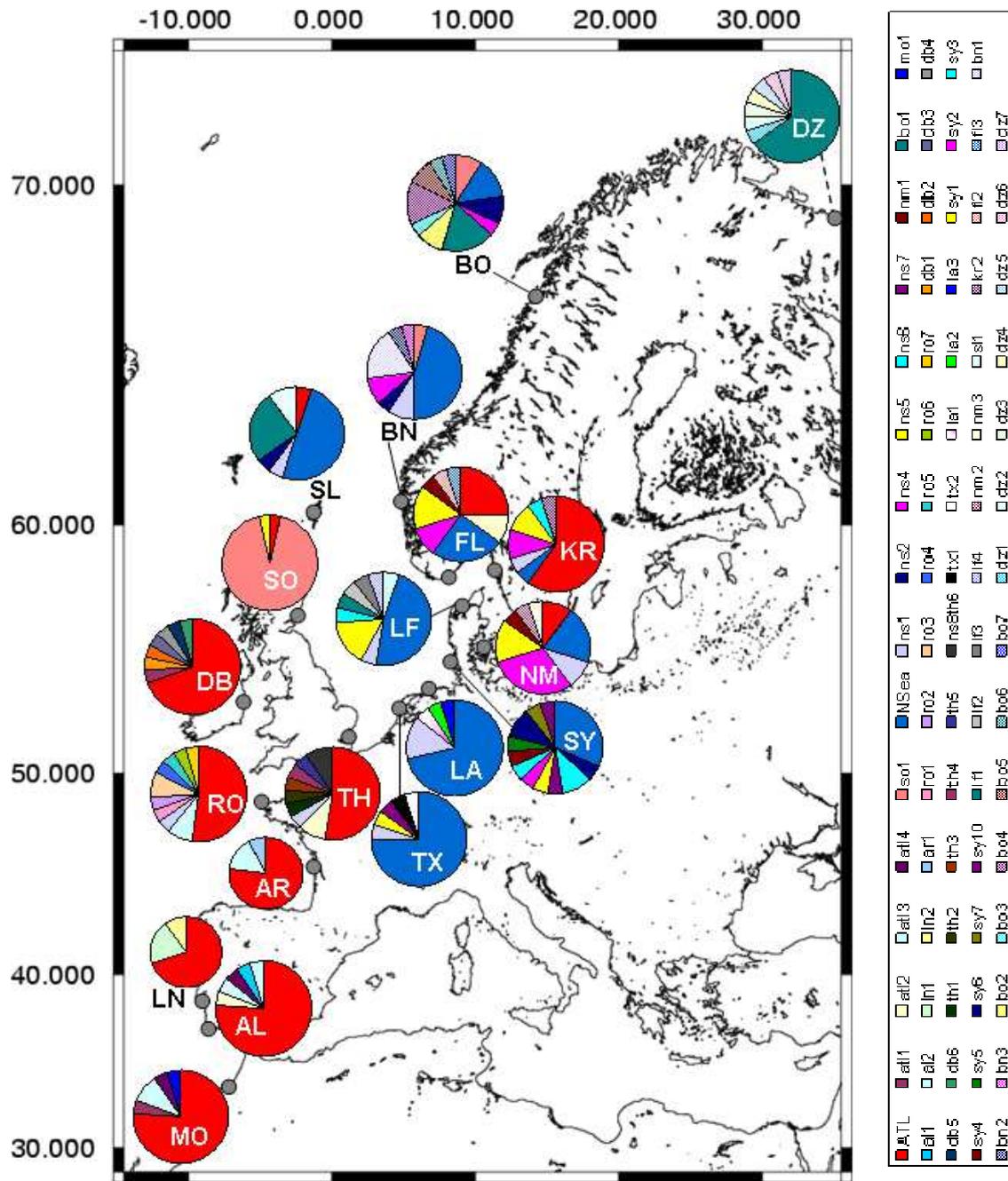


Figure 3.7: COI haplotypes of *C. edule* along the European shoreline. Same colours indicate same haplotypes as labelled in the legend. ATL and atl-types mean haplotypes occurring in the south-western (mainly Atlantic) sites. NSea and ns indicate shared haplotypes of the North Sea and Norwegian Sea area. Haplotype occurrence data are summarized in Appendix 3.4.

Geographic population structure

Phylogenetically proposed separation of haplotypes could be transferred to geographical structure which was supported by a test for differentiation among populations (Appendix 3.5). Furthermore, a significant amount of variation was revealed among the south-western and the northern populations (AMOVA: 43.6 %, $p < 0.001$). Differences between populations within each province contributed 13.1 % to variation. Additional separation of the peculiar sites, SO and DZ, which results in four statistically tested groups, showed the best fit for population division (AMOVA, among groups variation: 48.8 %, $p < 0.001$). Each of these two particular populations contained one haplotype which dominated the population and was shared only with maximum two more sites (Tab.3.2). These populations differed significantly from all other sites.

Further analysis on the spatial structuring of genetic diversity showed a strong clustering of the south-western group (including SO) in contrast to the northern populations (with DZ) by MDS plot set up using none-transformed K2P pairwise genetic distances data after Euclidian distance analysis (Fig.3.8; ANOSIM; Global R: 0.828; $p < 0.01$).

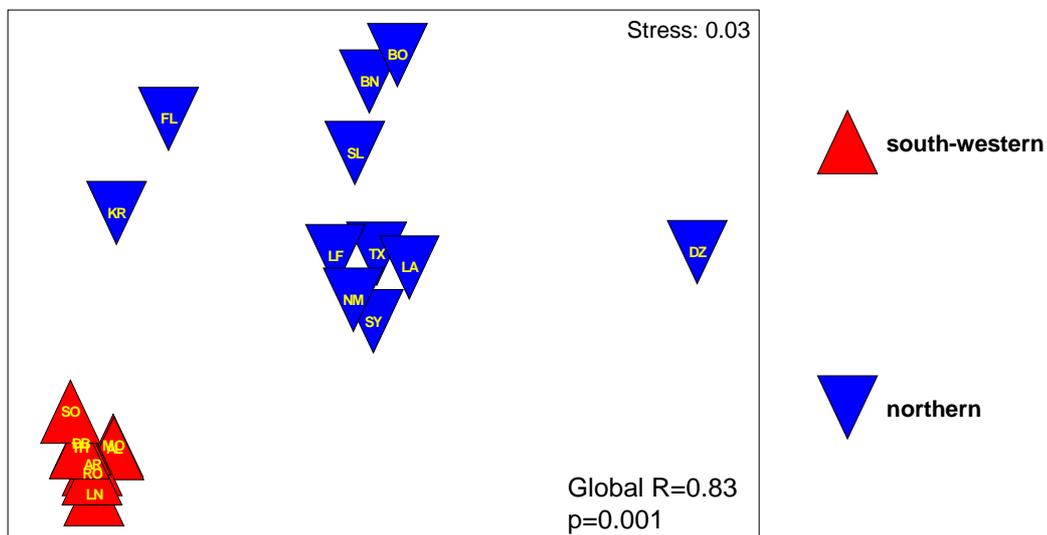


Figure 3.8: Geographic separation of cockle sites. For MDS analysis, inter-population distances were used. Highly significant correlation (Global R=0.83) is shown after applying Euclidian distance analysis for none-transformed pairwise genetic distance data.

For the northern populations, a more detailed grouping is detected (Fig.3.8). It separates a group of the Norwegian Sea (SL, BN and BO) from the Wadden Sea sites TX, LA, and SY, with the Danish sites LF and NM closeby (see Appendix 3.6). The Skagerrak populations KR and FL are more distant from the other northern populations. The outlier DZ, representing the Arctic Murmansk population, is the most different from all the other sites.

When focus lies on the relation of genetic and geographic distances between the 19 sample sites along the north-eastern Atlantic shoreline, a simple matrix correlation shows that about 20 % of the variation in pairwise genetic distances can be attributed to geographic distances between pairs of sites (Mantel test: 0.448, $p < 0.001$ under 5,000 permutations). Repeating the Mantel test with a subset of 11 populations from the northern group, the correlation coefficient increased to 0.62 ($p < 0.05$ with 5,000 permutations). This indicates an explanation that 38 % of variation is due to locations and significant isolation by distance for northern samples, while south-western populations did not show significant spatial relations in the test.

Population demographic history

Neutrality tests (Tajima's D and Fu's F statistics) indicate that the whole dataset is not in line with the expectation of neutral evolution (Tab.3.5). Tajima's D and Fu's F s statistics are negative for the south-western and the northern group and differ significantly from expectations under a neutral model of evolution assuming constant population size (Tab.3.5).

Table 3.5: Neutrality tests and population demographic parameters under a sudden expansion model.

τ : moment estimator of time to expansion; θ : mutation parameters for initial (θ_0) and final populations (θ_1); sw: south-western group; n: northern group.

group	Tajima's D	Tajima's D p	Fu's Fs	Fu's Fs p	τ	θ_0	θ_1
sw	-2.490	$p < 0.001$	-28.957	$p < 0.001$	0.857	0.225	99.999.000
n	-1.954	$p < 0.01$	-26.330	$p < 0.001$	3.562	0.011	9.395
ALL	-2.190	$p < 0.01$	-25.924	$p < 0.001$	3.863	0.000	8.452

However, the northern populations show a negative but non-significant value for Tajima's D (-1.749; $p > 0.05$; $N=184$) when DZ and SY are excluded (not shown). These two populations have the highest negative values within the northern group in Fu's F statistics with -3.862 and -6.693, respectively, and thus deviate strongly from equilibrium.

The neutrality tests (Tab.3.5), as well as the high number of singletons over large parts of the distribution area (Tab.3.3: H_U), indicate a sudden population expansion of the cockle *C. edule* in the recent past. Plotting of mismatch distribution for pairwise nucleotide differences reveals a peak in the number of mismatches (Fig.3.9) which does not fit into the constant population size model but into the growth-decline simulation of populations (Fig.3.9A+B). Testing the growth-decline model for the south-western populations, the curve is clearly unimodal (Fig.3.9C), which supports the assumption of a recent rapid population expansion proposed by the clustering of the southern haplotypes in one star-like pattern (cp. Fig.3.6). An earlier expansion is proposed for the northern population data as the right shifted peak shows (Fig.3.9D).

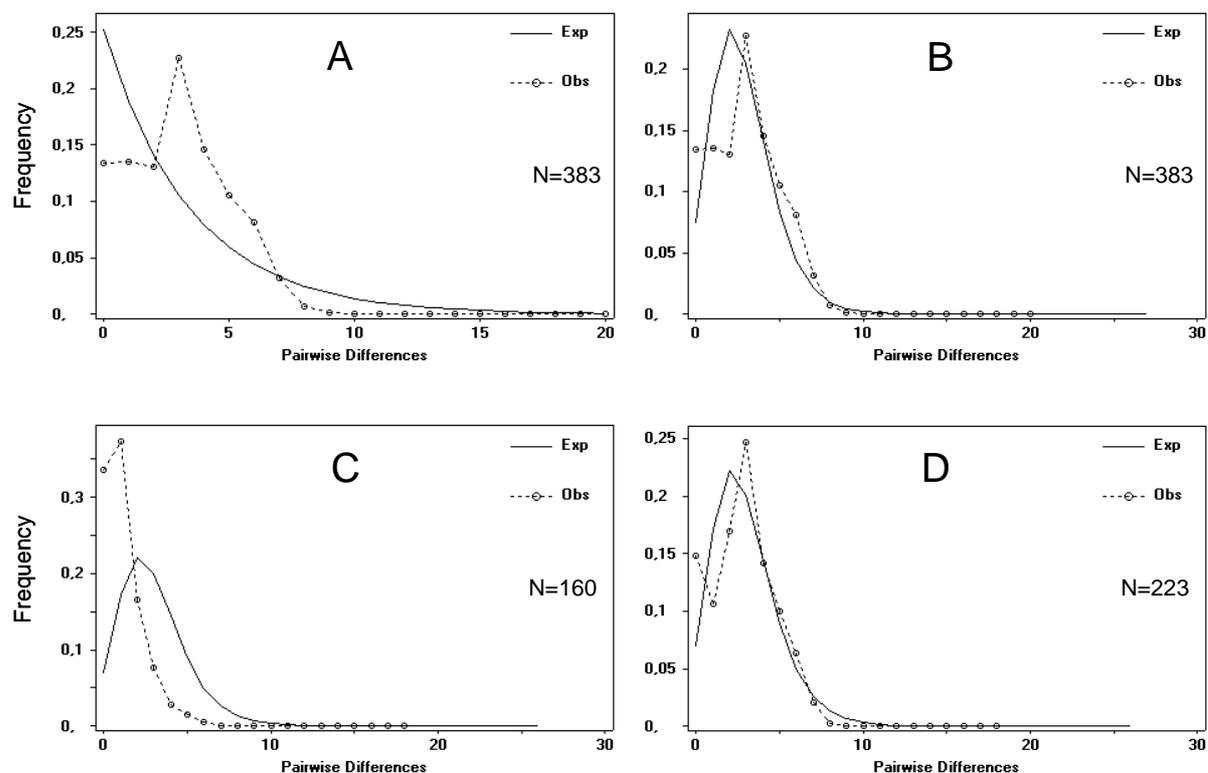


Figure 3.9: Mismatch distribution analyses. Upper panel: all sample sites: A. Constant population size model. B. Population growth-decline model. Lower panel: growth-decline model application for separated geographical groups: C. South-western populations, D. Northern populations. Exp: expected values, Obs: observed values. N: number of individuals.

Noticeably, only the Norsminde population displays a positive Tajima's D that does not significantly deviate from a neutral equilibrium model (0.131; n.s.; N=20), but small sample size may make this test bias. When data of the Skagerrak populations FL and KR are pooled with the NM population data for Tajima's D and mismatch distribution analysis, a non-significant, negative value (-0.401, $p > 0.1$; N=60), and the multimodal topology (Fig.3.10) indicate an equilibrium state of an old population or a potential secondary contact zone.

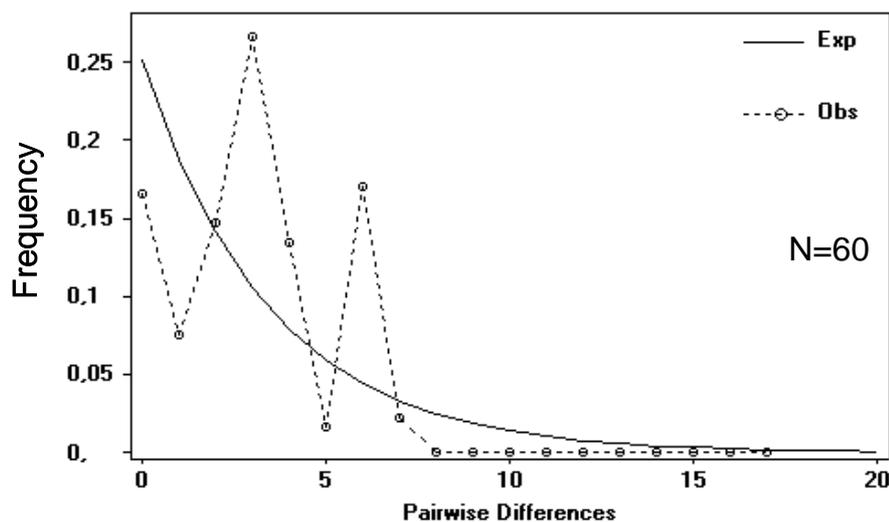


Figure 3.10: Mismatch distribution analysis for the Skagerrak/ Kattegat area. (N=60) Observed and simulated mismatch curves under constant population size.

Demographic parameters under a sudden expansion model are calculated in Arlequin after Schneider & Excoffier (1999) with the distance method for south-western and northern clades (Tab.3.5). When these values are combined with generally assumed mutation rates of invertebrate mtDNA (1.5 to 2 % per million years), the start for an expansion for the south-western group is calculated for a time frame between 0.3 and 0.2 million years before present (BP). As the shift of the mismatch distribution peak along the x-axis indicated (Fig.3.10D), the northern population expansion took place longer ago and is estimated to 1.5 and 1.1 million years BP.

Additionally, divergence time of the sister species *C. edule* and *C. glaucum* (~6 million years BP) was combined with information on current sequence differences (18 %,

Appendix 3.3) for estimation of the mutation rate to provide an impression of the coherence of these time ranges. Thus, the derived mutation rate for each cockle species is 1.5 % per million years. Using this value, the possible expansion time for the south-western group is set at 340,000 years BP considering the within group divergence of maximal 0.51 %. As this value of about 0.5 % nucleotide difference was also estimated for differences between the main haplotypes “ATL” and “NSea”, a divergence of the clades may coincide with the estimated age of the south-western group. For the northern populations, which show a nucleotide divergence of 1.71 % at most within the group, time estimation results in 1.14 million years BP. These datings are similar to the results above which are based on demographic model derived parameters.

Discussion

Genetic diversity and geographic structure of populations were found to be well developed within the bivalve species *C. edule*. The 383 individuals of *C. edule* showed up to 2.2 % nucleotide divergence resulting in 79 haplotypes distributed over the 19 sites. Cockle populations grouped roughly according to biogeographic provinces and showed highest genetic diversity at northern sites. Phylogenetically basal haplotypes occurred in the Kattegat/Skagerrak-region of the North Sea but dominated in the south-western province. Geographic variation along the latitudinal gradient of the Northeast Atlantic could be assigned to some extent to isolation by distance. Divergence and expansion of northern and southern clades were estimated to have happened before the last glacial episode of the Pleistocene.

High genetic diversity in the north

Sample sites of northern European seas showed high haplotype and nucleotide diversity within the mitochondrial DNA marker COI for the European cockle. The distribution range of this bivalve spans mainly from the intertidal of the Arctic Barents Sea coast to the subtropical Atlantic shore of Africa. Highest genetic diversity was expected in southern populations because of a lack of suitable marine coastal habitats in the north during Pleistocene ice ages (Hewitt 2004). The decrease in genetic diversity towards the Arctic

was reported before in studies on several other European species (e.g. bird: Merilä et al. 1996, fish: Bernatchez & Wilson 1996, Gysels et al. 2004). However, correlation of *C. edule* nucleotide diversity to latitude was positive and thus contradicts the common models of genetic diversity trends (Hewitt 2004, Maggs et al. in prep). These models summarized patterns of several marine studies on invertebrates and algae to generalize trends of latitudinal diversity and resulted either in constant or decreasing genetic heterogeneity for populations towards the north. Thus, the hypothesized genetic richness at southern sites has to be rejected for the intertidal cockle.

There are a few examples for this contradicting pattern. High genetic diversity in the north, especially in the eastern Wadden Sea, was recorded for the seagrass species *Zostera marina* and *Z. noltii* (Coyer et al. 2004, Olsen et al. 2004). However, the authors could not point out a plausible reason for this peculiar pattern. The ocean quahog *Arctica islandica* is a subtidal bivalve for which high genetic diversity was found in high latitudes (Dahlgren et al. 2000). In this case, the pattern was assumed to be influenced by recent Holocene warm periods instead of glaciation events when failed recruitment in the southern areas caused local extinctions.

The presence of subtidal populations may also have initiated and maintained high genetic diversity in the north for *C. edule* populations. For example, a subtidal source population is assumed to be responsible for the high portion of unique haplotypes found in the young Murmansk population (DZ) in addition to one dominant haplotype, as a cold winter before sampling reduced the intertidal cockle population after ice formation (E. Genelt-Yanovsky, pers. comm.). However, subtidal cockle beds are only verified from several sites of Atlantic coast up to the East Frisian North Sea coast (Wolff 1983, Dörjes et al. 1986, Bazairi et al. 2003, Cardoso et al. 2006, Sousa et al. 2006). Otherwise, young populations may display a bottleneck effect of recent colonisation (Nei et al. 1975) as shown for *C. edule* at St. Andrews (SO). Characteristic was the absence of private haplotypes and the low genetic diversity as one haplotype occurred in 93 % of the individuals. A potential source population for the recruitment of the Scottish site might be situated in the vicinity or also in the subtidal. The occurrence of haplotypes from Norway may indicate a connection, but Atlantic currents entering the North Sea leave this to speculation.

Low genetic diversity may also be caused by recent extinction events. Thus, present-day genetic pattern in *C. edule* populations of the south-western sites may be connected to human exploitation, as proposed for several southern populations of the common cuttlefish *Sepia officinalis* (Perez-Losada et al. 2007). Cockles are common food in the UK as well as in France, Spain and Portugal. Human-mediated disturbance by dredging (Ferns et al. 2000, Piersma et al. 2001, Leitao & Gaspar 2007) or mixing of individuals for harvesting sites may cause genetic homogenization in these populations. Stock collapses of fished bivalves in the 1990s (Beukema 1993, Beukema & Cadée 1996) may have caused lower genetic diversity in intertidal cockle populations of the Dutch Wadden Sea compared to the North Frisian Wadden Sea.

In contrast to other south-western sites, Roscoff and Southend populations which are located at both sides of the western Channel, showed high haplotype and nucleotide diversity. Studies on some shallow-water invertebrates and seaweeds propose the existence of refugial areas in the English Channel area extending to the tip of Brittany (Provan et al. 2005, Maggs et al. in prep) (Fig.3.11). Recent paleoclimatic dating of the area supports that the English Channel and coastline extending to the present Danish North Sea coast was ice-free during Last Glacial Maximum (Ménot et al. 2006). However, the lowering of the sea level made these areas to innercontinental sites (Fig.3.11).

High genetic indices and recent occurrence of subtidal populations over the whole distribution range suggest that the European cockle *C. edule* may have survived in deep waters in the north during ice ages. These refugial populations might have maintained genetic diversity in the north during and after glaciation for the last million years. This assumption raises the question whether a deep fjord next to the Skagerrak may have provided this refuge during glacial sea level dropping (Fig.3.11). Another refuge may have been next to the tip of Brittany (Fig.3.11). These potential source populations may be responsible for the present distribution of cockle haplotypes.

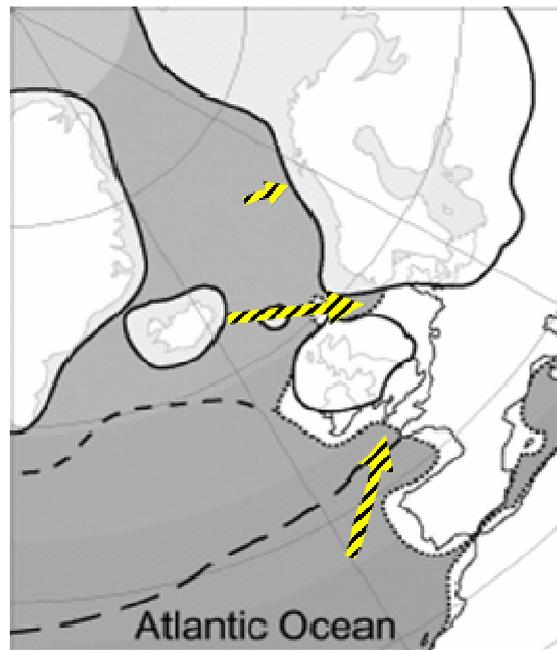


Figure 3.11: Maximum ice sheet extent of the Last Glacial Maximum (LGM) (after Frenzel et al. 1992, modified from Luttikhuizen et al. 2003a). Potential refugia are indicated by arrows. Land ice is shown by transparent surfaces, dotted lines are coastlines during the glacial maximum.

Phylogenetic clades assigned to northern and southern *C. edule* populations

The two sister species *C. edule* and *C. glaucum* showed 18 % nucleotide difference when COI sequences were compared. Similar numbers were gained for other mitochondrial studies on invertebrates. Five closely related shrimp species from Thai waters differed between 5.7 and 18.4 % (Hualkasin et al. 2003). Mayflies showed 18 % divergence between congeneric species and 1 % variation within species (Ball et al. 2005). Hare et al. (2005) showed subspecies of the bivalve *Spisula solidissima* to be diverged with 14 % while within the subspecies a difference of 1-5 % could be detected.

Regional separation of lineages forming a south-western and a northern group were defined by apparent phylogenetic structure of haplotypes within *C. edule*. Preliminary patterns of Amplified Fragment Length Polymorphism (AFLP) analyses point to similar regional affiliation (unpublished data). This finding is consistent with an emerging pattern indicating that several marine organisms may retain significant spatial population structure (e.g. Barber et al. 2002, Taylor & Hellberg 2003, Viard et al. 2006, Luttikhuizen et al.

2008). These structures were found in spite of long distance migration or potential for widespread larval dispersal.

Remarkably, only a few nucleotides segregate the two clades in *C. edule* indicating a rather recent separation or expansion of one of the lineages of about . Here, the star-like pattern of haplotype network in the southern clade contrasts with the intricately branched pattern within the northern sites. It seems likely that the source of southern haplotypes is an expansion out of the northern clade. However, this result would be dissonant with the phylogenetic analyses since “ATL” seems more basal in the phylogenetic analyses as defined by the vicinity to the outgroup species *C. glaucum*. Alternatively, the divergence between the Atlantic and the North Sea clades was reshaped by glacial and interglacial periods. Then, the Atlantic group may have experienced substantial loss in diversity, whereas the North Sea group outlived with high genetic diversity in refugial sites.

Biogeographic population structure resemblance

The biogeographic division of Lusitanian and Boreal region seems to apply roughly for genetic subdivisions of the bivalve *C. edule* along the Northeast Atlantic coast. However, the border is located east of the British Isles for *C. edule*, and not along the western edge of the English Channel as suggested by Briggs (1995) and Spalding et al. (2007) for marine organisms. Phylogeographic separation in Europe next to English Channel has been reported for the shore crab *Carcinus maenas* and the polychaete *Pectenaria koreni* (Roman & Palumbi 2004, Jolly et al. 2005). Stronger phylogenetic separation was shown for populations of the Atlantic and the Mediterranean Sea at the Oran-Almerian front (Dando & Southward 1981, Sanjuan et al. 1996, Daguin & Borsa 2000). However, there were no signs of an Atlantic-Mediterranean divide for e.g. the Norway lobster, *Nephrops norvegicus* (Stamatis et al. 2004). Unfortunately, no cockle population of the Mediterranean Sea could be considered in this study. As the European cockle *C. edule* is still mixed up with its sister species *C. glaucum*, it is not clarified up to now how far in the Mediterranean Sea *C. edule* occurs. An edge population, possibly sympatric with the Lagoon cockle *C. glaucum*, may be expected for the entrance of the Mediterranean, but there are also records of *C. edule* populations of the inner Mediterranean, e.g. in Adriatic waters of Slovenia (De Min & Vio 1997).

In the Baltic-North Sea transition area, the locality-level diversity in *C. edule* populations was rather high which is explained by a co-occurrence of the two biogeographic subgroups at these sites. This was also shown for the sister species *C. glaucum* as an overlap of genetic Baltic and North Sea types (Nikula & Väinölä 2003). In contrast, there are no directly neighbouring populations for *C. edule* which have an overlay zone, but a kind of inversion of biogeographic structure at the Skagerrak sites. This admixture of the two main haplotypes “ATL” and “NSea” is mainly responsible for generating a subcluster within this northern region. Enhanced connection of southern populations from the English Channel and the Irish Sea up to the Skagerrak by North Atlantic currents (Fig.3.12) would support the idea of a secondary contact zone due to present larval connectivity.

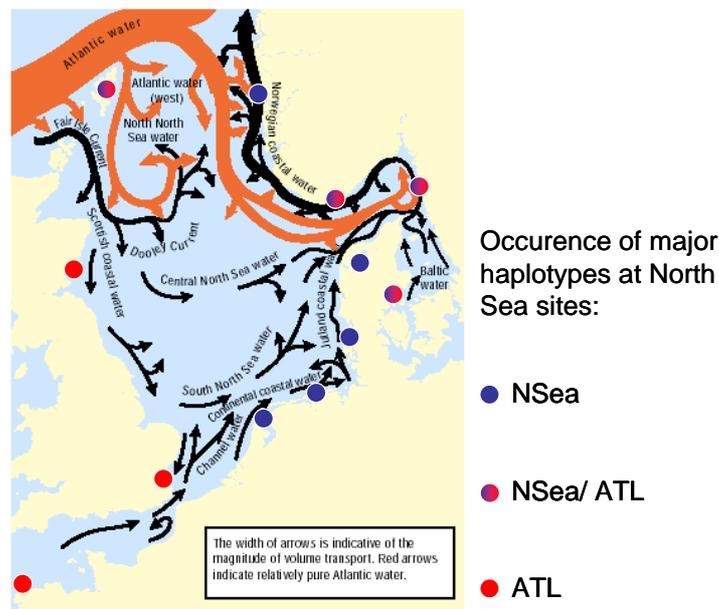


Figure 3.12: Water transport by currents in the North Sea. Larval bivalves drifting several weeks in the water column are assumed to follow the current directions. (Map modified after Turrell et al. 1992)

In the central and northern North Sea, larval dispersal may be retained by the internal counterclockwise, or cyclonic, circulation patterns (see Brown et al. 1999). Near the coast, counter currents may influence recent cockle dispersal in the back of barrier islands resulting in a closer connection of Wadden Sea populations against the Danish sites which are represented by populations sampled in Limfjord and in the Kattegat next to Norsminde. Two Norwegian locations plus the one from Shetland Islands form another subcluster due to highly differentiated haplotypes differing from the southern North Sea populations. The

lack of genetic connection by larval drift between the Channel and the Wadden Sea remains unclear (cp. Fig.3.12) but local gyres may play a role as hydrographic barrier.

In spite of the assumed larval stay of *C. edule* for several weeks in the water column, isolation by distance explains ~40 % of genetic variation for northern region and ~20 % for the whole distribution range of cockles. A closer look at larval ecology of *C. edule* may be necessary, as understanding pelagic larval transport and settlement variations for benthic species is clearly central to the further understanding of migration and gene flow between populations, as well as the evolution of marine invertebrates in general (see Gaines & Bertness 1992, Benzie & Williams 1997, Jessopp & McAllen 2007). The small, dispersing larval phase in marine invertebrate life cycles can control the distribution and abundance of populations of benthic adults with its spatial distribution and supply of propagules (Grosberg & Levitan 1992). Despite the occurrence of high abundance of *C. edule* adults next to the island of Sylt, relatively low densities of cockle larvae were found in a long-term plankton survey (M. Strasser, unpublished data). Thus, different adaptations of cockle larval mobility along the coastal gradient may influence the isolation by distance of populations and putative gene flow (cp. Todd et al. 1998) although Teske et al. (2007) found little effect of planktonic larval performance on connectivity among invertebrate populations along the South African coast.

Demographic history of population subdivision

The bivalve populations of *C. edule* were not in genetic equilibrium when analysed on this large scale from the Barents Sea to the African coast. This may be explained by a combination of dispersal potential and scaling of the survey, whereas less extensive dispersal potential or relatively large spatial scale let nonequilibrium processes prevail (Grosberg & Cunningham 2001). Thus, present-day genetic structure of many marine invertebrates often reflects the operation of both contemporary gene flow and historical factors with no equilibrium throughout their ranges (Grosberg & Cunningham 2001).

The divergence of the northern haplotype group seems to date back to the Early Pleistocene (~1 mio years BP) while expansion for the south-western clade is dated on the Middle Paleolithic (~300 kyears BP). Preglacial phylogeographic subdivisions in other European marine taxa, inferred from different geographical distributions (e.g.: Coyer et al.

2003, Nikula & Väinölä 2003, Provan et al. 2005), point to allopatric divergence as the cause. The current overlap of haplotype groups in the Skagerrak/Kattegat area may then represent a Holocene breakdown of previous isolation creating a secondary contact zone (cp. Jolly et al. 2005). As a point of comparison, similarly diverged mtDNA clades remain almost allopatric in the European bivalve *C. glaucum*, characteristic of enclosed lagoon habitats (Nikula & Väinölä 2003), in contrast to the overlap in *Macoma balthica rubra* that inhabits more open estuarine habitats (Nikula et al. 2007).

In respect to the estimated old age of the northern haplotype group and the low diversity in the south-western populations, the current genetic structure of *C. edule* populations probably originates from refugial areas during Pleistocenic ice ages. In this case, the Kattegat/Skagerrak populations, which displayed an equilibrium state, may have formed a source population not expected that far north. Small northern refugia were already suggested to have existed along the Norwegian coasts (Fig.3.11) (cp. Sutherland 1984, Vorren et al. 1988, Svendsen et al. 2004).

Conclusions and Outlook

The separation of *C. edule* populations into a northern European and a south-western clade seems to be the result of historically separated populations. Due to present current patterns, these groups may recently become more strongly connected. High genetic diversity in the northern group versus low differentiation in the southern populations supports the existence of northern refugia. In contemporary established *C. edule* populations, genetic differentiation may be affected by factors like range compression, habitat loss and human disturbance, which might be more intense in the southern regions. A shift towards the north or local extinctions may be expected along the distribution range with forecasted rising temperature. No definitive explanation for the diversity hot spot in the geologically young Wadden Sea habitat could be found. Studies employing molecular markers with greater resolving power, like AFLPs and microsatellites, may reveal whether the observed pattern of homogeneity in western Wadden Sea versus the high number of private haplotypes in the northern Wadden Sea are a result of varying current systems or maybe different human pressure on the cockle populations.

GENERAL DISCUSSION

European cockle populations exhibit a well structured pattern along the East Atlantic shore on two different levels: associated trematode assemblage composition and bivalve population genetics (CHAPTER 2+3, Fig.D.1). Morphological plasticity of cockle shells varied without a clear large-scale geographical pattern but showed strong relations to local factors (CHAPTER 1). However, the most northern *Cerastoderma edule* population of the Barents Sea (DZ) showed remarkable discrepancy to all other temperate populations in morphometrics and in mitochondrial sequences while parasite communities are unknown for this extraordinary site.

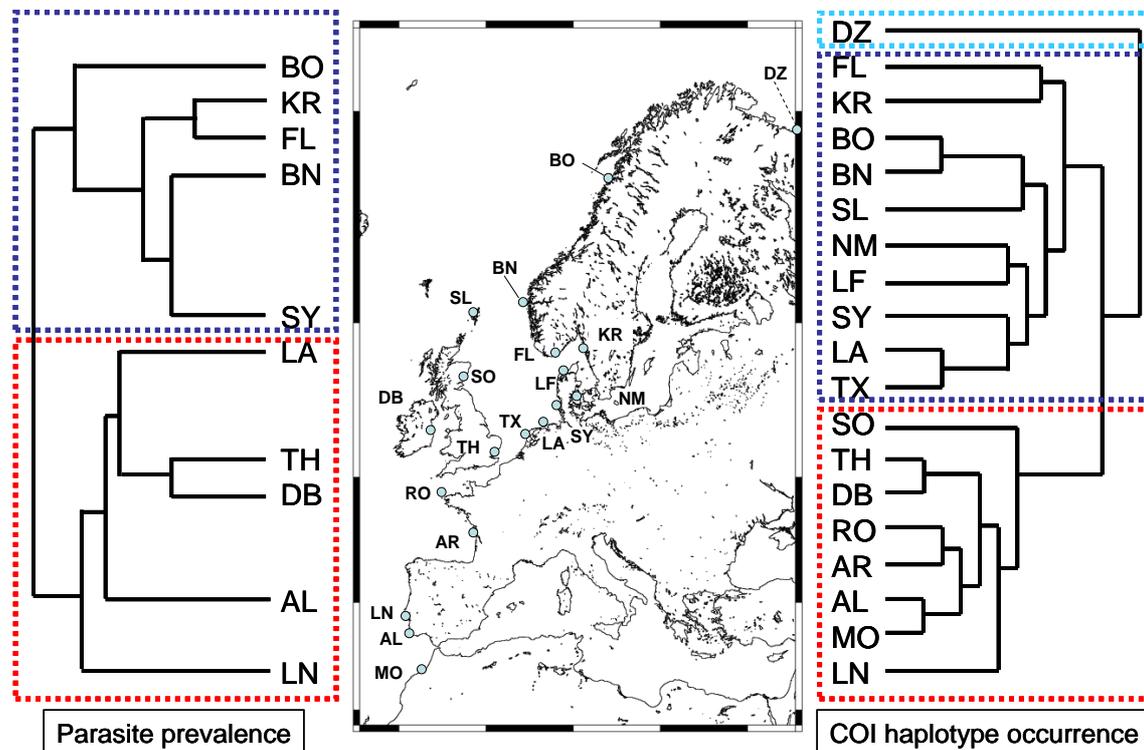


Figure D.1: Clusters of trematode species prevalence and of cockle haplotype occurrence. The geographic pattern along the European coast indicates three distinct groups for genetic subtypes of *C. edule*. Trematode prevalence data give hint on a change in species dominance more easterly within the German Bight (southern North Sea).

Data on the prevalence of trematode species form clusters for a northern and a southern group with a break between the Wadden Sea sites, Langeoog (LA) and Sylt (SY) (Fig.D.1). When only presence and absence of studied parasites is considered, defined marine

biogeographic provinces seem to be reflected, as the Portuguese sites front the more northern data sets (see CHAPTER 2). Both patterns of northern and southern groupings in cockle parasites are possibly caused by the preferential range and dominance of single trematode species. Their specific life cycles are often affected by environmental conditions and co-occurring species. Co-evolutionary processes between host and parasite may play a role for generating regionally distinct groups of host subpopulations but were not yet surveyed sufficiently. Phylogeographic structure of cockle populations was found to be well developed within the bivalve species *C. edule* (Fig.D.1). Again, grouping of the sites roughly resembles biogeographic provinces (sensu Briggs 1995). A specific feature of *C. edule* is the high genetic diversity at Boreal sites which contradicts general assumptions for diversity gradients (see CHAPTER 3). The northern genetic subgroup of *C. edule* populations is assumed to base on northern refugia that may have existed off the Norwegian coast. Low genetic differentiation in south-western populations indicates a postglacial expansion or recent diversity loss.

Local habitat characteristics and large-scale factors along the European coastline

In intertidal areas, marine organisms are known to be exposed to naturally changing stressors. High shell form variability of bivalves is most likely found in such environments. In order to adapt to tidal shallow waters, *C. edule* may have evolved a more tapering, light and laterally compressed shell form which facilitates burrowing (Mariani et al. 2002b). Local differences in shell form may thus be related to a different spectrum of predators, temperature, salinity regimes and tidal influence at the collection sites (Fig.D.2). Predation and parasitism may select for local morphological traits. Thickness of shells may vary with predation pressure (e.g. Dalziel & Boulding 2005). Size variants are also known to be caused by parasitic infections (e.g. Miura & Chiba 2007). Eschweiler et al. (subm.) showed that *Littorina littorea* originating from two distinct locations differ in population size structure due to presence or absence of predators and parasites. Plastic responses to heterogeneous environmental conditions, far from being a nuisance, are one of the most common phenomena characterizing the world (Pigliucci 2005). With changing climate, an increasing number of storms will disturb intertidal populations additionally and lead to a loss of sessile individuals in a population by drift or mortality. Local mean winter temperature and frost events strongly influence the survival of Wadden Sea invertebrates,

depending on shore-level position (Beukema 1985, Loo & Rosenberg 1989, Strasser et al. 2001). However, warm winters may reduce bivalve reproduction and survival rate of cockle spat due to enhanced predation (Strasser 2002, Dare 2004). High summer temperatures have been related to pronounced mortalities among recently spawned (Orton 1933) and heavily parasitized cockles (Jonsson & André 1992, Thieltges 2006b). Predation on cockles may provoke a local population breakdown enforced by human fishing (Beukema 1993, Beukema & Cadée 1996). This human disturbance may affect a loss of genetic diversity in contemporary established *C. edule* populations, which might be more intense in the south-western European regions (Ferns et al. 2000, Piersma et al. 2001, Leitao & Gaspar 2007). More indirectly, and over longer time scales, sediments lose fine silts during dredging events, and this leads to long-term reductions in settlement success in both cockles and baltic tellins (Piersma et al. 2001, Hiddink 2003).

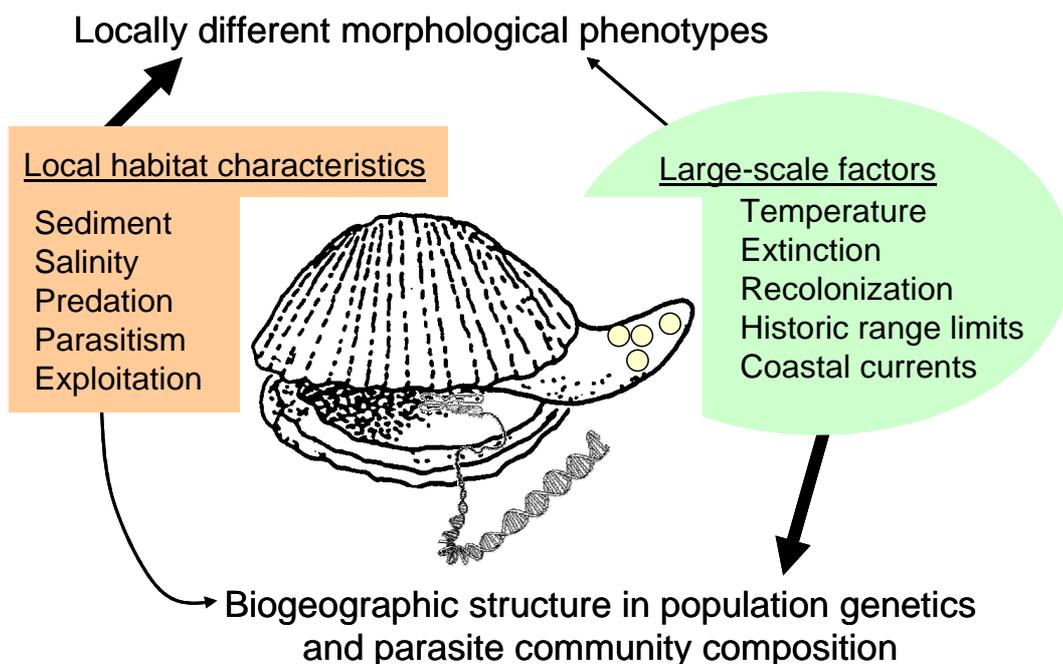


Figure D.2: External factors influencing the patterns surveyed for *C. edule*. Large scale factors have apparently less impact on morphological phenotypes which are mainly shaped by local habitat characteristics. These local factors seem to have less influence on parasite assemblages and genetic clades of cockles which results in biogeographic patterns.

Abiotic factors like temperature and hydrography seem to drive population genetics as well as trematode infection patterns for a latitudinal difference within populations or species assemblages (Fig.D.2). The interaction of climate and the timing of low tide along the

coasts create a complex mosaic of thermal environments, in which northern sites can be more thermally stressful than southern sites (Helmuth et al. 2002, Compton et al. 2007). Population size structure and genetic connectivity of populations are potentially formed by strong seasonal temperature regimes in the Boreal province due to extinction and recolonization events. Historic temperature changes most probably shaped current genetic diversity pattern of this intertidal bivalve *C. edule*. The separation of *C. edule* populations into a northern European and a south-western clade supports the existence of northern refugia in the northern seas during Pleistocene ice ages. Possible contemporary transport pathways of bivalve larvae were investigated in the southern North Sea using the North Sea hydrographic model NORSWAP (Fig.D.3) (Backhaus 1985, Darby & Durance 1989).

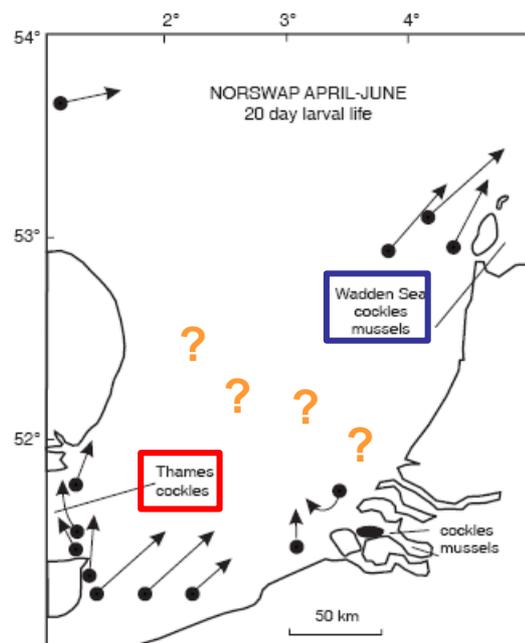


Figure D.3: Potential cryptic barrier east of the English Channel. Dispersal pathways for bivalve larvae predicted by the NORSWAP hydrographic model for a 20-day pelagic phase in the southern bight of the North Sea. Circles denote release points, arrows show length and direction of movement.

The prediction is based on a minimal 20-day pelagic phase; although 30 days or possibly even longer could be a more realistic period for colder waters (Darby & Durance 1989, Dare et al. 2004). According to this drift potential, entrance of southern genotypes into the North Sea should be possible. However, the missing “ATL” haplotypes in the Wadden Sea cockles may be related to local gyres which diminish larval drift from the Thames estuary or other near Channel populations towards the Dutch coast. However, these currents were

unfortunately not considered in the model (Fig.D.3). For southern European areas, larval drift is thought to be shortened with warmer water temperature due to enhanced larval development but high differentiation between sites was not found in *C. edule*.

Biotic factors are also important for the build-up of biogeographic pattern when co-existing species may influence population dynamics. Presence of pathogens e.g. trematodes, which infect host tissues and may completely destroy gonadal tissues, is strongly dependent on the presence of other hosts. As the distribution of host species is temperature dependent, the three-host life cycle was shown to be reduced in high latitude species to omit the free-living larval stage. However, predation on infective stages by non-host organisms may also influence the infection pattern and biogeographic distribution of trematode species. Noticeably, metazoan parasites and their life cycle characteristics are known to be important in determining the structure of a large marine food web (Thompson et al. 2005).

Outlook

Within this thesis study, I uncovered a biogeographic pattern of a coastal invertebrate species and its trematode parasites. The data contribute to improve the knowledge of large scale pattern of marine organisms endowed with pelagic larvae and may provide ideas for further studies on the changing coastal ecosystem.

The extraordinary genetic pattern of the European cockle *C. edule* with indication for a northern glacial refuge may provide a basis for further studies to clarify the influence of Pleistocene ice ages on coastal marine fauna and flora in Europe. These data demonstrate that the demographic histories of species in glaciated landscapes are often more complex and variable than one would intuitively expect. However, the genetic diversity in the geologically young Wadden Sea habitat could not be explained. Analyses of molecular markers with higher resolution power, like AFLPs and microsatellites, may indicate the influence of human exploitation of the coasts or separate larval drift by different coastal current systems. However, the understanding of how genetic discontinuities arise and evolve requires far more retrospective inference on historical biogeographical events as well as spatio-temporal series of population genetic data (cp. Barton & Hewitt 1985).

Studies on physiological characteristics of cockles should be set up to find out whether an adaptation of populations in the warm and cold temperate regions drives the separation of the clades as found at the level of mitochondrial DNA. Regression of similarity against distance may unite several ecological phenomena such as dispersal propensity and environmental structuring, and provides an effective approach for gauging the spatial turnover across sites (Soininen et al. 2007). An overlay of population genetics derived from host and closely associated parasite species may be considered to reveal a phylogeography based relationship, which would indicate a co-speciation of host and parasite with the possible development of specific subspecies. Further observations on coastal species may approve that climate change may not entail a poleward shift in the distribution of intertidal organisms, but instead will cause localized extinctions due to change in microhabitats of each individual species (Helmuth et al. 2002). However, Compton et al. (2007) showed that temperate invertebrate species maintain a broader capacity to survive changes in temperature as their thermal tolerance windows are greater than those of tropical species. Thus, intertidal species as the European cockle *C. edule* may cope well with direct effects of temperature rise, but populations could locally fail due to indirect effects like changes in e.g. habitat structures and co-existing species.

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APPENDIX

Appendix – CHAPTER 1

Appendix 1.1: Classification matrix for all site discriminant function analysis of *Cerastoderma edule*.

Appendix 1.2: Classification matrix for discriminant function of the sympatric sites of *C. edule* and *C. glaucum*

Appendix 1.3: Genetic structure of *C. glaucum* by COI mtDNA indicated on an European map plus unrooted tree

Appendix – CHAPTER 2

Appendix 2.1: Pictures of sample sites: Impressions of the local conditions during sampling

Appendix 2.2: Non-trematode findings of cockle survey

Appendix 2.3: Post hoc test for length variation (Scheffe's test within ANOVA)

Appendix 2.4: Length-trematode correlation. Examples for significance in *Meiogymnophallus minutus*

Appendix 2.5: Trematode species occurring in cockles (with references)

Appendix – CHAPTER 3

Appendix 3.1: 78 variable sites of 79 COI haplotypes of *Cerastoderma edule*.

Appendix 3.2: Variable sites with replacements on the aminoacid level of *C. edule*.

Appendix 3.3: Comparison of COI sequence of *C. glaucum* and *C. edule*.

Appendix 3.4: Frequency of all 79 haplotypes at the 19 sample sites.

Appendix 3.5: Significant F_{ST} values for the 19 sites.

Appendix 3.6: Cluster analysis of K2P pairwise genetic distances.

Appendix 1.1: Classification matrix for all site analysis

Classification matrix for *C. edule* at all 18 sites

Lines: surveyed classification

Columns: expected classification

	Percentage correct	MO p=,08576	AL p=,04460	LN p=,04288	AR p=,04974	RO p=,03774
MO	52.0	26	5	0	2	2
AL	61.5	2	16	0	1	0
LN	60.0	1	0	15	0	0
AR	69.0	2	1	0	20	0
RO	77.3	1	0	0	0	17
TH	71.9	1	0	1	0	0
DB	48.0	0	0	0	1	0
SO	79.2	1	1	1	4	0
SL	60.6	5	2	0	1	1
LA	100.0	0	0	0	0	0
SY	64.6	0	1	0	0	0
LF	40.0	4	0	0	1	0
NM	5.9	2	0	1	1	0
KR	39.1	0	0	2	0	1
FL	8.3	2	2	1	1	0
BN	72.0	0	1	0	0	0
BO	37.5	10	0	0	2	0
DZ	100.0	0	0	0	0	0
ALL	60.7	57	29	21	34	21

	TH p=,05489	DB p=,04288	SO p=,12350	SL p=,12178	LA p=,04288
MO	2	0	1	6	0
AL	0	0	3	2	0
LN	0	0	2	0	0
AR	0	0	0	5	0
RO	0	0	2	2	0
TH	23	0	4	0	0
DB	0	12	5	4	0
SO	4	2	57	2	0
SL	0	5	5	43	1
LA	0	0	0	0	25
SY	2	1	0	4	3
LF	1	0	1	3	2
NM	5	1	5	1	0
KR	1	0	4	0	0
FL	1	0	4	3	0
BN	0	0	1	4	0
BO	0	0	0	3	0
DZ	0	0	0	0	0
ALL	39	21	94	82	31

Appendix 1.1 (continued): Classification matrix for all site analysis**Classification matrix for *C. edule* at all 18 sites**

Lines: surveyed classification

Columns: expected classification

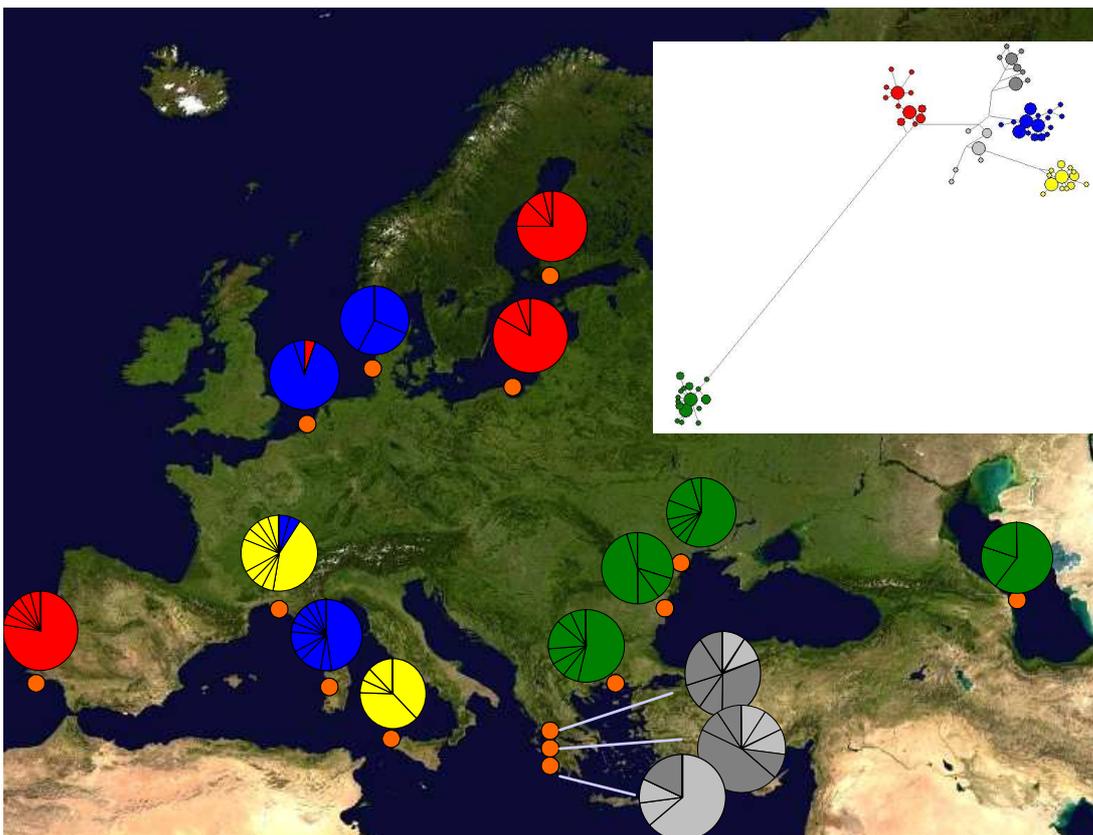
	Percentage correct	SY p=,08233	LF p=,04288	NM p=,02916	KR p=,03945	FL p=,04117
MO	52.0	2	2	0	0	0
AL	61.5	2	0	0	0	0
LN	60.0	2	0	2	1	1
AR	69.0	0	0	0	0	0
RO	77.3	0	0	0	0	0
TH	71.9	2	0	0	1	0
DB	48.0	0	0	0	0	1
SO	79.2	0	0	0	0	0
SL	60.6	2	0	0	0	1
LA	100.0	0	0	0	0	0
SY	64.6	31	4	0	1	0
LF	40.0	2	10	0	0	0
NM	5.9	0	0	1	0	0
KR	39.1	3	0	0	9	0
FL	8.3	4	0	0	1	2
BN	72.0	0	0	0	0	1
BO	37.5	0	0	0	0	0
DZ	100.0	0	0	0	0	0
ALL	60.7	50	16	3	13	6

	BN p=,04288	BO p=,04117	DZ p=,03431
MO	0	2	0
AL	0	0	0
LN	1	0	0
AR	0	1	0
RO	0	0	0
TH	0	0	0
DB	2	0	0
SO	0	0	0
SL	2	3	0
LA	0	0	0
SY	1	0	0
LF	1	0	0
NM	0	0	0
KR	3	0	0
FL	3	0	0
BN	18	0	0
BO	0	9	0
DZ	0	0	20
ALL	31	15	20

Appendix 1.2: Classification matrix for the sympatric sites

Classification matrix for <i>C. edule</i> and <i>C. glaucum</i> at three northern sites							
Lines: surveyed classification							
Columns: expected classification							
	Percentage correct	SYG N=25	SYE N=19	NMG N=17	NME N=9	KRG N=8	KRE N=22
SYG	92.0	23	0	1	1	0	0
SYE	78.9	0	15	1	0	0	3
NMG	82.4	1	0	14	2	0	0
NME	66.7	0	1	1	6	1	0
KRG	37.5	0	0	1	1	3	3
KRE	77.3	0	1	0	0	4	17
all	78.0	24	17	18	10	8	23

Appendix 1.3: Preliminary genetic structure of *C. glaucum* by COI mtDNA (K. Tarnowska, pers. comm.)





Appendix 2.1: Pictures of sample sites. Upper panel: Bergen, Norway; Kristineberg, Sweden. Middle panel: Sylt, Germany; Dublin, Ireland. Lower panel: Lagos (Algarve), Portugal

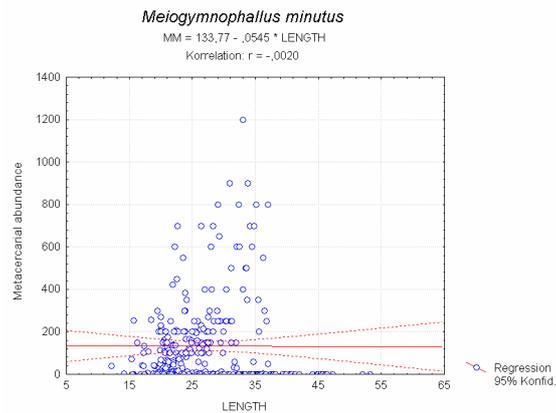
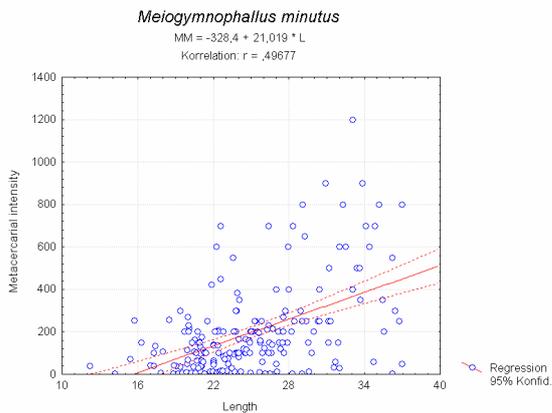
Appendix 2.2: Overview of all non-trematodes found.

site	<i>Mytilicola</i> sp.		Metazoa				Nematodes		<i>Trichodina</i> sp.
	Prev [%]	mean Int ± SD	Prev [%]	mean Int ± SD	Prev [%]	mean Int. ± SD	Prev [%]	mean Int ± SD	Prev [%]
BO							3.3	1	
BN									
FL							3.3	1	
KR							3.3	2	
SY					33.3	1.3 ± 0.7			13.3
LA	3.3	1	90.0	6.9 ± 3.2	17.1	1.2 ± 0.4	26.7	1.5 ± 1.1	43.3
DB					16.7	1.8 ± 1.8	16.7	3.4 ± 5.4	76.7
TH					17.1	1.2 ± 0.4			
LN	20.0	2.2 ± 1.6			3.3	1	6.7	1	3.3
AL					43.0	3.1 ± 2.4			3.3

Appendix 2.3: Post hoc test for length variation.

Scheffe-Test; Variable: LOG_L
coloured differences are significant

	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	
	M=1,4050	M=1,3382	M=1,4746	M=1,3114	M=1,3886	M=1,5188	M=1,3482	M=1,4582	M=1,4310	M=1,6028	
LN {1}			0,2126	0,1621	0,0058	0,9998	0,0001	0,4618	0,5679	0,9936	0,0000
AL {2}			0,2126	0,1621	0,9918	0,6475	0,0000	1,0000	0,0000	0,0068	0,0000
DB {3}			0,0000	0,0000	0,0000	0,0203	0,8052	0,0000	0,9998	0,8189	0,0000
TH {4}			0,0058	0,9918	0,0000	0,0675	0,0000	0,9294	0,0000	0,0000	0,0000
LA {5}			0,9998	0,6475	0,0203	0,0675	0,0000	0,8790	0,1624	0,8432	0,0000
SY {6}			0,0001	0,0000	0,8052	0,0000	0,0000	0,0000	0,3562	0,0153	0,0270
KR {7}			0,4618	1,0000	0,0000	0,9294	0,8790	0,0000	0,0002	0,0322	0,0000
FL {8}			0,5679	0,0000	0,9998	0,1624	0,3562	0,0002	0,9911	0,0000	0,0000
BN {9}			0,9936	0,0068	0,8189	0,0000	0,8432	0,0153	0,0322	0,9911	0,0000
BO {10}			0,0000	0,0000	0,0000	0,0000	0,0270	0,0000	0,0000	0,0000	0,0000



Appendix 2.4: Length-trematode correlation. Examples of length dependence of metacercarial intensity (without zero values) in comparison to all data relation. No other correlation could be detected.

Appendix 2.5: Trematode species occurring in cockles (with references)

Echinostomatids:

- cercariae develop in rediae in gastropods, adults parasitize the intestine of shore birds
- *HIMASTHLA* (Loos-Frank 1967)
 - Little host specificity
 - At least four unidentified European *Himasthla* cercariae, i.e. *C. littorinae obtusata*
- *Himasthla quissetensis*, defined in Woods Hole, America; develops in nassarid snails; occurs in mantle, gills and foot of *Mya arenaria*, *M. edulis*, ... (Miller & Northup 1926, Stunkard 1938, Uzmann 1951), in cockles in foot and byssal gland (Desclaux 2003, Desclaux et al. 2004)
 - 31 head spines!!! All other have 29 head spines
 - Size range: 140 to 190µm in diameter
- *H. elongata*: encysts primarily in the distal portion of the foot musculature of *C. edule*, *M. edulis*, and rarely in other bivalves like *C. lamarcki*, *Mya arenaria* and *Macoma balthica* (Loos-Frank 1967, Werding 1969, Lauckner 1971, Dethlefsen 1972), and *N. diversicolor* (Reimer 1971); =*H. secunda*!
 - final host: e.g. oystercatcher *Haematopus ostralegus*.
 - Cercariae from *Littorina littorea* = *Cercaria proxima* (Werding 1969, Lauckner 1980), miracidia hatch in open water and actively invade snails; density defines *C. edule* infestation intensity (Bowers 1969)
 - Size range: 247.5 ± 8.8µm (Lauckner 1971)
 - Records of adult worms in *Larus* sp. also from Spain and Iceland (Sanmartín et al. 2005, Skirnisson & Galiaktionov 2002)
- *H. continua* prefers the proximal part of the foot
 - *Hydrobia ulvae/ventrosa* as 1st intermediate host (Loos-Frank 1967)
 - Size range: 194.7 ± 5.1µm (Lauckner 1971)

- cercariae exhibit positive phototaxis (Loos-Frank 1967)
- ***H. interrupta*** prefers mantle margin
 - *Hydrobia ulvae* as 1st intermediate host, cercariae exhibit positive phototaxis (Loos-Frank 1967)
 - Size range: 164.2 ± 7.1 μm (Lauckner 1971)
- ***Curtuteria arguinae*** (Desclaux et al. 2006)
 - First and final host are unknown
 - Records from France, Portugal and Morocco

Psilostomatids:

- ***Psilostomum brevicolle***
 - *Hydrobia ulvae* as 1st intermediate host, various shore birds as final hosts (Loos-Frank 1968)
 - Size: 200-230 μm, encysts in digestive gland of *C. edule*, *C. lamarcki* and *M. edulis*; Baltic Sea record (Reimer 1964)
 - = *Cercaria mytili*, recorded in Northumberland (Northeast England and Scotland (Lebour 1911), cercariae also found in *Hydrobia ventrosa* in Iceland (Skirnisson et al. 2004)

Renicolidae:

- ***Renicola roscovita*** (≠ *Cercaria parvicaudata* in Woods Hole *Littorina* spp.)
 - *L. littorea* and *L. saxatilis* (Dietvorst 1972, western WAD) as 1st intermediate, egg has to be swallowed
 - Prefers palps, mantle margin and visceral mass of *C. edule*, *C. lamarcki*, *M. edule* and *Mya arenaria*
 - Final host: gulls, e.g. *Larus argentatus*, in renal tubulus (Werdning 1969)
 - Fjälling et al (1980) recorded high infestation in *M. edulis* from exposed locality at Tjärnö, Sweden

Gymnophallidae (exclusively marine):

- many workers failed to distinguish between cercariae and metacercariae
- distribution and abundance of their metacercariae seem to be controlled by subtle differences in ecological factors (Lauckner 1983)
 - ***G. gibberosus*** occurs in *C. edule*, *C. lamarcki* and *Macoma balthica* (Lauckner 1971, Loos-Frank 1971), 1st int. host: *Macoma balthica*, final host: duck
 - In mantle portion surrounding the insertions of the anterior adductor and siphon retractor muscles on the inner surface of the valves, sometimes in adductor muscle
 - ***Meiogymnophallus minutus***
 - Final host: oystercatcher (Cobbold 1859, first species description)
 - 1st intermediate host: *Scrobicularia plana* (0.3%, James et al. 1977)
 - highly host specific in *C. edule* in South Wales (100% of 1 year olds, Bowers and James 1967); East Frisian coast (50%, 5-50 metacs, Loos-Frank 1971), Sylt (100%, Lauckner 1971), Dutch commercial beds (high, Pistor 1969); recent records include the south down to Morocco (Gam et al. in press)
 - Never found in *C. lamarcki*! But in Mediterranean *C. glaucum* (Bowers et al. 1990)
 - Hyperparasite (microsporan) could be found (Bowers & James 1967, Canning & Nicolas 1974, Goater 1993)
 - ***Meiogymnophallus fossarum***
 - 1st intermediate is *Scrobicularia plana* recorded first in the Mediterranean (Bartoli 1965/1974/1981); there it is abundant and infects *C. glaucum* and *Venerupis aurea* (Bartoli 1973, 1976)
 - 2nd host are also *Tapes*, *Spisula*, *Solen*, *Ensis* and others
 - final host: *Haematopus ostralegus*
 - ***Gymnophallus choledochus*** = '*Cercaria fulbrighti*' (Hutton 1952)
 - Typically with forked-tail cercariae, strictly host-specific; but metacs show weak host specificity (e.g. *Nereis diversicolor*, *Nephtys hombergi* and *Arenicola marina*)
 - Alternative life cycle: motile sporocysts in gonads of *C. edule* produce cercariae during summer, in winter transform into metacs without leaving the sporocysts (Loos-Frank 1969, Russell-Pinto et al. 2006)

- May induce surfacing of cockles and enhance mortality (Lauckner 1983, Thieltges 2006)
- Final hosts: *Larus* spp., *Tadorna tadorna* [Brandgans, shelduck], Anatidae, Limicolidae; gall bladder infection (Odhner 1900, 1906)
- Records from Plymouth (1.2%, Hutton 1952), Wales (0.3%, James & Bowers 1967; 0.1%, Richards et al. 1970), Sylt (7%, Loos-Frank 1969)
- In *C. glaucum*(?): low abundance (Camargue, Bartoli 1974)

Bucephalidae:

- fairly uniform life cycle patterns: sporocyst and cercariae occur in bivalves, metacs live in cysts in various parts of the nervous system or in musculature of teleost fishes, and adults inhabit alimentary tract of predatory fishes
- conspicuous dichotomously branching sporocysts infiltrate every organ except the foot
 - ***Bucephalus (Labratrema) minimus*** = *Cercaria bucephalopsis haimeana* (Pina et al. In press-a)
 - Sporocysts in *C. glaucum*, metacs in gobies, *Pomatoschistus microps*, and smelt, *Atherina mouchon*, adults parasitize in the intestine of bass *Dicentrarchus labrax* (Maillard 1976); plaice *Pleuronectes platessa* (0-group) are highly susceptible to cercarial infection, high mortality possible (Matthews 1973)
 - Early records in *C. edule* in England (2-10%, Lebour 1912), Plymouth (26.4%, Hutton 1952), North Wales (~2%, Cole 1956), Burry estuary in Wales (18%, Hancock & Urquhart 1965; 9.5%, James & Bowers 1967), Arcachon (40.7%, Deltreil & His 1970)
 - Very common in the Mediterranean

Monorchiiidae:

- ***Asymphylogora demeli***; p.692 (within Monorchidae?-stayed open (Cable 1956))
 - encysted in the kidney of *C. glaucum* (Markowski 1936, Reimer 1970, Lauckner 1971, Vaes 1974)
 - Rediae and tail-less cercariae=*Cercariaeum hydrobiae ventrosae* (Hel, Poland; Markowski 1936)
 - Adults in fishes, *Gobius niger* and *Pomatoschistus* spp., in North and Baltic Sea (Reimer 1970, Fonds 1973), but also in intestine of *Nereis diversicolor* (Reimer 1970, Vaes 1974) =natural definitive host!?
- ***Monorchis parvus*** = *Cercaria cerastodermae* I; p.659
 - Cercariae transform into metacercariae within the sporocyst during winter time (oct-apr) probably triggered by temperature change or host's reproductive cycle (Sannia & James 1978, Sannia et al. 1978, Bartoli et al. 2000)
 - Final host: *Diplodus* spp. (Bartoli et al. 2000)
 - Invades the haemocoel, the gonads and the foot of *C. edule*, kills within one year after infection?! (Sannia & James 1978)
 - Infested cockles exhibit growth reduction
 - Reports of infested *C. edule* from Thames estuary (mean prevalence 1.18%, max. 4%, Sannia & James 1978), North Scotland (0.5%, Bowers 1965), England (5.7%/ 2.3%/ 0.07%, Boyden 1969), records also for Sweden (Jonsson & André 1992) and Morocco (Gam et al., in press)
 - Also recorded in *C. lamarcki/glaucum* (0.1%/ 2.4%, Boyden 1969)

Zoogonidae:

- ***Diphtherostomum brusinae***
 - Metacercariae around the palps (often less than 10 individuals)
 - Final host: fish, 1st intermediate host: *Nassarius reticulatus* (Pina et al. in press-b)

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	10	20	30	40	50	60	70
ATL	TTCTGTCGTCGTCTATTGCTTTTCTCGCTTCTGCTTCCGACCCCAAGTACACTTTCCCGGCTCTTATTCCCCTGATTA						
at12	A.....
al1	T.....
at13	C.....
al2	C.....	C.....
at14	C.....	T.....
ar1	A.....	T..T.....
ns1	C.....	C.....
bn1	C.....	AC.....	G.....	C.....
ns4	C.....	C..G.....	A.....	C.....
NSea	C.....	G.....	C.....
bn3	C..G.....	C.....	T.....
so1	A.....
ns2	C..T.....	G.....	C.....
bn2	C.....	C.....
bo1	C.....	G.....	C.....	C..T.....
bo2	C.....	C.....	A.....	C.....
bo3	C..T.....	T.....	G.....	C.....
bo4	C..T.....	T.....	G.....	T.....	C.....
bo5	C.A.....	G.....	C.....
bo6	C..T..C.....	G.....	C.....
bo7	C.....	C.....	C..G.....	A.....	C.....
dz1	C.....	G.....	C.....	C..T.....
dz2	T.....	C..T.....	T.....	G.....	C.....
dz3	C.....	G.....	C.....	C..TA.....
dz5	C.....	G.....	G..C.....	CA.....	T.....
dz6	C.....	C.....	C..T.....
dz4	C.....	G.....	C.....	C.....	C..T.....
dz7	C.....	G.....	GT.....	C.....	C..T.....
db1	A.....
db2	C.....
db3	C.....
db4	T.....
at11	T.....
db5	C.....	T.....	C.....
db6	C.....
ns5	T.....	C.....
fl2	C.....	G.....	C.....	C.....
fl3	C..C.....	T.....	C.....	C.....
nm1	C.....	G.....	G.....	C.....
ns6	C.....
kr2	T.....	G.....	C.....
la1	C.....	G.....	C..A.CAT
la2	T.C.....	G.....	A.....	C.....
la3	C.....	C.....	C.....	C.....
lf4	C.....	G.....	G.....	C.....
lf1	G.....	C.....	C.....
lf2	C.....	GC.....	C.....	C.....
lf3	T.....	C.....	C.....
ln1	T..T.....
ln2	AA.....	C.....
mo1	C.....	C.....
nm2	C..T.....	T.....	C..T.....
nm3	T.....	T.....	C.....
ro1	C.....	T.....	T..C..T.....
ro2	G.....
ro3	T.....
ro4	C.....	T.....	C.....
ro5	A.....
ro6	T.....
ro7	C.....
sl1	T.....
sy2	C.....	G.....	T.....	C..C.....
sy6	C.....	G.....	C.....
sy10	C.....	C.....	G.....	T.....
ns7	C.....	T.....	G.....	C.....
sy1	C.....
sy3	C.....	G.....	C..T.....
sy4	C.....	G..C.....	C.....
sy5	C.....	G.....	C.....	A..C..T.....

Appendix 3.1: 78 variable sites of the 79 COI haplotypes of *C. edule*.

```

sy7 .....C.....G.....C.....C.....
tx1 .....G.....
tx2 .....T.....T.....C.....
th6 .....T.....C.....
th1 .....A.....
th4 .....A...C...G.....C.....
th5 .....A.....
th2 .....C.....
th3 .....A.....T.....

```

Appendix 3.1 (continued): 78 variable sites of the 79 COI haplotypes of *C. edule*.

```

          10          20          30          40          50          60          70
ATL SRPSSWLHNHGLYNIITSHALIIFFIVMPVMIGGFNWLPLILIVPDIHFRLNNISFWFVFNALIL
sol .....
dz2 .....L.....
dz3 .....
db4 .....
kr2 .....
lal .....
la2 .....F.....
lf4 .....
rol .....
th1 .....
th3 .....
th4 .....I.....

          80          90          100          110          120          130          140
ATL LAFSGFVEGGVGAGWTIYPPLTSEFLGDPSMDLAIFALHLGGISSIAASLNFCSTAINIRQSORVHNI
sol .....
dz2 .....
dz3 .....
db4 .....
kr2 .....
lal .....
la2 .....
lf4 .....G.....
rol .....V.....
th1 .....
th3 .....
th4 .....

          150          160          170          180          190
ATL PILPISLAITALLLIITAMPVLAGALTILLDRNFATSFDPVGGDPILFMHLF
sol .....I.....
dz2 .....
dz3 .....Y.....
db4 .....L.....
kr2 .....M.....
lal .....I.H.....
la2 .....
lf4 .....
rol .....S.....
th1 .....T.....
th3 .....S.....
th4 .....

```

Appendix 3.2: Non-neutral variation on the aminoacid level of *C. edule*. There are 12 replacement positions in the 194 fragment length.

```

      10      20      30      40      50      60      70
Cglaui TCTCGTCCGTCCTCTTACTTTCATAATCATGGGCTATACAATCTTATTATTACAAGGCACGCTTTGATTA
ln2    ..C..C..T..T..A..T..G.....T.....C..C.....T.....A...
ATL    ..C..C..T..T..A..T..G.....T.....C..C.....T.....A...
NSea   ..C..C..T..T..A..T..G.....T.....C..C.....T.....C..A...
la2    ..C..C..T..T..A..T..G.....T.....T.....C..C.....T.....C..A...

      80      90      100     110     120     130     140
Cglaui TAATTTTCTTCATAGTGATGCCAGTGATAATGGGTGGTTTTGGTAATTGGTTAGTTCGGTTAATATTGAT
ln2    .....T.....G..T..G..A..A..G.....C.....G..A..T.....C..A..
ATL    .....T.....G..T..G..A..A..G.....C.....G..A..T.....C..A..
NSea   .....T.....G..T..G..A..A..G.....C.....G..A..T.....C..A..
la2    .....T.....G..T..G..A..A..G.....C.....G..A..T.....C..A..

      150     160     170     180     190     200     210
Cglaui GGTACCTGACATACACTTCCCCGGTTAAATAACATAAGATTCTGGTTTGTCCAAATGCTCTAATCCTA
ln2    A..C.....T..T...T.....T.....T.....C.....T..TT..
ATL    A..C.....T..T...T.....T.....T.....C.....T..TT..
NSea   A..C.....T..T...T.....T.....T.....C.....T..TT..
la2    A..C.....T..T...T.....T.....T.....C.....T..TT..

      220     230     240     250     260     270     280
Cglaui TTAGCATTTTCTGGGTTCTAGAAAGGTGGTGTGGGGCTGGTTGAACATTTTACCCGCCTTTAACTTCGA
ln2    C...G...C..C..T..G...G..G..A..C.....C.....A..AC.C...A.
ATL    C...G...C..C..T..G...G..G..A..C.....C.....A..AC.C...A.
NSea   C...G...G..C..T..G...G..G..A..C.....C.....A..AC.C...A.
la2    C...G...G..C..T..G...G..G..A..C.....C.....A..AC.C...A.

      290     300     310     320     330     340     350
Cglaui TTGAATTCTTGGGGGACCCCTTCAATGGACTTGGCTATTTTTCGCTGCACCTAGGGGGAATTTCTTCTAT
ln2    .....T.....T...G..C...T.....CT.A..T.....T.....
ATL    .....T.....T...G..C...T.....CT.A..T.....T.....
NSea   .....T.....T...G..C...T.....CT.A..T.....T.....
la2    .....T.....T...A..C...T.....CT.A..T.....T.....

      360     370     380     390     400     410     420
Cglaui TGCGGCAAGTCTGAATTTCTGTTCTGACTGCTATTAAATATGCGACAGAGGCAACGATGGGTTCACAAAATC
ln2    .....C..G..A..C..T...A..C...C..C..A..C..A...G..G...
ATL    .....C..G..A..C..T...A..C...C..C..A..C..A...G..G...
NSea   .....C..G..A..C..T...A..C...C..C..A..C..A...G..G...
la2    .....C..G..A..C..T...A..C...C..C..A..C..A...G..G...

      430     440     450     460     470     480     490
Cglaui CCAATACTACCGATTTCCTTAGCAATTACAGCTCTTTTATTGATTATTGCTATGCCGGTTCCTGGCTGGAG
ln2    ..T.....T...T...T.....C..AC.....C..A.....A.....T.
ATL    ..T.....T...T...T.....C..AC.....C..G.....G.....T.
NSea   ..T.....T...T...T.....C..AC.....C..G.....G.....T.
la2    ..T.....T...T...T.....C..AC.....C..G.....G.....T.

      500     510     520
Cglaui CTTTAACTATGCTTCTTAGATCGAAATTTTGCTACAA
ln2    .GC.T...A..C.....T.....
ATL    .GC.T...A..C...T.....T.....
NSea   .GC.T...A..C...T.....C..T.....
la2    .GC.T...A..C...T.....C..T.....

```

Appendix 3.3: Comparison of COI sequence of *C. glaucum* and *C. edule* (below). There are 93-96 differing nucleotide positions in 529 bp fragment length.

Appendix 3.4: Frequency of all 79 haplotypes at the 19 sample sites.

sample code	N	ATL	at1	at2	at3	at4	so1	NSea	ns1	ns2	ns4	ns5	ns6	ns7	bo1	nm1	mo1
MO	21	16	1	-	2	1	-	-	-	-	-	-	-	-	-	-	1
AL	21	16	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-
LN	10	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AR	12	9	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
RO	20	12	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-
DB	23	16	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH	21	11	-	2	-	-	-	-	1	-	-	-	-	-	-	-	-
TX	20	-	-	-	-	-	-	15	1	-	-	1	-	1	-	-	-
LA	21	-	-	-	-	-	-	15	3	-	-	-	-	-	-	-	-
SY	16	-	-	-	-	-	-	6	-	1	-	-	2	1	-	-	-
LF	19	-	-	-	1	-	-	9	1	-	-	3	1	-	-	-	-
NM	20	2	-	-	-	-	-	4	2	-	6	3	-	-	-	1	-
KR	20	11	-	1	-	-	-	1	1	-	1	2	1	-	-	-	-
SO	20	1	-	-	-	-	17	-	-	-	-	1	-	-	-	-	-
SL	20	1	-	-	-	-	-	10	1	1	-	-	-	-	5	-	-
FL	20	5	-	2	-	-	-	5	-	-	2	3	-	-	-	1	-
BN	22	-	-	-	-	-	1	10	2	1	2	-	-	-	-	-	-
BO	22	-	-	-	-	-	2	3	-	2	1	-	-	-	4	-	-
DZ	20	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-
sample code	N	al1	al2	ln1	ln2	ar1	ro1	ro2	ro3	ro4	ro5	ro6	ro7	db1	db2	db3	db4
MO	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AL	21	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LN	10	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-
AR	6	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
RO	20	-	-	-	-	-	1	1	2	1	1	1	1	-	-	-	-
DB	23	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1
TH	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TX	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LA	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SY	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LF	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NM	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FL	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BN	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BO	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DZ	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX

sample code	N	db5	db6	th1	th2	th3	th4	th5	th6	tx1	tx2	la1	la2	la3	sy1	sy2	sy3
MO	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AL	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LN	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AR	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DB	23	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH	21	-	-	1	1	1	1	1	2	-	-	-	-	-	-	-	-
TX	20	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
LA	17	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-
SY	11	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1
LF	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NM	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FL	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BN	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BO	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DZ	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

sample code	N	sy4	sy5	sy6	sy7	lf1	lf2	lf3	lf4	nm2	nm3	kr2	sl1	fl2	fl3	bn1	bn2
MO	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AL	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LN	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AR	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DB	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TX	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LA	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SY	11	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
LF	14	-	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-
NM	20	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-
KR	20	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
SO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL	20	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
FL	20	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-
BN	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1
BO	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DZ	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

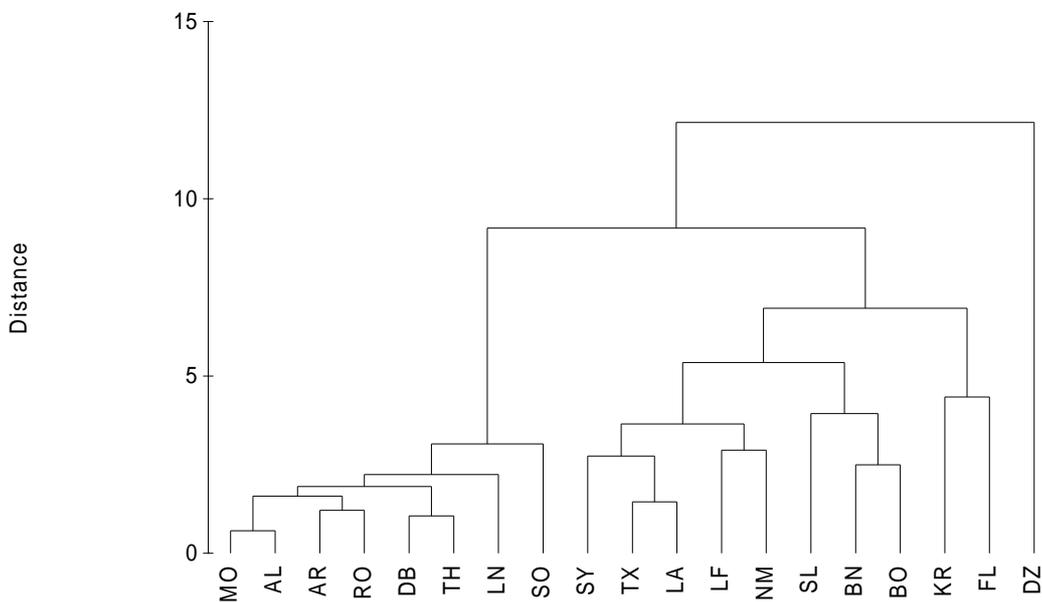
sample code	N	bn3	bo2	bo3	bo4	bo5	bo6	bo7	dz1	dz2	dz3	dz4	dz5	dz6	dz7
MO	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AL	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LN	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AR	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DB	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TH	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TX	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LA	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SY	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LF	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NM	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KR	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SO	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FL	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BN	20	1	-	-	-	-	-	-	-	-	-	-	-	-	-
BO	22	-	2	1	3	2	1	1	-	-	-	-	-	-	-
DZ	20	-	-	-	-	-	-	-	1	1	1	1	1	1	1

Appendix 3.5: Significant differences in F_{ST} values for the 19 populations. The strong connectivity of the south-western sites is marked by non-significant differences in F_{ST} values. The outstanding position of SO and DZ is shown by significant differences from all other sites. Significance level was Bonferroni-corrected for multiple comparisons ($p < 0.003$). Proposed geographical groups were indicated by coloured site codes (red: south-western, blue: northern).

Matrix of significant F_{ST} P values
 Significance Level=0.0030

 Number of permutations : 506

Label	Population name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1:	MO	1	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
2:	AL	2	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
3:	LN	3	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
4:	AR	4	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
5:	RO	5	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
6:	DB	6	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
7:	TH	7	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
8:	TX	8	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
9:	LA	9	+	+	+	+	+	+	-	-	-	-	+	+	+	-	+	-	-	+
10:	SY	10	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
11:	LF	11	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
12:	NM	12	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+
13:	KR	13	+	+	-	-	-	-	+	+	+	+	-	+	+	-	-	+	+	+
14:	SO	14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15:	SL	15	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-	+
16:	FL	16	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+
17:	BN	17	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	+
18:	BO	18	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	-	-	+
19:	DZ	19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+



Appendix 3.6: Hierarchical cluster analysis of K2P pairwise genetic distances. Grouping of south-western and northern sites is supported but the Russian site DZ is placed out of the northern clade. Differentiation within the northern group indicates three smaller clusters.

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Erklärung

List, den 27.05.2008

Hiermit versichere ich, dass diese Abhandlung – abgesehen von der Beratung durch meine akademischen Lehrer – nach Inhalt und Form meine eigene Arbeit ist und dass ich keine anderen als die angegebenen Hilfsmittel und Quellen verwendet habe. Die Arbeit hat bisher weder ganz noch zum Teil an anderer Stelle im Rahmen eines Prüfungsverfahrens vorgelegen.

Manuela Krakau