What is natural?

The scale and consequences of marine bioinvasions in the North Atlantic Ocean

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1

The scale and consequences of marine bioinvasions: General introduction and thesis outline

Deniz Haydar

1

Introduction

Ecosystems are constantly changing: species diversity and community assembly change due to species additions and deletions, populations of single species fluctuate in size and genetic diversity and changing circumstances may cause spread or retreat of species and communities. This also holds for marine benthic ecosystems. The geographical distributions of marine benthic flora and fauna are determined by species-specific tolerance to environmental factors, habitat preferences and natural dispersal potential. Species distributions have therefore changed over geological time as land masses and marine basins appeared and disappeared, and temperatures and sea-levels fluctuated. The resulting modern-day distributions of species are still subject to change.

Changing distributions and diversity patterns of marine communities are natural phenomena, but may also be inflicted by man through the introduction of non-indigenous species. **The extent to which natural patterns of diversity and natural distributions in coastal waters have been affected by anthropogenic species introductions as a result of human activities, such as trans-oceanic shipping and transfers of shellfish from one region to another, is the subject of this thesis.**

Based on current species distributions, species assemblages, degree of endemism, physical conditions and the presence of geographical boundaries to spread, biogeographic regions or provinces have been defined by various authors (Ekman 1953; Briggs 1974; Vermeij 1978; Spalding *et al.* 2007). In this thesis the main realms of concern are the temperate and Arctic North Atlantic Ocean (Fig. 1.1).

Invasion biology

The early naturalists and biologists already noted the possibility of translocation of species outside their native range by human activities (e.g. Darwin in "the Origin of Species", see Ludsin & Wolfe 2001). However, "invasion biology" as a field is relatively young; the impact of biological invasions has only been acknowledged since the 1950s (Elton 1958), and invasions in the sea have only received widespread attention since the 1980s after publication of the first regional overview of introduced species in coastal waters (Carlton 1979). Since then, the scientific and public interest in marine bioinvasions has increased substantially, and biological invasions are now acknowledged to be an important component of global change (Carlton 2000; Occhipinti Ambrogi & Savini 2003; Harley *et al.* 2006) and globalization (Sax & Gaines 2003; Ehrenfeld 2005), and are considered to be a major threat to coastal marine biodiversity (Chapin *et al.* 2000; Bax *et al.* 2003; Molnar *et al.* 2008).

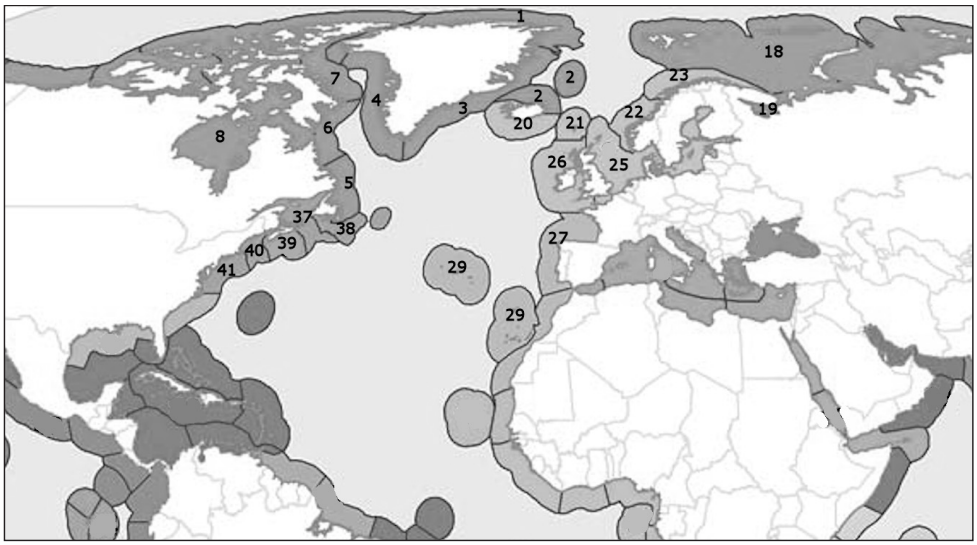


Figure 1.1 The marine ecoregions of main interest in this thesis: *Arctic* - 1. North Greenland, 2. North and East Iceland, 3. East Greenland Shelf, 4. West Greenland Shelf, 5. Northern Grand Banks–Southern Labrador, 6. Northern Labrador, 7. Baffin Bay–Davis Strait, 8. Hudson Complex, 18. North and East Barents Sea, 19. White Sea. *Northern European Seas* - 20. South and West Iceland, 21. Faroe Plateau, 22. Southern Norway, 23. Northern Norway and Finnmark, 25. North Sea, 26. Celtic Seas. *Lusitanian province* - 27. South European Atlantic Shelf, 29. Azores, Canaries, Madeira. *Cold Temperate Northwest Atlantic* - 37. Gulf of St. Lawrence–Eastern Scotian Shelf, 38. Southern Grand Banks–South Newfoundland, 39. Scotian Shelf, 40. Gulf of Maine/Bay of Fundy, 41. Virginian region. Redrawn from Spalding *et al.* (2007).

Terminology

In the literature, many denotations for introduced species are used, some of these being aliens, exotics, invaders, non-indigenous species, immigrants, translocated species, naturalized species and adventives. The definition of invasive species varies among authors, taxonomic groups and geographic regions (Richardson *et al.* 2000; Carlton 2002). The term "invasion" is being used for the natural process of range expansion by natural dispersal in geological time (Briggs 2000). More commonly, invasive species are considered by many to be introduced species that have established self-sustaining populations, have undergone rapid spread, and have negative ecological or economic impact. However, "invasive" is also used for species with an unknown impact, which is the majority of introduced species. In this thesis I use the terms invasive, non-indigenous, non-native, introduced and exotic species as alternative terms to indicate those species introduced by humans to a biogeographic region or province outside their natural range.

Invasion process in seven steps

A successful biological invasion-event can be simplified to a seven-step model (Fig. 1.2). At a certain point in time, in the donor region a species pool of a number of species is present. Some of these species may actually interact with a vector: the physical means or agent by which a species is transported to another biogeographic region. Not all species are potentially transported by a vector, only those that have certain habitat preferences or life-history traits that enable them to be taken up will actually interact with a vector (1). After uptake by a vector (2), a species will have to survive transport (3). Surviving transport depends on species characteristics as well as vector characteristics (e.g. duration of transport, physical conditions, interaction with other species). After successful transport, a species will have to be released (e.g. by crawling or swimming away, or by releasing gametes or larvae) in the recipient region (4). In the recipient region, the environmental variables have to allow the species to survive (5) (e.g. presence of hard substrates to settle on, salinity, availability of food). Starting a new population from a single individual is not always possible, and in order to establish an initial population (6) the presence of other individuals of the same species is often necessary. For long-term establishment (7) competition over resources and space with native species is an important factor, and some species are capable of outcompeting native species and may spread and develop into a pest or nuisance. A lag-phase between initial establishment and population growth is common (Mack *et al.* 2000), and can explain the delayed observation of novel species after the initial introduction event.

This invasion process has a number of steps that have to be overcome by a number of individuals in order to secure establishment, and therefore not all introductions are successful. Of the successfully established introductions, not all develop into a pest (Williamson & Fitter 1996; Parker *et al.* 1999).

Determinants of the success of introductions

The success-rate of introductions is dependent on species characteristics, vector characteristics and characteristics of the recipient region. Introductions are more successful in disturbed environments (Cohen & Carlton 1998; Occhipinti Ambrogi & Savini 2003), and the presence of already established introductions may facilitate the establishment of newly introduced species, increase the magnitude of impact and potentially result in an increasing rate of introductions; known as 'invasional meltdown' (Simberloff & Von Holle 1999). Accumulation of introduced species in coastal systems may also transform historical low-impact introductions into rapidly expanding pests (Grosholz 2005). The relationship between diversity and invasibility of the recipient region is debated. The classical view is that more diverse communities become invaded less easily because of biotic resistance, but this is not always demonstrated in experimental studies (Levine & D'Antonio 1999). Revealing the mechanisms behind biotic resistance of communities is one of the challenges in invasion biology (Britton-Simmons 2006).

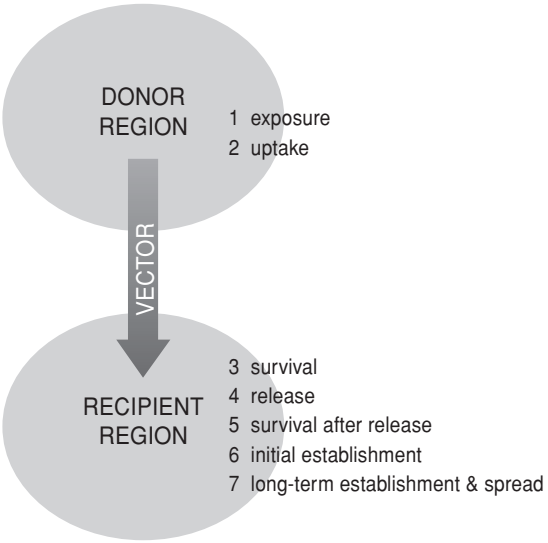


Figure 1.2 The invasion process in seven steps. See text for explanation.

Many studies have tried to link species characteristics to successful introductions, but these efforts do not result in a general set of biological characteristics that are applicable to all successful invaders (Heger & Trepl 2003). Instead, they vary across taxonomic groups, which makes it hardly possible to predict future invasions based on biological characteristics of species. Physiological tolerance, niche breadth and fecundity are critical, but by themselves are inaccurate predictors of successful introductions (Sakai *et al.* 2001; Hayes & Barry 2008). The only accurate species-level predictors of the success of introductions are the degree of climate and habitat matching of the donor and recipient regions, and a history of successful introductions in other parts of the world (Hayes & Barry 2008). This has lead to the creation of 'worst invaders' lists, e.g. the 100 worst invaders (Global Invasive Species Database 2005), and the development of rapid (molecular) tools to detect (propagules of) these unwanted species in an early stage of the invasion process, preferably before release by a vector, in order to prevent establishment (e.g. Harvey *et al.* 2009).

A vector characteristic that is a good determinant of the success of introductions, is the number of individuals that is released, which is positively correlated with the probability of establishment (Hayes & Barry 2008). Coupled with this, higher genetic diversity of the introduced species also increases colonization success, possibly due to the fact that a larger number of genotypes makes adaptation to local circumstances easier (Crawford & Whitney 2010).

In summary, the success rate of introductions is dependent on donor- and recipient region characteristics, taxonomic group, species characteristics and vector characteristics, which may all change over time (Carlton 1996b), and because of this complexity, the establishment and potential impact of introductions are highly unpredictable.

Vectors

Characteristics of the vector by which species are introduced, such as numbers of individuals released, the type of community transported and the pathway of introduction, are determinants of the success of introductions and can explain patterns in changing diversities and distributions. In Table 1.1, the predominant vectors for the introduction of non-indigenous species are listed with some examples of associated introductions from the literature. The predominant vectors world-wide are shipping- and aquaculture-associated vectors, in particular hull fouling, ship ballast and oyster transports.

Table 1.1 Human activities and associated vectors of introduction for non-indigenous species. Gray-shaded vectors are discussed in detail in this thesis; in Chapter 4 historical hull fouling and boring and associated historical introductions are dealt with. In Chapter 3 oysters as a vector for associated species introductions are analyzed.

activity	vectors	some examples and references
shipping	hull fouling and boring	shipworm *Teredo navalis* (Hoppe 2002)
	solid ballast	*Fucus serratus* and *Littorina littorea* introduced to America from Europe with rock ballast (Blakeslee *et al.* 2008; Brawley *et al.* 2009)
	ballast water	(Carlton 1985; Mills *et al.* 1993)
	sea-chests	(Lee & Chown 2007)
	anchor	*Codium fragile* ssp. *tomentosoides* possibly transported in anchor chains (Schaffelke & Deane 2005)
aquaculture	deliberate translocation of fish and shellfish	Oyster translocations (Ruesink *et al.* 2005)
	accidental with deliberate translocation of fish and shellfish	*Crepidula fornicata* (Blanchard 1997)
	accidental with seaweed packing of fish and shellfish	(Mills *et al.* 1993)
canal construction	natural range expansion through man-made canals	*Cordylophora caspia* invasion in the Baltic Sea via canals from the Caspian sea (Nehring & Leuchs 1999), Lessepsian migrants in the Mediterranean (Por 1978)
	with ships through man-made canals	mussel *Brachidontes pharaonis*, did not naturally migrate but was introduced by ships (Shefer *et al.* 2004)
plant introductions	deliberate translocation of plants and associated introductions	*Spartina* (Gray *et al.* 1991)
individual activities	bait	(Weigle *et al.* 2005)
	accidental with bait or seaweed packing of bait	*Carcinus maenas* in San Francisco Bay (Cohen *et al.* 1995)
	release from aquaria	reef fishes in Florida (Semmens *et al.* 2004), *Caulerpa taxifolia* in the Mediterranean (Meinesz *et al.* 2001)
release as a result of research activities		*Crassostrea gigas* in the Wadden Sea (Tydeman 2008)

Ships transport non-indigenous species on and in their hull (hull-fouling and boring organisms) and have done so since ancient times. The organisms that colonize ship hulls are mostly of subtidal origin, and may be sessile, boring, encrusting and mobile species. Hull fouling increases drag of sailing ships, and historically ships had to be careened periodically to manually remove fouling. Since the use of steel in ship construction, the development of motorized vessels and the increase of ship speed (giving organisms less opportunity to settle on the hull and to become released in the recipient region, and increasing the chance of organisms being swept off the hull during transport), and the wide-spread use of effective anti-fouling paints the number of inter- or trans-oceanic hull-fouling introductions has been reduced. However, pleasure craft travelling intra-regionally and at slower speeds still form an important vector for the secondary spread of introduced species (Floerl & Inglis 2005). With the ban of TBT-containing paints (due to the detrimental effects of TBT on marine fauna) and improving water quality in ports and harbors this vector may experience a revival in the future, since more abundant and diverse fouling communities will be able to colonize the hulls of visiting ships (Carlton 1996b; Nehring 2001).

Ships that do not carry cargo use ballast to secure their stability. In the past (before 1900), dry or solid ballast, in particular rocks and sand, were used to stabilize ships. Ballast was taken up in the port of departure, mostly from the lower shore, and was dumped in ports of arrival before loading cargo. The organisms associated with solid ballast were mostly of intertidal origin and were introduced with their substrate.

From 1870 onwards, the use of dry or solid ballast was gradually replaced by ballast water for stability, thus creating a new mode of dispersal for organisms (Carlton 1985). Ballast water is taken up in the port of departure and dumped in the port of arrival. Ballast water may contain organisms from all phyla and of all life-stages, and these may occur in high numbers due to the large volume of ballast water in a single ship. Ballast water is a potent vector, not only because of the large number of individuals and taxa that are contained in ballast tanks and sediments on the bottom of the tanks, but also because rates of survival in ballast tanks are high due to the fairly stable conditions. The chance of survival upon release in the recipient coastal region is also high, as environmental conditions are often similar to those in the donor region (Carlton & Geller 1993). Ballast water exchange in the open ocean is used as a measure to decrease the risk of introducing coastal non-indigenous species at the other side of the ocean.

In order to restock or start local fisheries, non-indigenous species of fish, crustaceans and shellfish have been introduced outside their native ranges. These transfers of live aquaculture species, mostly shellfish, resulted in the establishment of these species, but also of species that are associated with these organisms. The most commonly introduced shellfish are oysters, in particular the Pacific oyster *Crassostrea gigas*, which originally occurred in the northwest Pacific, and now has established naturally reproducing populations in the coastal zones of all continents (except Antarctica) (Ruesink *et al.* 2005; Molnar *et al.* 2008). When live, adult oysters are translocated, the oyster shells provide a habitat for a large number of sessile and mobile fouling organisms, and this epiflora and -fauna of the oyster shell is introduced

with its substrate. Large-scale oyster transports occurred in the past, and continue to take place in the present. They are therefore still an important vector for primary introductions and secondary dispersal of associated species.

The transport mechanisms elaborated on above are the most important vectors in terms of numbers of species that were introduced with them. However, smaller vectors can also result in invasions with enormous impact, such as the accidental release from an aquarium of the alga *Caulerpa taxifolia* in the Mediterranean (Meinesz *et al.* 2001). The number of acting vectors is increasing over time, as is the frequency of transport due to increased global trade (Carlton 1999a; Carlton & Cohen 2003).

Rates of invasions

The growing magnitude of global trade and associated transport vectors, and the multiplied number of acting vectors (Carlton & Cohen 2003) are greatly accelerating the rate of non-indigenous species introductions in coastal communities, especially in the past 30 years (Ruiz *et al.* 1997; Galil 2000; Leppäkoski *et al.* 2002; Hewitt *et al.* 2004; Streftaris *et al.* 2005; Chapter 3). On a global scale, only 17% of the marine bioregions have no introduced species, which most likely may be due to underreporting (Molnar *et al.* 2008).

Some regions are more invaded than others, and some major receiver and donor areas have been identified (Carlton 1987). This directionality of invasions can be explained by different factors. Invasions tend to occur from high-diversity regions to regions of lower diversity, similar to the exchange of biota in geological history (e.g. the trans-Arctic migration of Pacific species into the North Atlantic Ocean). This has been attributed to longer evolutionary history resulting in more stable and diverse communities (Vermeij 1991) and high rates of prior extinction (Vermeij 2005). Systems with low species richness, such as brackish waters, are hypothesized to have many empty niches, which can be occupied by arriving exotic species (Wolff 1999; Paavola *et al.* 2005), resulting in high rates of invasions.

The impact of invasions

The impact of introduced species can be studied on different levels and scales. Ecological consequences of introduced species can be the alteration of fundamental processes such as nutrient cycling, primary and secondary production, disruption of key ecological interactions, habitat alteration and competition with native species for resources, resulting in (functional) extirpation of native species (Mack *et al.* 2000). The general success of invaders over native species has been attributed to the absence of parasites (Torchin *et al.* 2003) or natural predators in the newly invaded range - the 'Enemy Release Hypothesis' (Williamson 1996; Keane & Crawley 2002), and anthropogenic disturbance in the recipient region that disrupts native communities, making them vulnerable to invasions (Mack *et al.* 2000).

Invaders that have great economic consequences, e.g. by negatively impacting fisheries, are considered a pest. In the marine realm, introduced species are hard to combat once they have established self-sustaining populations, and in most cases, once they are found, they have already established. There are only two examples of a successful eradication of nuisance species in the sea. *Caulerpa taxifolia* was successfully eradicated from southern California, where it was only locally present and was manually removed and treated with chlorine (Anderson 2005). The dreissenid mussel *Mytilopsis sallei* was eradicated from localized introduction sites in Australian marinas by dumping chlorine in the marina and eradicating not only the introduced mussel, but all organisms that were there (Bax *et al.* 2002). The intentional introduction of exotic species in order to control or decimate non-indigenous pest species has been used in terrestrial and freshwater systems only and is known as biocontrol. The organisms that are introduced parasitize, eat, infect or compete with the pest species, and are ideally host-specific. Although biocontrol has been successful in some cases, it can also result in unexpected and unintended development of the introduced species into a new pest (Secord & Kareiva 1996). Marine ecosystems have important contrasting attributes compared to terrestrial ecosystems, such as life-history characteristics, dispersal strategies and the relative openness of the system (Strathmann 1990). This creates a higher risk of unwanted side-effects of biocontrol, and because of the unpredictability of these introductions biocontrol has not been carried out in the marine environment (Secord 2003).

There are no records of extinctions of marine organisms due to the introduction of non-indigenous species (Wolff 2000; Gurevitch & Padilla 2004) and therefore negative consequences of invasions are debated. Most introductions appear harmless and are thus by some authors concluded to increase local biodiversity and thereby positively affect the receiving ecosystem (Reise *et al.* 1999; Briggs 2000). However, there are many examples of invasions with negative consequences, and more importantly, the impact of most invasions (90-95%) remains unknown (Parker *et al.* 1999; Carlton 2003b). It can therefore not be said that introduced species in general have positive effects, and biological invasions are acknowledged to be a major threat to coastal marine biodiversity (Chapin *et al.* 2000; Bax *et al.* 2003).

Underestimation of invasions

In addition to the lack of studies on the impacts of invasions, we may also be greatly underestimating the number of introduced species world-wide. This is likely due to underreporting; in some regions novel species are not documented.

Recognized invaders more commonly belong to well-studied taxonomic groups, such as crustaceans, mollusks, macroalgae and fish, and less often to marine bacteria, meiofauna and microalgae, which are typically of smaller size (the 'small's rule' (Carlton 2003b; Carlton 2008). In general, less conspicuous and taxonomically problematic taxa or small organisms typically have larger proportions of species with

cosmopolitan distributions. The 'everything is everywhere' hypothesis states that small organisms (bacteria, protists, small invertebrates), due to their high dispersal potential, have cosmopolitan distributions and do not show biogeographical patterns (Pommier *et al.* 2007). This hypothesis has been challenged in the past decades by molecular genetic studies, which have resulted in the discovery of cryptic species complexes and diversity patterns across all taxonomic groups and from all habitats. The marine realm appears to harbor an especially high number of cryptic species because of the high species richness and complex inter-specific interactions (Bickford *et al.* 2007), which is one explanation for the high share of cosmopolitan species in these groups. However, because of their cryptic life-histories or small sizes, anthropogenic dispersal and introduction of these groups is highly likely, and current numbers of introduced species of these less-conspicuous groups are greatly underestimated, resulting in an overall underestimation of the scale of invasions in the sea.

Another fundamental issue that has resulted in the under-reporting of introduced species is the fact that species are assumed to be native by default, ignoring the fact that trans-oceanic shipping started long before the first comprehensive biological studies were carried out in coastal systems. These early ships carried rich fouling communities, used solid ballast, travelled at slow speeds and had long harbor residence times, increasing the risk of introducing exotic species. Historical invasions are known to have occurred, famous examples being the soft-shelled clam *Mya arenaria*, which was introduced to Europe from America by the Vikings (Petersen *et al.* 1992), and the Portuguese oyster *Crassostrea angulata*, which was likely introduced by merchant vessels from the Northwest Pacific in the 16th century (Ó Foighil *et al.* 1998). These are only two examples, from a well studied taxonomic group that additionally has a fossil record, but we have certainly overlooked many more historical invasions. These unrecorded invasions are part of today's cryptogenic species: species that are neither demonstrably native, nor introduced (Carlton 1996a). Cryptogenic species are often included in regional lists of introduced species, but the cryptogenic category is mostly used conservatively: only those species are included for which there is evidence that they are introduced, but for which the region of origin remains unknown. Instead, all species that are potentially transported by anthropogenic vectors and have a distribution pattern that cannot be explained by natural dispersal mechanisms should be included in the cryptogenic category (Carlton 1996a; 2008). The underestimation of the number of invasions has great consequences for our view of natural distributions and diversity patterns in the sea and our understanding of the magnitude and consequences of biological invasions (Carlton 2003b).

Thesis outline

The general topic of this thesis is the extent of marine bioinvasions, including historical invasions, in a temperate coastal system. In order to investigate the extent of invasions, I researched established invasions and cryptogenic species in the North Sea, analyzed

oyster transports (one of the predominant vectors) in detail, estimated the scale of cryptogenesis in the North Atlantic Ocean and analyzed the phylogeography of a cryptogenic ascidian. This thesis consists of two parts, that differ in the temporal, geographical and diversity scales that are investigated, which is illustrated in Fig 1.3.

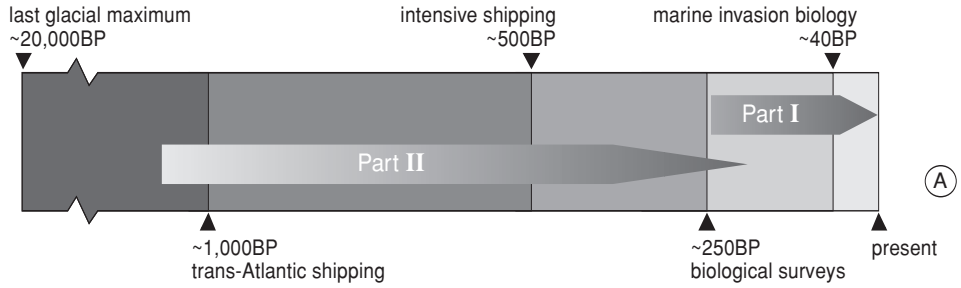
Part I: Vectors

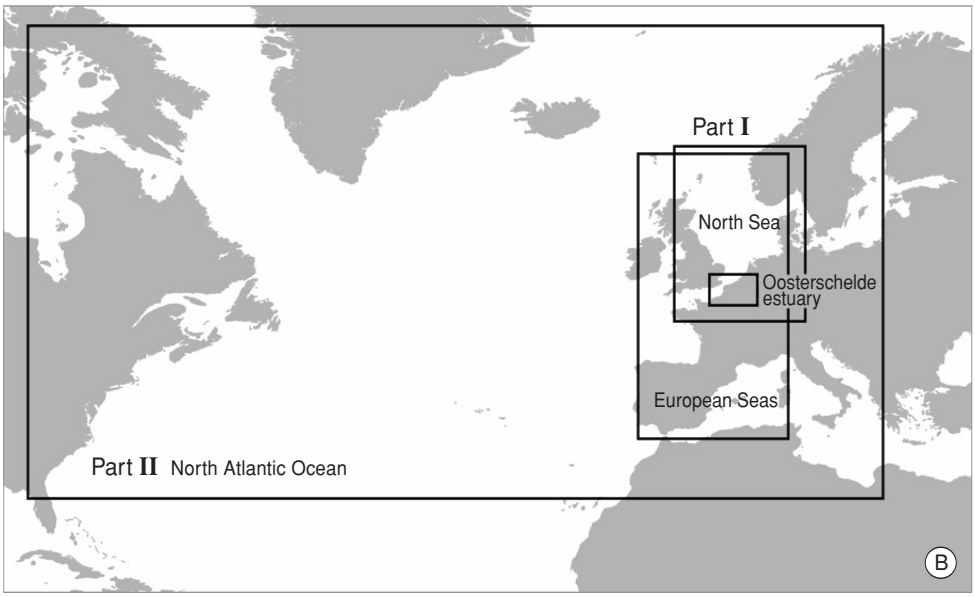
In **Chapter 2** an up-to-date overview is given of the known introduced and cryptogenic species of the North Sea. Previous studies in countries bordering the North Sea have created national lists of invaders in their coastal waters, (e.g. Eno *et al.* 1997; Nehring & Leuchs 1999; Wolff 2005b; Jensen & Knudsen 2005). A previous list of invaders in the North Sea (Reise *et al.* 1999) is updated here. In addition, aspects of the invasion history of three notorious introduced species are described in detail. Established introductions are analyzed for donor regions and taxonomic groups introduced, and for the relative importance of vectors, in terms of their contribution to the number or proportion of established invasions, which is known as vector strength (Carlton & Ruiz 2005). By determining vector strength and analyzing current invasions in different parts of the North Sea we can indicate which human activities are of risk of introducing novel species, and which species are to be expected. The cryptogenic species listed together with the known historical invasions constitute a first estimate of the extent of historical invasions.

One of the predominant vectors in the North Sea region, as well as in other parts of the world (Molnar *et al.* 2008), is the translocation of adult and seed oysters for aquaculture purposes (Table 1.1). Oyster transports as a vector are analyzed in detail in **Chapter 3**. The success rate and timing of introductions with oysters were estimated by compiling an overview of species introduced with this vector from the literature. Furthermore, we reconstructed commercial oyster shipments to The Netherlands, and tried to link species introductions to these shipments. Propagule pressure is a vector characteristic that is composed of the absolute number of individuals released in one introduction event and the number of discrete release events (Carlton 1996b; Lockwood *et al.* 2005). It is an important determinant of the success of an introduction. By identifying macroalgae growing on Pacific oyster shells from the Oosterschelde estuary in the Southwest Netherlands and combining this with the reconstruction of commercial oyster shipments, the propagule pressure exerted by this vector was determined.

Part II: The scale of cryptogenesis

In general, geographic distributions of marine organisms are shaped by dispersal and vicariance events, as described in the introduction. In the North Atlantic, the Pleistocene glaciations have had a profound impact on species diversity and distributions of marine biota (Hewitt 1999; 2000). Temperature and sea-level fluctuations and the formation and retreat of ice-sheets resulted in repeated compression and expansion of species distributions. During the Last Glacial Maximum (LGM), ~21,000 years BP, the continental shelf was exposed in parts of the North Atlantic Ocean, including the North Sea, and large parts of the American and Eurasian continents and parts of the





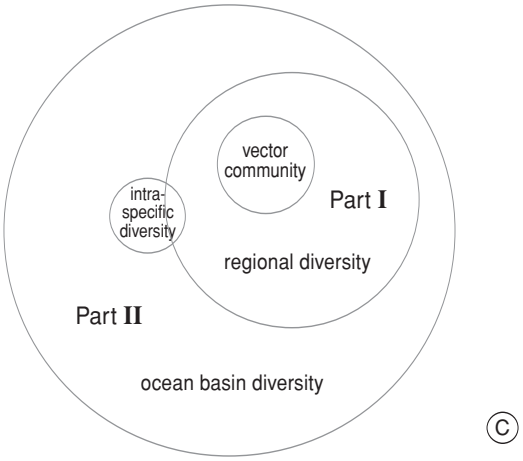


Figure 1.3 Different temporal (a), geographic (b) and diversity (c) scales addressed in this thesis.

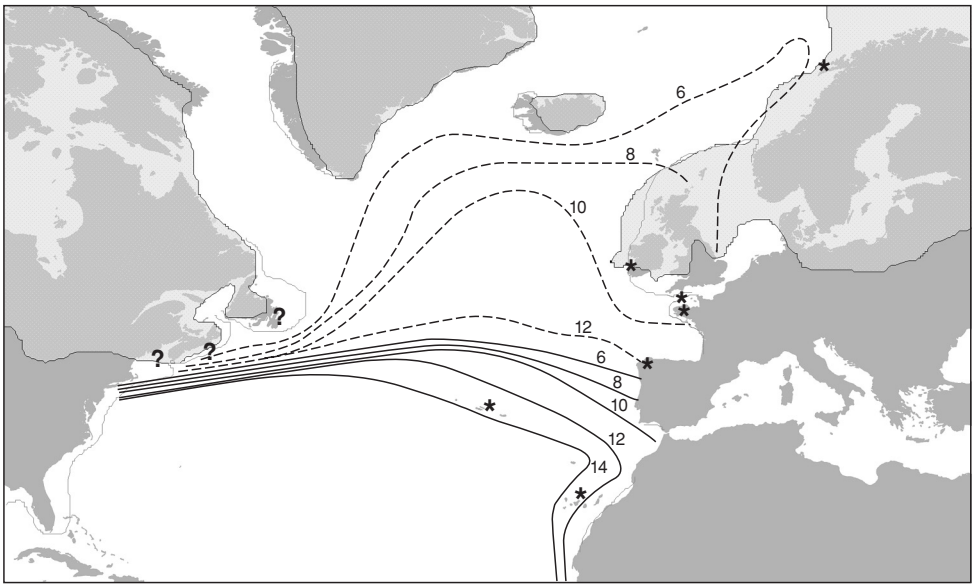


Figure 1.4 The North Atlantic Ocean during the LGM (~21,000 BP). The extent of ice sheets is shown, as well as the location of (putative) glacial refugia (indicated with an asterisk for demonstrated refugia, and a question mark for putative refugia). The exposed continental shelf is shown by the thin gray lines. In addition, the Sea Surface Temperatures during the LGM and in modern times are indicated. The lower set of solid contour lines reflects sea surface temperature (SST) isotherms (6–14°C) during the LGM; the upper set of dotted contour lines reflects SST isotherms in modern times during summer. Note the compression of isotherms on the West Atlantic coast. Redrawn from Olsen *et al.* (2010).

coastal ocean were covered by a thick ice sheet (Fig. 1.4). Species were restricted to refugia, from where they colonized their modern ranges after the LGM, when the ice sheets retreated and sea-levels and temperatures rose, resulting in modern-day distributions of benthic flora and fauna. Based on geological and mostly genetic evidence, several southern refugia have been identified on European and American coasts, and several northern periglacial refugia have been postulated to have existed, such as on the Icelandic coast (reviewed in Maggs *et al.* 2008; Fig. 1.4). Due to the severity of the effects of the Pleistocene Glaciations in the North Atlantic Ocean, the current distributions of shallow-water benthic invertebrates and algae in the North Atlantic are relatively recent when compared to their North Pacific counterparts.

Within the North Atlantic Ocean basin, there are some marked differences between the European and North American Atlantic coasts. The Summer Sea Surface Temperature Isotherms are more compressed on the North American coast when compared to Europe, thus creating a smaller potential range for temperate species (Fig. 1.4). On the American side, hard substrates are absent south of Cape Hatteras, further restricting potential distributions of rocky-shore species. During the LGM American

hard substrate species would not have been able to retreat to refugia in the south, and were thus restricted to a few putative refugia in the north. Refugia in Europe were more numerous, and hard substrates are present all along the European Atlantic coast. The result of this is that the European coast is generally more diverse in species than the American coast (Briggs 1974; Vermeij 2005). Recolonization of the American coast is assumed to have taken place by natural dispersal from local refugia, as well as from refugia in Europe (Wares & Cunningham 2001).

The possibility of anthropogenic dispersal with ships (mainly as hull fouling) is rarely being considered. However, trans-Atlantic shipping started with the Vikings reaching Newfoundland around 1000 BP. After the (re)discovery of the Americas in 1492 large-scale shipping did occur across the North Atlantic, and there are many examples of historical introductions from those times which are only now being revealed by molecular studies (e.g. Blakeslee *et al.* 2008; Brawley *et al.* 2009).

In order to distinguish between natural distribution patterns and distribution patterns that are the result of anthropogenic introduction, in **Chapter 4** an overview of all species of Hydrozoa, Bivalvia and Ascidiacea present in the North Atlantic Ocean is presented. These taxonomic groups were chosen because they vary in their natural and anthropogenic dispersal potential and have different life-history traits. Disjunct amphi-Atlantic distribution patterns (i.e. species that occur in shallow waters on European and North American Atlantic coasts, but are absent from Arctic or sub-Arctic waters) are used as a proxy for cryptogenic species plus known introductions. Distribution patterns and dispersal potential of individual species are investigated, and species are assigned to native, introduced or cryptogenic categories. By reviewing natural dispersal potential, cryptic speciation, the effects of the LGM and anthropogenic dispersal, an estimate of the number of possibly unrecorded introductions in the North Atlantic is obtained. The aim of this chapter is <u>not</u> to demonstrate that species are introduced, but the history of species is questioned and the number of cryptogenic species is compared across taxonomic groups. Identifying cryptogenic species is a first step in acknowledging the true scale of marine bioinvasions, which is crucial to our understanding of rates of evolution in the sea and the influence of introduced species on ecosystem functioning.

In **Chapter 5**, one of the cryptogenic species identified in Chapter 4 is investigated in detail. The ascidian *Molgula manhattensis* has a disjunct amphi-Atlantic distribution pattern, has limited natural dispersal capacities, is associated with hull fouling and has a recent history of world-wide invasions. mtDNA COI sequence data have been demonstrated to be a successful tool in ascidian species identifications and determining previously unrecognized or cryptic ascidian invasions (Tarjuelo *et al.* 2001; Castilla *et al.* 2002; Turon *et al.* 2003; Tarjuelo *et al.* 2004; López-Legentil *et al.* 2006; López-Legentil & Turon 2006). This molecular tool is used to analyze the phylogeography of *M. manhattensis* in the North Atlantic Ocean, in order to distinguish between anthropogenic and natural causes for its current disjunct distribution pattern, and to determine the effect of anthropogenic dispersal on genetic diversity patterns.

PART I - Vectors

Introduced aquatic species of the North Sea coasts and adjacent brackish waters

2

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2

Introduction

Introduced aquatic species have received more attention in north-western Europe following the summaries from the German North Sea coast (Gollasch 1996; Nehring & Leuchs 1999), Britain (and Ireland) (Eno *et al.* 1997; Minchin & Eno 2002), Norway (Hopkins 2002) and a more general account for the North Sea (Reise *et al.* 1999). Since then, several inventories have appeared: for the German coast (Nehring 2005), the Dutch coast (Wolff 2005b) and the Danish coast (Jensen & Knudsen 2005). In this account we review, summarize and update all those previous accounts. We have also included non-indigenous introduced species which were known from the North Sea but most probably are extinct in this area today, and species that have been recorded, but for which we have no proof of self-sustaining populations. For the purpose of this account:

- The North Sea is defined from a line between Dover and the Belgian border in the south-west to a parallel line from the Shetland Islands to Norway in the north, and includes the Skagerrak in the east (modified after North Sea Task Force 1993). The boundary between the North- and Baltic Seas, as defined by the Helsinki Commission (www.helcom.fi), is the parallel of the Skaw in the Skagerrak at 57°44.43'N.
- We define marine and brackish-water species as those aquatic species which do not complete their entire life cycle in freshwater (modified after ICES 2005). Marine species are those that have their main distribution in salinities higher than 18 psu; brackish-water species have their main distribution in salinities between 1 and 18 psu.
- Introduced species (= non-indigenous, exotic or alien species) are species transported intentionally or accidentally by a human-mediated vector into habitats outside their native range. Note that secondary introductions may be transported by human-mediated vectors or by natural means (ICES 2005).
- A vector is any living or non-living carrier that transports living organisms intentionally or unintentionally (ICES 2005).

Non-indigenous aquatic species in the North Sea region

In total, 167 introduced and cryptogenic species were reported in the North Sea. There appear to be more records from The Netherlands than from other parts (Fig. 2.1) which may be explained by the most intensive shipping (Port of Rotterdam) and aquaculture (Oosterschelde Estuary) activities in the North Sea region (Wolff 2005b). The lower number of records for the British North Sea coast is more difficult to explain. With respect to red algae, Maggs and Stegenga (1999) suggest that the prevailing alongshore currents from the north are less likely to spread introduced species compared to the eastward currents from Norfolk and the Channel which pass the continental shores of the North Sea.

Figure 2.1 Introduced species in the North Sea region. Pie charts show relative importance of likely introduction vectors for non-indigenous species (excluding cryptogenic species) per country. The total number of non-indigenous species per country is given.

The dominant introduction vectors are shipping and intentional introductions for stocking or aquaculture purposes (Table 2.1, Fig. 2.3). The most recently recorded non-indigenous species are *Rapana venosa* and *Neogobius melanostomus*, which were both recorded for the first time in the North Sea and adjacent waters in 2005 (Kerckhof *et al.* 2006; van Beek 2006). Shortly after the first version of this manuscript was submitted a new non-indigenous species of great concern was found in the North Sea (<2006): *Mnemiopsis leidyi*. This comb jelly was also introduced in other European Seas and contributed to the decline of fisheries.

Table 2.1 Introduced and cryptogenic marine and brackish-water species from the North Sea and distribution records in countries bordering the North Sea. For cryptogenic species, no vector is indicated, and all cryptogenic species are established.. Note added in proof: This table reflects the situation up to March 2006 and only a few remarkable species such as *Mnemiopsis leidyi* and new records of *Urosalpinx cinerea* were added.

Species	Taxonomic group	Country							Status	Habitat	Ballast water	Hull fouling	Aquacult. or stock.	Other	Unknown	Unclear
Coscinodiscus wailesii	Bacillariophyceae	NO	SE		GE	NL	BE	UK	establ	marine	x		x			x
Chaetoceros calcitrans f. *pumulis*	Bacillariophyceae							UK	uncertain	marine			x			
Odontella sinensis	Bacillariophyceae	NO	SE	DK	GE	NL	BE	UK	establ	marine	x					
Pleurosigma planctonicum (= *simonsenii*)	Bacillariophyceae					NL		UK	establ (extinct in NL)	marine	x					
Thalassiosira punctigera	Bacillariophyceae	NO	SE		GE	NL	BE	UK	establ	marine	x		x			x
Thalassiosira tealata	Bacillariophyceae	NO					BE	UK	establ	marine	x		x			x
Alexandrium angustitabulatum	Dinophyceae		SE						uncertain	marine	x					
Alexandrium leei	Dinophyceae					NL			establ	marine	x		x			x
Alexandrium minutum	Dinophyceae	NO	SE	DK					crypto	marine						
Alexandrium tamarense	Dinophyceae	NO	SE			NL		UK	crypto	marine						
Dicroerisma psilonereiella	Dinophyceae		SE						establ	marine	x					
Gyrodinium corallinum	Dinophyceae		SE						establ	marine	x					
Karenia (= *Gymnodinium*) *mikimotoi* (= *aureolum*) (= *Gymnodinium* cf. *nagasakiense*)	Dinophyceae	NO	SE	DK	GE	NL	BE	UK	establ	marine	x					
Prorocentrum minimum	Dinophyceae		SE			NL			crypto	marine						
Prorocentrum redfieldii	Dinophyceae				GE	NL			crypto	marine						
Thecadinium yashimaense (= *mucosum*)	Dinophyceae				GE				uncertain	marine	x					
Verrucophora cf. *fascima*	Dinophyceae		SE						establ	marine	x					
Chattonella antiqua	Rhaphidophyceae				GE	NL			establ	marine	x					
Chattonella marina	Rhaphidophyceae				GE	NL			establ	marine	x					
Fibrocapsa japonica	Rhaphidophyceae				GE	NL			establ	marine	x					
Heterosigma akashiwo (= *carterae*) (= *Olisthodiscus luteus*)	Rhaphidophyceae	NO				NL		UK	crypto	marine						
Verruca (= *Chattonella*) cf. *verruculosa*	Rhaphidophyceae	NO	SE	DK					crypto	marine						
Isochrysis sp. (*Tahitian strain*)	Haptophyte							UK	uncertain	marine			x			
Codium fragile ssp. *atlanticum*	Chlorophyta	NO						UK	establ	marine		x				
Codium fragile ssp. *scandinavicum*	Chlorophyta	NO	SE	DK					establ	marine		x				
Codium fragile ssp. *tomentosoides*	Chlorophyta	NO	SE	DK	GE	NL	BE	UK	establ	marine		x	x			x

Ulva pertusa	Chlorophyta					NL			establ	marine				x		
Acrochaetium densum	Rhodophyta	NO				NL			crypto	marine						
Agardhiella subulata	Rhodophyta					NL			establ	marine	x					
Aglaothamnion halliae	Rhodophyta	NO	SE		GE				establ	marine	x					
Anotrichium furcellatum	Rhodophyta					NL			extinct	marine	x					
Antithamnionella spirographidis	Rhodophyta					NL	BE		establ	marine	x					
Antithamnionella ternifolia	Rhodophyta				GE	NL	BE		establ	marine	x					
Asparagopsis armata	Rhodophyta					NL		UK	establ	marine	x					
Bonnemaisonia hamifera	Rhodophyta	NO	SE	DK	GE			UK	establ	marine	x					
Colaconema dasyae	Rhodophyta					NL			establ	marine	x					
Dasya baillouviana	Rhodophyta	NO	SE	DK	GE	NL			establ	marine	x					
Gracilaria gracilis	Rhodophyta	NO							crypto	marine						
Gracilaria vermiculophylla	Rhodophyta		SE	DK	GE	NL			establ	marine	x					
Grateloupia turuturu (= *doryphora*)	Rhodophyta					NL			establ	marine	x	x				x
Heterosiphonia japonica	Rhodophyta	NO	SE			NL		UK	establ	marine				x		
Mastocarpus stellatus	Rhodophyta			DK	GE				crypto	marine						
Neosiphonia (= *Polysiphonia*) *harveyi*	Rhodophyta	NO	SE	DK	GE	NL	BE	UK	establ	marine	x					
Polysiphonia senticulosa	Rhodophyta					NL	BE		establ	marine		x				
Porphyra miniata	Rhodophyta			DK					establ	marine	x					
Sphaerococcus coronopifolius	Rhodophyta	NO	SE						crypto	marine						
Asperococcus scaber	Phaeophyta					NL			establ	marine	x	x				x
Colpomenia peregrina	Phaeophyta	NO	SE	DK	GE	NL		UK	establ	marine				x		
Elachista sp.	Phaeophyta					NL			establ	marine			x			
Fucus evanescens	Phaeophyta	NO	SE	DK					establ	marine	x					
Laminaria ochotensis	Phaeophyta				GE				uncertain	marine	x					
Leathesia verruculiformis	Phaeophyta					NL			establ	marine	x					
Sargassum muticum	Phaeophyta	NO	SE	DK	GE	NL	BE	UK	establ	marine	x	x				x
Scytosiphon dotyi	Phaeophyta							UK	establ	marine		x				
Undaria pinnatifida	Phaeophyta					NL	BE		establ	marine	x	x				x
Cotula coronopifolia	Anthophyta				GE	NL			establ	brackish				x		
Spartina anglica	Anthophyta				GE	NL	BE	UK	establ	marine			x			
Spartina x townsendii	Anthophyta			DK	GE	NL	BE	UK	establ	marine			x			
Bonamia ostreae	Protozoa					NL			establ	marine		x				
Haplosporidium armoricanum	Protozoa					NL			extinct	marine		x				
Marteilia refringens	Protozoa					NL			extinct	marine		x				
Haliclona (= *Acervochalina*) *loosanoffi*	Porifera				GE	NL			crypto	marine						
Haliclona cf. simplex	Porifera					NL			crypto	marine						
Haliclona rosea	Porifera					NL			crypto	marine						
Hymeniacidon perlevis	Porifera					NL			crypto	marine						
Mycale micracanthoxea	Porifera					NL			crypto	marine						
Scypha scaldiensis	Porifera					NL			crypto	marine						

Species	Taxonomic group	Country							Status	Habitat	Ballast water	Hull fouling	Aquacult. or stock.	Other	Unknown	Unclear
Bougainvillia macloviana	Cnidaria				GE				extinct	marine		x				
Clavopsella (= *Thieliana*) *navis*	Cnidaria				GE	NL			uncertain	brackish		x				
Cordylophora caspia	Cnidaria	NO	SE	DK	GE	NL	BE		crypto	brackish						
Diadumene cincta	Cnidaria				GE	NL			crypto	marine						
Diadumene lineata	Cnidaria				GE	NL		UK	establ	marine		x	x			x
Garveia (= *Bimeria*) *franciscana*	Cnidaria				GE	NL	BE		establ	brackish		x				
Gonionemus vertens	Cnidaria	NO	SE		GE	NL	BE	UK	establ	marine	x	x	x			x
Nemopsis bachei	Cnidaria	NO			GE	NL			establ	brackish	x	x				x
Ostroumovia inkermanica	Cnidaria					NL			extinct	brackish	x	x				x
Rhizogeton nudum	Cnidaria	NO							crypto	marine						
Mnemiopsis leidyi	Ctenophora			DK	GE	NL	BE		establ	brackish	x					
Cercaria sensifera	Trematoda							UK	uncertain	marine			x			
Gyrodactylus salaris	Trematoda	NO							establ	brackish			x			
Pseudobacciger harengulae	Trematoda		SE						establ	marine			x			
Pseudodactylogyrus anguillae	Trematoda	NO		DK					establ	brackish			x			
Pseudodactylogyrus bini	Trematoda	NO							establ	brackish			x			
Anguillicola crassus	Nematoda	NO	SE	DK	GE	NL	BE	UK	establ	marine			x			
Euplana gracilis	Turbellaria					NL			uncertain	brackish		x				
Imogine necopinata	Turbellaria					NL			uncertain	brackish			x			
Stylochus flevensis	Turbellaria					NL			crypto	brackish						
Alkmaria romijni	Polychaeta	NO		DK		NL			crypto	brackish						
Aphelochaeta (= *Tharyx*) *marioni*	Polychaeta				GE	NL			crypto	marine						
Boccardia ligerica (= *Polydora redeki*)	Polychaeta		SE		GE	NL	BE		crypto	brackish						
Clymenella torquata	Polychaeta							UK	uncertain	marine	x	x	x			x
Ficopomatus enigmaticus	Polychaeta				GE	NL	BE	UK	establ	brackish	x	x	x			x
Hydroides elegans	Polychaeta				GE	NL			establ	brackish	x	x				x
Janua brasiliensis	Polychaeta					NL			uncertain	marine		x	x			x
Marenzelleria neglecta	Polychaeta	NO		DK	GE	NL	BE	UK	establ	brackish	x					
Marenzelleria viridis	Polychaeta		SE	DK	GE	NL		UK	establ	brackish	x					
Microphthalmus similis	Polychaeta				GE	NL			crypto	marine						
Proceraea cornuta	Polychaeta					NL			crypto	marine						
Scolelepis cf. *bonnieri*	Polychaeta	NO				NL			establ	marine					x	
Tharyx killariensis	Polychaeta				GE				establ	marine	x	x				x
Tubificoides heterochaetus	Oligochaeta				GE	NL	BE		crypto	brackish						
Corambe obscura (= *batava*)	Gastropoda					NL			extinct	brackish					x	

Crepidula fornicata	Gastropoda	NO	SE	DK	GE	NL	BE	UK	establ	marine			x			
Rapana venosa	Gastropoda					NL		UK	uncertain	marine					x	
Urosalpinx cinerea	Gastropoda					NL		UK	uncertain	marine			x			
Aulacomya ater	Bivalvia							UK	unestabl	marine		x				
Crassostrea gigas	Bivalvia	NO		DK	GE	NL	BE	UK	establ	marine			x			
Crassostrea virginica	Bivalvia				GE	NL		UK	unestabl	marine			x			
Ensis directus (= *americanus*)	Bivalvia	NO	SE	DK	GE	NL	BE	UK	establ	marine	x					
Mercenaria mercenaria	Bivalvia					NL	BE	UK	establ	marine			x	x		x
Mya arenaria	Bivalvia	NO	SE	DK	GE	NL	BE	UK	crypto	marine						
Mytilopsis (= *Congeria*) *leucophaeta*	Bivalvia				GE	NL	BE		establ	brackish	x					
Petricola pholadiformis	Bivalvia	NO	SE	DK	GE	NL	BE	UK	establ	marine			x			
Ruditapes (= *Tapes*) *philippinarum*	Bivalvia	NO						UK	establ	marine			x			
Teredo navalis	Bivalvia	NO	SE	DK	GE	NL	BE	UK	crypto	marine						
Limulus polyphemus	Xiphosura			DK	GE	NL			unestabl	marine					x	
Artemia salina	Branchipoda							UK	unestabl	marine			x			
Eusarsiella zostericola	Ostracoda							UK	establ	brackish			x			
Acartia tonsa	Copepoda		SE	DK	GE	NL	BE	UK	establ	marine	x					
Eurytemora americana	Copepoda					NL		UK	establ	brackish	x					
Myicola ostreae	Copepoda					NL			uncertain	marine			x			
Mytilicola intestinalis	Copepoda			DK	GE	NL		UK	establ	marine		x	x			x
Mytilicola orientalis	Copepoda					NL			establ	marine			x			
Nucellicola holmanae	Copepoda							UK	crypto	marine						
Balanus amphitrite amphitrite	Cirripedia					NL	BE	UK	establ	marine		x				
Balanus eburneus	Cirripedia					NL			uncertain	marine		x				
Balanus improvisus	Cirripedia	NO	SE	DK	GE	NL	BE	UK	crypto	brackish						
Elminius modestus	Cirripedia			DK	GE	NL	BE	UK	establ	marine		x				
Megabalanus coccopoma	Cirripedia					NL	BE		establ	marine		x				
Megabalanus tintinnabulum	Cirripedia						BE		establ	marine		x				
Solidobalanus fallax	Cirripedia	NO					BE	UK	establ	marine		x				
Caprella mutica	Amphipoda	NO			GE	NL	BE	UK	establ	marine	x	x		x		x
Chelicorophium curvispinum	Amphipoda				GE	NL			establ	brackish	x					
Corophium (= *Monocorophium*) *sextonae*	Amphipoda	NO			GE	NL		UK	crypto	marine						
Gammarus tigrinus	Amphipoda		SE		GE	NL			establ	brackish			x			
Incisocalliope aestuarius	Amphipoda					NL	BE		establ	brackish	x					
Melita nitida	Amphipoda					NL			establ	marine	x	x				x
Orchestia cavimana	Amphipoda				GE	NL	BE		establ	brackish	x					
Platorchestia platensis	Amphipoda				GE	NL			crypto	marine						
Callinectes sapidus	Decapoda				GE	NL	BE		establ	marine	x					
Chionoecetes opilio	Decapoda	NO							uncertain	marine					x	
Eriocheir sinensis	Decapoda	NO	SE	DK	GE	NL	BE	UK	establ	marine	x					
Hemigrapsus penicillatus	Decapoda					NL	BE		establ	marine	x	x				x

Species	Taxonomic group	Country							Status	Habitat	Ballast water	Hull fouling	Aquacult. or stock.	Other	Unknown	Unclear
Hemigrapsus sanguineus	Decapoda					NL			uncertain	marine	x	x				x
Homarus americanus	Decapoda	NO						UK	unestabl	marine				x		
Marsupenaeus (= *Penaeus*) *japonicus*	Decapoda	NO							unestabl	marine				x		
Palaemon macrodactylus	Decapoda					NL	BE	UK	establ	marine	x					
Rhithropanopeus harrisii	Decapoda				GE	NL	BE		establ	brackish		x				
Telmatogeton japonicus	Insecta	NO		DK	GE	NL	BE	UK	establ	marine					x	
Bugula simplex	Bryozoa					NL	BE		crypto	marine						
Bugula stolonifera	Bryozoa					NL	BE	UK	crypto	marine						
Smittoidea prolifica	Bryozoa					NL			establ	marine			x			
Tricellaria inopinata	Bryozoa					NL	BE		establ	marine		x				
Victorella pavida	Bryozoa		SE		GE	NL			crypto	marine						
Botrylloides violaceus	Tunicata					NL			establ	marine		x				
Botryllus schlosseri	Tunicata	NO	SE	DK	GE	NL	BE	UK	crypto	marine						
Didemnum sp.	Tunicata					NL			establ	marine					x	
Diplosoma listerianum	Tunicata					NL			crypto	marine						
Molgula manhattensis	Tunicata	NO				NL		UK	crypto	marine						
Styela clava	Tunicata			DK	GE	NL	BE	UK	establ	marine		x	x			x
Lebistes reticulatus	Pisces				GE	NL			establ	brackish			x			
Micropogonias undulatus	Pisces					NL			uncertain	marine	x					
Neogobius melanostomus	Pisces					NL			establ	brackish	x					
Oncorhynchus gorbuscha	Pisces	NO						UK	extinct	marine			x			
Oncorhynchus keta	Pisces	NO						UK	extinct	marine			x			
Oncorhynchus kisutch	Pisces					NL			extinct	marine			x			
Oncorhynchus mykiss (= *Salmo gairdneri*)	Pisces	NO		DK	GE				establ	brackish			x			
Trinectes maculatus (= *Achirus fasciatus*)	Pisces					NL			unestabl	marine	x					

BE = Belgium, DK = Denmark, GE = Germany, NL = The Netherlands, NO = Norway, SE = Sweden and UK = United Kingdom. "Aquaculture" includes species imports for stocking; "hull fouling" includes ships, vessels and pleasure craft; "other" includes ornamental species, species imported for direct human consumption, but released into the wild; "unclear" refers to species for which no single vector could be assigned (in these cases possible vectors are indicated). Key references: Gollasch (1996); Eno *et al.* (1997); Maggs & Stegenga (1999); Reise *et al.* (1999); Wolff (1999); Weidema (2000); Minchin & Eno (2002); Jensen & Knudsen (2005); Nehring (2005); Wolff (2005); Kerckhof *et al.* (2007).

Most introduced species in the North Sea are benthic species, of which most are animal taxa (Table 2.2). More than two thirds of the known introductions have established self-sustaining populations. For others the population status is unknown. For some species there are only single specimen records or occurrences in small numbers and some populations may have been present over varying time periods, although there are no recent records (Fig. 2.2). The majority of introduced species have local distributions (Table 2.3), although 18 taxa were found in six of the seven North Sea countries (i.e. Belgium, Denmark, Germany, The Netherlands, Norway, Sweden and United Kingdom). Many native species are widely distributed on the coasts of North Sea countries and this pattern is generally found for many species that were introduced at an early time and had the ability to become dispersed. Table 2.3 shows that many recent introductions, as well as cryptogenic species, were recorded in one or two North Sea countries, which may indicate a comparatively recent arrival.

Of the total number of introduced species, 136 were marine taxa (81.9%). However, the proportion of marine vs. brackish water invaders varied by country, and marine species dominated. Investigations on alien species will have different levels of effort according to the degree of nuisance a species causes, its size, the available taxonomic expertise and diligence of monitoring surveys in each country. There will almost certainly be other introduced species that have as yet not been recognized. The absence

Table 2.2 Numbers of non-indigenous species in the North Sea per functional group.

Group	Number
Zoobenthos	84
Phytobenthos	36
Phytoplankton	22
Parasite/pathogen	12
Nekton	8
Zooplankton	5
Total	167

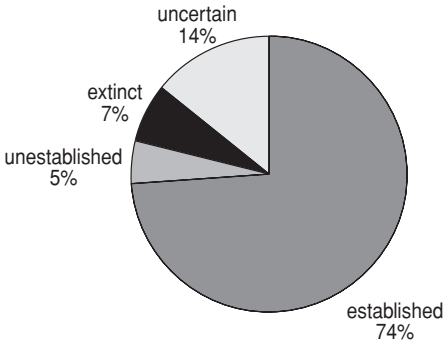

Figure 2.2 Invasion status of non-indigenous and cryptogenic species in the North Sea.

Table 2.3 Occurrence of all non-indigenous and cryptogenic species per number of North Sea countries.

Number of Countries	All non-indigenous species	Cryptogenic species
1	48	11
1	25	11
3	23	8
4	6	3
5	9	
6	8	1
7	10	4
total	129	38

of a species in neighboring countries may reflect some of these issues. For several species, the invasion vector cannot be easily determined, for example, Pacific oysters may be introduced either as adults attached to ship hulls, as larvae carried in ballast water of ships, with imports of stock for aquaculture purposes, or for direct human consumption but released into the environment. We have selected the most likely vector, which in this case we believe to be stock movements of Pacific oysters because the evidence for this is strongest. For species that are most frequently associated with hull fouling, this form of transport was assumed to be the responsible vector. For planktonic taxa and microscopic resting stages we have deemed ballast water to be the most likely vector since such species that are associated with hull fouling might be expected to become flushed away during ship journeys at sea (Table 2.1). The human activities near to the site of the first records generally are assumed to be responsible for an introduction event. However, such deductions are not always secure and for this reason we have indicated where the likely vector remains unclear (Table 2.1, Fig. 2.3).
In summary, the dominant vectors of introductions are shipping-associated vectors (i.e. hull fouling of ships and small craft and ships' ballast water and its sediments) and live aquaculture products, including their associated biota (Fig. 2.3).

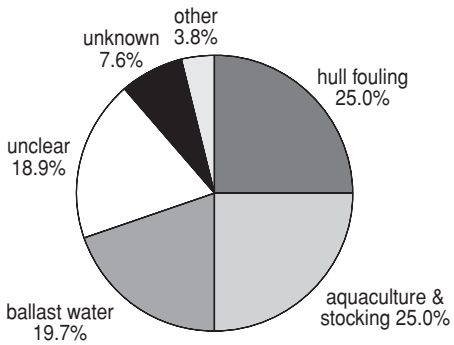

Figure 2.3 Vectors of first introduction for non-indigenous species in the North Sea.

Cryptogenic Species

Some species (n = 38) for which the origin remains unknown or undecided may be identified as introduced species at some future time with, for example, the use of genetic markers. In the meantime these species are deemed to be species of uncertain status, i.e. they are neither demonstrably native nor introduced, and these are assigned to the cryptogenic category (Carlton 1996a). These species may have been introduced during the time of the early sea voyages, and they may have either deliberately or inadvertently become imported to Europe on return from these trans-oceanic voyages. Some of these almost certainly became established and spread within Europe and may today be considered native. The soft-shelled clam *Mya arenaria* is such a species thought to have been introduced to Europe by returning Viking expeditions in the 1200s (Petersen *et al.*1992; Strasser 1999) and the Portuguese oyster *Crassostrea angulata*, may have been carried with returning sailing ships from Taiwan in the 1500s. It is because the study of taxonomy and ecology developed at a later time, from the eighteenth century onwards, that the changes in distributions have been more carefully recorded. During the years preceding ecological and taxonomic studies, ships will have had wooden hulls, which may have been subject to intensive fouling and boring, and they travelled at low speeds and remained immersed in the water over long periods, increasing the possibility of introducing associated species.

There are potentially many overlooked introductions, often belonging to the less conspicuous, and less studied groups, such as interstitial fauna, polychaetes, microalgae, protozoans, hydroids, and bryozoans (Carlton 2003b). Estimating the total number of cryptogenic species in the North Sea is almost impossible, although some indication may be obtained by examining each taxon and its ability to foul or bore in ship hulls or to survive voyages with solid ballast. Indications of a non-indigenous origin may be provided by identifying species with disjunct distributions, low dispersal potential, high fouling capacity and the likelihood of interacting with a human mediated vector and route that may have occurred at some point in time.

Non-indigenous species recorded in the North Sea as a result of natural dispersal

In the introduction, an overview was given of the published accounts of introduced species in the North Sea region. These accounts tend to list only those species that have known impacts or are commonly encountered. Species recorded as non-indigenous in these country reports may actually be native to another North Sea country, or to the biogeographic region encompassing the North Sea and may have spread by human activities. Natural events, such as exceptional water inflow due to rare hydrodynamic events or storms, can result in (mostly) temporary occurrence of species outside their normal ranges (e.g. Berge *et al.* 2005). Vagrant species such as fishes (i.e. *Mola mola* and *Carcharinus longimanus*), neustonic species (i.e. *Lepas anatifera*) and planktonic species

occasionally appear in the North Sea under such natural circumstances. Wiltshire (pers. comm.) and Franke & Gutow (2004) have indicated that many species newly found in the North Sea previously had eastern distribution limits in the British Channel, but these have been extended into the North Sea in recent decades most probably due to climate change (Stachowicz *et al.* 2002; Beare *et al.* 2004; Perry *et al.* 2005). Some species native to warmer climate regimes have colonized lagoons or docks that generally have higher temperatures, or appear in some areas in summer or in areas where there are thermal plumes. For example, the polychaete *Ficopomatus enigmaticus* was first recorded at the London Docks (United Kingdom) in 1922 (Eno *et al.* 1997), in the port of Vlissingen (The Netherlands) in 1967 near a power plant (Wolff 2005b) and also in the port of Emden (Germany) in close proximity to a power plant (Kühl 1977). Today, the species is widespread in the south-western North Sea and is established in four countries (Belgium, Germany, The Netherlands and The United Kingdom).

Case Histories

Three introduced species that have a significant impact in the North Sea and are found in all seven countries are selected as case studies. These are the slipper limpet *Crepidula fornicata*, the Chinese mitten crab *Eriocheir sinensis* and the shipworm *Teredo navalis*. These species have varying social, economic and ecological effects within the North Sea region.

Crepidula fornicata – the Slipper Limpet

This snail has a thin white shelf inside the shell aperture that protects the visceral mass, giving it a slipper-like appearance and it can attain a size of 5 cm. Individuals are most often found in a 'chain' with the oldest, female individual at the base. Following its planktonic phase the crawling male seeks to attach to the last member of a chain where it will remain confined. Over time the male gradually transforms to a female to which other wandering males may become attached to extend the chain to as many as twelve individuals. Those that do not find chains may self-fertilize (Cole 1952). In temperate waters *C. fornicata* can produce more than one brood a year and survive up to ten years. This species is a successful invader because of its persistent recruitment success and ability to colonize a wide range of habitats. Its first known occurrence in Europe was in 1872 in Liverpool Bay, England. It did not form an established population at this time but did so at a later time on the south-east coast of Britain following introductions of half-grown American oysters *Crassostrea virginica* relaid on estuarine shores.

Once introduced, a population can develop to nuisance levels within ten years. *C. fornicata* is tolerant of a wide range of conditions within its native range where it occurs from the Gulf of St Lawrence to northern Mexico. It occurs in shallow bays, estuaries and lagoons where temperatures range from –6°C when exposed to frosts to

>25°C, and salinities of 25–35 psu, but it can endure short periods of lower salinity (Walne 1956). Should mortalities arise from extreme weather events, recruitment from planktonic larvae can take place from deeper waters. There is evidence that slipper limpet populations declined during cold winter periods (Thieltges *et al.* 2004). However, the current trend of warmer winters may have aided in its continued northward expansion. It now occurs as far north as 59°N on the Norwegian coast but has also extended its range southwards to the Spanish rias. For some reason it has not become abundant in the shallow Baie de Arcachon in France (Montaudouin *et al.* 2001). *C. fornicata* is also known to occur in Sicily in the Mediterranean Sea.

The routes and modes of spread of the slipper limpet are varied. It reached Europe tucked with American oysters inside wooden barrels dispatched as deck cargo on steam-ships from Long Island Sound (Minchin *et al.* 1995). These oysters were laid on shores and the limpets among them colonized an estuary on the southeast coast of Britain, first found there in 1893. It then spread, partly aided by its planktonic larval stage, to become established along the south British coast. It has also been spread with flotsam. Specimens were stranded on Belgian shores in 1911 and soon after became established there. Korringa (1942) found many specimens attached to a stranded wreckage on the Dutch coast in 1926. A few years later the species was found in the Oosterschelde estuary; in 1930 it had become common. On 'D-Day' in 1944 during the Second World War, large numbers of *C. fornicata* were carried to Normandy, France, as hull fouling on the undersides of Mulberry Harbors used to deliver military equipment ashore. These floating units had acquired sufficient limpet fouling while awaiting deployment in sheltered British estuaries (Blanchard 1997).

Much of the slipper limpet expansion along North Sea coasts has involved the movement of oysters between estuaries and lagoons such as the Wadden Sea (Thieltges *et al.* 2003) and the Limfjord. It has even spread to isolated islands such as Helgoland. Scallops often bear the slipper limpet and stocking with sowing sized scallops may also result in its secondary spread.

Off the coast of Brittany, this limpet has become associated with maerl deposits which are important for conservation. In some areas, such as Marennes-Oleron, *C. fornicata* populations are culled by dredging to reduce their competition with oysters (Deslous-Paoli 1985). Abundant slipper limpets change sediment structure by the accumulation of vast numbers of their vacant shells and fine particles from faeces and pseudofaeces accumulated within these shells. In the 1980s, *C. fornicata*'s biomass in Europe probably exceeded one million tonnes (Quiniou & Blanchard 1987). Although during the Second World War 4000 tonnes of *C. fornicata* were processed for human consumption, it has not been marketed since.

Soon after its arrival in Europe it was declared an 'oyster pest', although the evidence is somewhat equivocal. In field experiments, Montaudouin *et al.* (1999) could not find any effect on the growth of the Pacific oyster *Crassostrea gigas*, and by use of carbon and nitrogen isotopes, Riera *et al.* (2002) found differences in food sources; however, competition was shown between the slipper limpet and the mussel *Mytilus edulis*. Thieltges (2005a) found negative effects of the slipper limpet on mussel growth

and survival. However, mussels with attached slipper limpets were also found to have higher survival rates than mussels without slipper limpets, which suffered from higher levels of predation by sea-stars (Thieltges 2005b). Chauvaud *et al.* (2000) have suggested that the impact of harmful algal blooms can be reduced where the slipper limpet is abundant. Apparently, there is a complex series of interactions within an ecosystem that results in both negative and positive effects of this invader on other components of the ecosystem (Thieltges *et al.* 2006).

Outside of Europe, *C. fornicata* occurs on the North American Pacific coast, Japan and Uruguay. It has the ability to colonize other temperate estuaries and inlets of the world, such as on the southern coastline of Australia, Tasmania and New Zealand, South Africa and South America. It is possible that it is distributed to these regions by oyster transports or as hull fouling on ships. Vigilance in the monitoring of oyster transports should aid in preventing their establishment in these regions.

In areas where *C. fornicata* has become abundant, individuals or some small chains were first found. Early reporting, if soon acted on, may thus lead to elimination. Following the 1993 European Trade agreement, the Pacific oyster, subject to some conditions, may be transported within European waters. This is likely to lead to the further spread of the slipper limpet and of other species unless consignments are carefully monitored. Despite management measures, the high dispersal ability of the slipper limpet has ensured that it spread within Europe following its establishment over a century ago. This spread has been due to the variety of vectors it is associated with, but also to natural spread of larvae and settled stages. The eastern oyster drill *Urosalpinx cinerea*, native to the northwest Atlantic coast, was introduced along with the slipper limpet to the south-east coast of Britain at about the same time. This predatory snail has no pelagic life-history stage, which reduces its natural dispersal potential. The close regulation of the movement of oysters in Britain from the areas where it occurs and the prevention of its spread within Europe even after a century appeared to demonstrate that some control measures do work. However, *U. cinerea* was recently introduced to The Netherlands with mussel imports from the United Kingdom and Ireland and appears to have established in the Oosterschelde estuary (Faasse & Ligthart 2007; 2009).

Eriocheir sinensis – the Chinese Mitten Crab

This crab's life-cycle is characterized by migration between waters of different salinities. Larvae develop in marine waters and juveniles and young adults actively migrate upstream into freshwater habitats. Two-year-old adults migrate downstream to marine conditions, which may take several months and during this they become reproductively mature. There is no native crab in Europe with a similar catadromous mode of life. *E. sinensis*'s area of origin are waters in temperate and tropical regions between Vladivostock (Russia) and southern China (Peters 1933; Panning 1938). The centre of occurrence is the Yellow Sea, a temperate region off northern China (Panning 1952). The mitten crab was first recorded outside its native range in 1912 in the German river Aller. It has been suggested that *E. sinensis* was introduced to Germany with ballast

water. In Europe, it is most abundant in estuaries adjacent to the North Sea. The first mass development was documented during the 1930s – and was followed by other mass occurrences in the 1940s, 1950s, 1980s and 1990s (Schnakenbeck 1924; Boettger 1933; Sukopp & Brande 1984; Anger 1990; Reise 1991; Michaelis & Reise 1994; Clark *et al.* 1998; Fladung pers. comm.). After the last mass occurrence the crab population declined in Germany (Strauch pers. comm.). Soon after it was first found, it spread to the Baltic coast of Germany (1926) and Poland (1928), probably via the Kiel Canal. Today it is frequently found along southern and eastern Baltic coasts up to the eastern Gulf of Finland. This is >1500 km from the German Bight, its main centre of abundance (Ojaveer *et al.* 2007). While it seems unlikely that self-sustaining populations occur in the central and eastern Baltic due to low salinities, which are unsuitable for larval development, an egg-carrying female was recently found in Lithuanian waters at very low salinity (Olenin pers. comm.). Other records of the crab in Europe are from the White Sea, Norway, Ireland, Portugal, Black and Caspian Seas, and even the French Mediterranean coast without any indication of establishment. Mitten crabs also invaded other regions of the world. They were first found in San Francisco Bay in 1992 and have since spread up and down the coast (Cohen & Carlton 1995; Rudnick *et al.* 2000). Individuals were collected in the Great Lakes from 1965 to 1994 (Nepszy & Leach 1973) and from Quebec, in the St Lawrence River (de Lafontaine 2005). A single Chinese mitten crab was collected in the Mississippi River delta in 1987 (Felder pers. comm.).

When abundant, there is considerable impact. The mitten crab preys on native species, fishes caught in traps and nets, and cultured fishes in ponds. It also has habitat structuring effects, mainly by burrowing in river embankments, causing erosion and damage to dikes. Crabs also aggregate on water-intake filters of industrial cooling water supplies and drinking water plants.

In its native range in Asia, the Chinese mitten crab is the second intermediate host for the human lung fluke parasite. The oriental lung fluke is a parasite which uses a snail as its primary host, freshwater crayfish and crabs as intermediate hosts, and a variety of mammals (including humans) as the final hosts. The fluke settles in the lungs and other parts of the body, and can cause severe bronchial illness (Ichiki *et al.* 1989). The disease is not known in Europe, but it may become established in the future.

Since its first occurrence in 1912, the crab's economic impact in Germany is estimated at €80 million (based on modified calculations of Fladung pers. comm.). These costs include catchment gear installation and maintenance, impact on bank erosion and loss in commercial fisheries and pond-aquaculture (estuaries and in-land). Chinese mitten crabs can be marketed at €1–3 per kg for industrial use and for direct human consumption on the Asian markets. During 1994–2004, crabs to the total value of approximately €3–4.5 million were sold in Germany (Gollasch & Rosenthal 2006). However, this is still much less than the costs of mitigation.

***Teredo navalis* – the Shipworm**

The description of *Teredo navalis* by Linnaeus in 1758 was based on material collected by Sellius in The Netherlands in 1730–1732. Its massive occurrence during these years

(Vrolik *et al.* 1860; Van Benthem Jutting 1943) suggests a non-indigenous origin. Mass occurrences have often been observed for non-indigenous species some years after their introduction and in several cases this resulted in their discovery (e.g. Ostenfeld 1908).

The classical authors Aristotle, Ovid, and Pliny (Vrolik *et al.* 1860), living by the Mediterranean Sea, knew of shipworms, but the species involved are not known. Almost a thousand years later, from 1516 onwards, shipworms were reported from the West Indies and Atlantic Europe (Moll 1914). Vrolik *et al.* (1860) record fossil finds from northwest Europe, but it is unclear whether these are of Holocene age and belong to this species. Moll (1914) lists only fossil finds belonging to other species. There seem to be no records of damage to Viking vessels in northern Europe (Hoppe 2002). However, in the historical museum of Haithabu (Germany), wood with boreholes from the stem of a Viking ship is on display (Minchin pers. obs.). Since this vessel was found in a freshwater environment, later colonization by marine borers can be excluded. It is unclear, however, which species created these boreholes. The first confirmed accounts of *T. navalis* in Atlantic Europe are from The Netherlands.

Van Benthem Jutting (1943) states that, before 1730, *T. navalis* occurred sporadically along the Dutch coast. She refers to Hooft (1580) who recorded damage to seawalls in Zeeland, but without identifying the cause (Moll 1914). Vrolik *et al.* (1860) cite the 'Journal des Savants de l'an 1665' and state that vessels in the IJ estuary at Amsterdam were virtually destroyed by the shipworm (however, this may be due to a different species, e.g. *Psiloteredo megotara* or *Teredo norvegica*, and the "worms" may have colonized the ships elsewhere). Martinet (1778) also records heavy damage to herring fishing vessels in 1714 and 1727. Any records before 1730 concern either unspecified damage or the occurrence of shipworms in vessels. Hence, it seems that until the eighteenth century we have no clear indication that *T. navalis* occurred in wooden structures in The Netherlands. In 1730 considerable damage to wooden constructions along seawalls was recorded from Zeeland and West-Friesland in The Netherlands (van Benthem Jutting 1943). Vrolik *et al.* (1860) record damage to seawalls in 1730, 1731, 1732, 1770, 1827, 1858 and 1859, and found a relationship between the outbreaks of *Teredo* and dry, warm summers and periods of higher salinities. In the eighteenth century, however, its occurrence in the wood constructions protecting Dutch seawalls was considered a disaster which enforced a radical and costly switch to new dike protection methods. The former wooden poles at the seaward side of the dike had to be replaced by stones imported from abroad. In the eighteenth and nineteenth century, damage to the wooden tide gates and locks was also widespread in The Netherlands and Germany. In The Netherlands a special governmental 'shipworm committee' was even installed to study causes of the problem and suggest solutions (Vrolik *et al.* 1860). The construction of the German naval base at Wilhelmshaven was seriously delayed when a protective dam constructed out of parallel pilings with earth in between them was damaged by a shipworm infestation and collapsed during a storm in January 1860 (Blackbourn 2006). Thereafter the occurrence of *Teredo* gradually declined because wood was no longer used for commercial ship building and dike construction and more resistant tropical hardwoods were being used for the doors of locks.

Recently, *T. navalis* showed up for the first time in the brackish waters of Bremerhaven in the Weser estuary, where it was most abundant in fir floating fenders (>10,000 m^2) but less abundant in fir and oak pier posts (Tuente *et al.* 2002). It is also common in Dutch coastal waters today (Wolff 2005b) and is apparently increasing in wooden coastal defense structures in the northern Wadden Sea (Reise pers. obs.). Elsewhere in the North Sea *T. navalis* still causes minor economic damage occurring in driftwood, wrecks, and wooden poles.

Van Benthem Jutting (1943) considers *T. navalis* to be a cosmopolitan species probably originating from the North Sea area, whereas eighteenth-century authors believed that ships returning from the East Indies were responsible for their introduction (e.g. Martinet 1778). However, during this period, North Sea states were trading worldwide and *T. navalis* may have been introduced from anywhere. It is for these reasons that this species is considered to be cryptogenic.

Conclusions

We presented a checklist of 167 non-indigenous and cryptogenic species in the North Sea. Shipping associated and aquaculture vectors are considered to be the dominant vectors. More than two thirds of the recorded non-indigenous species have established self-sustaining populations. The majority of non-indigenous species have localized distributions; only ten of these are known from all of the seven countries bordering the North Sea.

Crepidula fornicata, Eriocheir sinensis and *Teredo navalis* are examples of non-indigenous and cryptogenic species that have a significant impact on coastal systems of the North Sea. Reise *et al.* (1999) concluded that in the North Sea introduced species in most cases increase biodiversity without having major unwanted economic or ecological impacts. However, nowadays the introduced Pacific oyster *Crassostrea gigas* is spreading in the coastal waters of the North Sea (Reise *et al.* 2005) and is replacing the native blue mussel *Mytilus edulis*. This rapid spread is probably promoted by the recent warm summers which support the recruitment of the Pacific oyster (Diederich *et al.* 2005) and also due to the lack of cold winters which are required for good recruitment of *M. edulis*. It is assumed that the current abundance of *C. gigas* may become reduced should water temperatures decline (Nehls *et al.* 2006). However, this is unlikely because of a continuing trend of rising seawater temperatures in the region.

In the North Sea region paleoenvironmental history as well as strongly transformed modern coastal environments have contributed to a relatively low species richness. Many of the species that were introduced and tolerated the physical regime became established, increased local diversity and together considerably modified ecosystem functioning in the nearshore zone (Reise *et al.* 2006).

Plants like the introduced cordgrass *Spartina anglica* and the Japanese seaweed *Sargassum muticum* altered structural complexity, while abundant benthic filter feeders like the molluscs *Ensis directus*, *Crassostrea gigas* and *Crepidula fornicata* can be assumed

to impact regional plankton dynamics in the coastal waters. Some introduced species have the capability of re-organizing trophic relationships within an ecosystem and influencing economies both negatively and positively. Though potentially enormous, the impacts of introduced species are highly unpredictable. Those with noted impact in other temperate regions are likely to have impact in the North Sea. Others may develop unexpectedly high levels of abundance or cause disease and harm that could not be predicted. Since ballast water can carry millions of propagules which are being discharged into North Sea harbors each day, and because other vectors may further spread these species, we need an improved understanding of the vector mechanisms involved in order to reduce unwanted species introductions in the future.

The rate of invasions has increased in the North Sea (Reise *et al.* 1999), as it has increased worldwide, and it will probably continue to increase as a consequence of climate change and globalization. For each individual species, the potential number of transport vectors has also increased, e.g. the European shore crab *Carcinus maenas* is potentially dispersed by ten different vectors today, whereas 200 years ago there were two possible modes of transport and dispersal (Carlton & Cohen 2003).

Knowledge of the invasion process is essential in designing management plans to cope with the potential detrimental effects of invasive species, and to attempt to prevent their large-scale spread. The checklist of introduced species in the North Sea provided here can serve as a basis for future studies of introduced species and design of management plans in this region, but as the list will inevitably continue to grow longer, it will need to be periodically updated.

Oysters as a vector for native, cryptogenic and introduced species

Deniz Haydar
Wim J. Wolff

3

Summary

Oyster transports are one of the leading vectors of introduction of non-indigenous species worldwide. In Dutch coastal waters, oysters are the most important vector. We here investigate characteristics of this vector in order to explain its high share of introductions into The Netherlands.

We reviewed literature on oyster-associated species introductions, analyzed commercial oyster imports to The Netherlands, and collected and identified epiflora from Pacific oyster shells.

In total, 35 species are known to have been introduced to The Netherlands with oysters; the Rhodophyceae are the dominant taxonomic group introduced by this vector. The number of introductions and quantity of oysters imported are not necessarily positively correlated, particularly in the past 20 years, when quantities of imported oysters decreased but the rate of introductions increased. The epiflora of oyster shells was dominated by red seaweeds, and we found 42 taxa of introduced, native and cryptogenic macroalgal species.

This study demonstrates the high potential of oyster transports for introducing non-indigenous species. The discrepancy between oyster imports and associated introductions can be explained by unreported imports and the characteristics of this vector: a single oyster may harbor a large number of species which are introduced with their substrate, facilitating establishment. Furthermore, the recent establishment of extensive Pacific oyster reefs now provides a suitable substrate for associated species, further facilitating successful establishment even after low propagule-pressure introduction events. Ongoing shellfish translocations within Europe increase rates of introduction of non-indigenous species, promote their secondary spread and result in mixing of native and cryptogenic populations.

Introduction

Exploitation of wild oyster stocks as a food source has a long history. Due to overexploitation of wild stocks oyster cultures were established world-wide, and in order to maintain high productivity of cultures native and non-indigenous oysters were imported (Wolff 2005a). Overall, non-indigenous oysters have been introduced and established permanently in at least 24 countries outside their native ranges (Ruesink *et al.* 2005). These oyster translocations have since long been acknowledged to be one of the major vectors for the introduction of associated species (Elton 1958).

The Dutch wild stocks of the native oyster *Ostrea edulis* have extensively been fished since at least the 17th century (Smallegange 1696; Dijt 1961; Dijkema 1997). Overexploitation, introduced parasites, severe winters and changing hydrographic conditions resulted in the near extirpation of *O. edulis* from the Wadden Sea and the estuaries in the Southwest Netherlands (Drinkwaard 1999; Wolff & Reise 2002). To compensate for depleted *O. edulis* stocks, oysters of various species were imported from other regions into The Netherlands as early as the 18th century (Wolff & Reise 2002). *O. edulis* and the Portuguese oyster *Crassostrea angulata* were imported from other European countries and the American oyster *Crassotrea virginica* was imported from the USA. Imports of Portuguese and American oysters did not result in establishment of these species in Dutch coastal waters (Wolff & Reise 2002; Wolff 2005b). The Pacific oyster, *Crassostrea gigas*, was imported as spat or seed (newly settled oysters) into the Oosterschelde estuary from British Columbia, Canada, and from Japan, starting in 1964 (Shatkin *et al.* 1997; Drinkwaard 1999) and adult Pacific oysters were imported in 1971 (Wolff & Reise 2002). These imports were successful; nowadays the Pacific oyster is the most important cultured oyster in The Netherlands. *C. gigas* is no longer restricted to the culture plots: it has established reproducing populations in the Oosterschelde estuary, the center of oyster culture in The Netherlands, after strong spatfalls in 1976 and 1982. *C. gigas* has also spread to the Wadden Sea, possibly as a deliberate introduction to the island of Texel in the western Wadden Sea (Tydeman 2008), from where it spread east. The Pacific oyster was also deliberately introduced to the island of Sylt in the German Wadden Sea, from where it spread north and south, and since the year 2000 the entire Wadden Sea has been colonized (Wehrmann *et al.* 2000). The Pacific oyster now forms extensive and dense intertidal and subtidal beds and reefs in Dutch coastal waters (Dankers *et al.* 2006; Smaal *et al.* 2009; Troost 2010).

Although only the Pacific oyster was successful in establishing in Dutch coastal waters, imports of this and other oyster species into Europe are known to have resulted in the introduction of a number of associated species, e.g. the slipper limpet *Crepidula fornicata*, introduced with American oysters (Blanchard 1997, Chapter 2), and the brown seaweed *Sargassum muticum*, imported with Pacific oysters (Critchley *et al.* 1990). Ongoing movements of oysters within European waters are believed to have resulted in rapid secondary spread of introduced species (Grizel & Héral 1991). Oyster imports are suggested to be one of the most important single vectors for the introduction of non-indigenous marine and estuarine species in The Netherlands (Wolff 2005b).

Many first records of introduced species are from the Oosterschelde estuary, near areas of extensive oyster culture (Maggs & Stegenga 1999; Wolff 2005b). Possible explanations for the high share of oyster-associated introductions are the underestimation of the number of non-indigenous species that were historically introduced as hull fouling (Chapter 4), and the fact that The Netherlands mostly export ballast water rather than receive large quantities of ballast water from other regions (AquaSense 1998). More significantly, the frequency and scale of oyster translocations and the characteristics of this vector - the rugged shells of oysters offer ample opportunities for epiflora and –fauna to settle and survive transport to other regions - may also explain the relatively high number of oyster-associated introductions.

This suggested importance of oyster transports for the introduction of non-indigenous species in The Netherlands is in contrast with several studies from other regions, which suggest that shipping-associated vectors, either hull fouling or ballast water, are the most important anthropogenic vectors of introduction (Ruiz *et al.* 2000; Hewitt *et al.* 2004). This higher relative importance of shipping in the introduction of non-indigenous species has also been postulated for the North Sea region (Gollasch 2002). Therefore, in this paper we aim to determine the potential of oyster transports as a vector for the introduction of associated species into Dutch coastal waters.

First, we review literature on oyster-associated species introductions into The Netherlands to quantify the importance of this anthropogenic vector and to look for patterns. Second, we investigate data on commercial oyster imports into The Netherlands to see if their frequency and magnitude supports the putative high importance of oyster transports for associated species introductions. Third, by collecting and identifying epiflora from the shells of live Pacific oysters from the Oosterschelde estuary we investigate the capacity of individual Pacific oysters to act as a vector for the introduction of non-indigenous species.

Materials and Methods

Oyster-associated introductions

Based on published literature, an overview was created of marine flora and fauna hypothesized or known to have been introduced to Dutch coastal waters with oyster imports. We included species that were directly imported from other biogeographic regions - outside the Northern European Seas and the South European Atlantic Shelf (Spalding *et al.* 2007) - into The Netherlands with oysters, as well as those that were first introduced elsewhere in Europe with oysters and may have spread to The Netherlands by natural dispersal or with anthropogenic vectors. This overview includes the year of first observation, current distribution, the native range, and possible vectors of introduction. For each species, the year of first observation in Europe and in The Netherlands is given, as well as the supposed vector of introduction into The Netherlands and Europe.

Oyster imports in The Netherlands

Quantitative data on oysters imported commercially from various countries from 1960 to 2008 were obtained from Statistics Netherlands (CBS). The obtained data had been categorized differently over the years. From 1960 to 1974 imported oysters were characterized as either "seed oysters" or "other oysters". After 1974 a distinction was made between "live *Ostrea* spp. smaller than 40g" and "other oysters". In both periods the category of "other oysters" included all oyster species that are imported alive, fresh, cooled, frozen, dried, salted, or pickled, and thus included oysters intended for direct consumption as well as oysters that were relaid in recipient waters for storage and/or growth. To show the amount of oysters imported we present the quantity of each category of imported oysters per year. For the analysis of origins of annual oyster imports we pooled all data, not making a distinction between the "seed oysters", "live *Ostrea* spp. smaller than 40g", and "other oysters", since the data did not always allow to make this distinction.

Oyster epiflora

Pacific oysters (*C. gigas*) were dredged from culture plots in the Oosterschelde estuary by a commercial oyster grower. Nine samples of oysters were collected in the months May 2003, September to December 2003, February to March 2004 and June 2004. After dredging by the commercial oyster grower oysters were handled as if they were to be transported to other regions, either for direct sale, or for (temporary) relaying in other estuaries. This meant that the oysters were superficially cleaned by hand. Next they were transported to the laboratory in a cooler and kept at 11°C for three days in a moist environment in order to mimic transport conditions as they are encountered when oysters are moved between culture sites in different parts of Europe.

After this, epiflora was collected from 30 individual oysters per sample under a stereo-microscope, fixed in 4% formalin and mounted for permanent preservation on microscopic slides in a syrup medium (Stevenson 1984). Some algae were stained as reference material using Fast Green FCF. Slides were examined microscopically (magnification 100–200×) and species were identified with the help of a phycological expert. Furthermore, we obtained distributional data for encountered algae from AlgaeBase (Guiry & Guiry 2010). The epiflora of a total number of 270 oysters was analyzed. Data were grouped for all oysters, resulting in the percentage of the total number of oysters carrying individual species of algae, and seasonal occurrence of the most common species on oyster shells. Epifauna was collected as well but the data were not analyzed in detail because of major taxonomic uncertainties presented by the often tiny specimens, and therefore these data are not presented here.

In order to evaluate our sampling effort, we created a species accumulation curve for all 270 oysters analyzed. To this end we assigned random numbers to individual oysters and plotted cumulative numbers of species by adding the new species found in consecutive randomly-numbered oysters. The species accumulation curve was fitted and extrapolated to calculate expected species richness using the equation

$$Cum. = \frac{aN}{(1 + bN)}$$

where *Cum.* is the cumulative number of species, *N* is the number of oysters and *a* and *b* are constants; *a/b* is the maximum species richness for a very large number of oysters (Bunge & Fitzpatrick 1993). The equation was rewritten as

$$\frac{N}{Cum.} = \left(\frac{b}{a}\right)N + \left(\frac{1}{a}\right)$$

and was fitted by linear regression with least squares to estimate *a* and *b*.

In order to check whether any propagules or individuals were present on the shells that would be too small and would be missed by only visually checking and collecting epiflora, an additional sample of about 30 oysters was taken in February 2005. The animals were removed from their shells, and the valves were kept in experimental tanks at 12°C. We analyzed the total epiflora growing on the valves after one month for presence of species that we had not recorded in our monthly samples of oysters that were analyzed three days after collection.

Results

Oyster-associated introductions

Table 3.1 presents a list of established non-indigenous plant and animal species in The Netherlands that are known or supposed to be associated with oyster translocations. A total of 35 non-indigenous species associated with oyster translocations have become established in The Netherlands. The time elapsed between the first observation elsewhere in Europe and the first observation of the same species in The Netherlands ranges from 1 year (*Coscinodiscus wailesii*) to 128 years (*Polysiphonia harveyi*). The average time between the first introduction elsewhere in Europe and the first record of the same species in The Netherlands is 29 years; 57% are recorded within 20 years after their first introduction in Europe. For four species the primary northwestern European introduction occurred in The Netherlands (*Smittoidea prolifica, Colaconema daviesii, Dasya baillouviana* and *Polysiphonia senticulosa*).

The Rhodophyceae represent the largest taxonomic group introduced by this vector with 39% of all introductions belonging to this taxonomic group. Of all associated introductions 45% originate from the Northwest Pacific and these are assumed to have been introduced with Pacific oyster imports, either directly from Japan or via British Columbia and/or France. The Northwest Atlantic is the origin of 20% of oyster-associated introductions; these species are supposed to have been introduced with shipments of the American oyster *C. virginica*.

The numbers of first observations of oyster-associated introductions changed over time. In Figure 3.1 the number of established introductions is presented per decade from 1891 to 2009. There is an increasing trend in the rate of introductions, with a peak of 9 species that were first observed between 1991 and 2000. These all originate from the Northwest Pacific and were presumably introduced with *C. gigas*.

Table 3.1 List of (supposed) oyster-associated introduced species in The Netherlands (NL). For each species, the year of first introduction in Europe and in The Netherlands is given, as well as the supposed vector of introduction to The Netherlands and Europe. Detailed distributional information was obtained from the literature; key references are indicated in the last column. The "origin" column gives the presumed region this species originates from.

taxonomic group	species	distribution	origin	1st record Europe	vector	1st record NL	vector	comments and key references
Protista	*Bonamia ostreae* Pichot, Comps, Tigé, Grizel & Rabouin, 1979	Netherlands, Spain, France? NE Pacific, California	NE Pacific	1977	oysters	1980	oysters	(Wolff 2005b)
Coscinodiscophyceae	*Coscinodiscus wailesii* Gran & Angst, 1931	North Sea, English Channel, Atlantic coast of France, Indo-Pacific	Indo-Pacific	1977	oysters, ballast water	1978	natural	(Gollasch *et al.* 2008) dispersal
Bacillariophyceae	*Thalassiosira punctigera* (Castracane) Hasle, 1983	North Sea, English Channel, S Atlantic, Indo-Pacific	S Atlantic, Indo-Pacifc	1978	oysters	1981	natural dispersal	(Wolff 2005b)
Phaeophyceae	*Undaria pinnatifida* (Harvey) Suringar, 1873	North Sea, Spain, Portugal, Mediterranean, California, Mexico, Argentina, NW Pacific, Australia, New Zealand	NW Pacific	1983	oysters, deliberate introduction	1999	oysters	(Verlaque 2001)
	Colpomenia peregrina Sauvageau, 1927	NE Atlantic S to Morocco, Mediterranean, N Pacific, New Zealand, Australia	NE Pacific	1906	oysters	1921	oysters, natural dispersal	introduced with American oysters from Pacific coast US (Wolff 2005b)
	Sargassum muticum (Yendo) Fensholt, 1955	North Sea, Baltic Sea, S to Spain and Portugal, Mediterranean, Hawaii, N Pacific	NW Pacific	1966	oysters	1980	natural dispersal	(Critchley & Dijkema 1984)
Rhodophyceae	*Polysiphonia senticulosa* Harvey, 1862	Netherlands, Belgium, S England, Washington State, British Columbia, Japan, New Zealand, S Australia	NW Pacific	1993	oysters, hull fouling	1993	oysters, hull fouling	(Maggs & Stegenga 1999; Guiry & Guiry 2009)
	Polysiphonia harveyi J. Bailey 1848	North Sea, Atlantic Europe, Newfoundland to South Carolina, California, Japan, New Zealand	NW Pacific	1832	oysters	1960	oysters, natural dispersal	suggested introduction with *Crassostrea angulata* or with *Codium fragile* (McIvor *et al.* 2001)

Grateloupia turuturu Yamada, 1941	NW Atlantic, NE Atlantic, Mediterranean, Japan	NW Pacific	1969	oysters	1993	oysters, hull fouling	initially described as *G. doryphora* (Maggs & Stegenga 1999; Gavio & Fredericq 2002)
Heterosiphnia japonica Yendo, 1920	North Sea, Atlantic coast France, Spain, Mediterranean, Alaska, California, NW Pacific	NW Pacific	1990	oysters	1994	oysters	initially described as *Dasysiphonia* sp. (Maggs & Stegenga 1999; Verlaque 2001; Guiry & Guiry 2009)
Dasya baillouviana (S.G. Gmelin) Montagne, 1841	cosmopolitan	NW Atlantic	1950	oysters, hull fouling	1950	oysters, hull fouling	(Maggs & Stegenga 1999; Guiry & Guiry 2009)
Colaconema daviesii (Dillwyn) Stegenga, 1985	cosmopolitan	NW Atlantic	1950	oysters, hull fouling	1950	oysters, hull fouling	introduced with *D. baillouviana*, epifytic
Bonnemaisonia hamifera Hariot, 1891	NE Atlantic, Mediterranean, NW Atlantic, New Brunswick, California, Mexico, S Africa, Japan	NW Pacific	1890	oysters	1983	oysters, natural dispersal	(Maggs & Stegenga 1999; Guiry & Guiry 2009)
Lomentaria hakodatensis Yendo, 1920	The Netherlands, Atlantic coast France, Mediterranean, Japan	NW Pacific	1984	oysters	2004	oysters	(Stegenga 2004)
Asparagopsis armata Harvey, 1855	NE Atlantic S to Senegal, Mediterranean, Adriatic, California, S Africa, Australia, New Zealand	S Pacific	1925	oysters	1983	natural dispersal	(Maggs & Stegenga 1999; Guiry & Guiry 2009)
Antithamnionella spirographidis (Schiffner) E.M. Wollaston, 1968	NE Atlantic, Mediterranean, Adriatic, S Africa, Australia, N Pacific	N Pacific	1906	hull fouling	1974	oysters	(Maggs & Stegenga 1999; Wolff 2005b; Guiry & Guiry 2009)
Antithamnionella ternifolia (J.D. Hooker & Harvey) Lyle, 1922	NE Atlantic Scotland S to Portugal, Chile, China, S Africa, Australia, New Zealand	S Pacific	1906	hull fouling	1951	oysters	(Eno *et al.* 1997; Maggs & Stegenga 1999; Wolff 2005b; Guiry & Guiry 2009)
Anotrichium furcellatum (J. Agardh) Baldock, 1976	The Netherlands, France, Spain, Portugal, Atlantic islands, Mediterranean, California, S Africa, Japan, Korea	Medi-terranean?	1922	oysters?	1968	oysters?	unknown whether it is established in the North Sea (Wolff 2005b; Guiry & Guiry 2009)

Table 3.1 Continued

taxonomic group	species	distribution	origin	1st record Europe	vector	1st record NL	vector	comments and key references
	Agardhiella subulata (C. Agardh) Kraft & M.J. Wynne, 1979	N and S Atlantic, Mediterranean, Indian ocean, N Pacific	NW Pacific or NW Atlantic	1973	oysters, hull fouling	1998	oysters, hull fouling	cryptogenic, but introduced from Japan (Wolff 2005b; Guiry & Guiry 2009)
Polychaeta	*Polydora hoplura* Claparède, 1869	cosmopolitan	unknown	1869	oysters, hull fouling	1949	oysters	cryptogenic (Korringa 1951)
	Proceraea cornuta (Agassiz, 1884)	North Sea, British Isles, NW Atlantic, NE Pacific	NW Atlantic	1929	hull fouling	1941	oysters	(Wolff 2005b)
Anthozoa	*Diadumene cincta* Stephenson, 1925	Netherlands, England, Germany, warm-temperate Atlantic?	unknown	1920	oysters, hull fouling	1925	oysters, hull fouling	cryptogenic (Gollasch *et al.* 2008)
	Diadumene lineata (Verrill, 1869)	cosmopolitan N Atlantic, N Pacific, Indonesia, New Zealand, Hawaiian Island	NW Pacific	1896	oysters, hull fouling	1912	oysters, hull fouling	(Carlton 1979; Gollasch & Riemann-Zürneck 1996)
Hydrozoa	*Gonionemus vertens* Agassiz, 1862	North Sea, British Isles, Brittany, Portugal, NW Atlantic, San Francisco Bay, NW Pacific	NW Pacific	1913	oysters, hull fouling	1960	oysters, hull fouling	(Chapter 4)
Gymnolaemata	*Smittoidea prolifica* Osburn, 1952	The Netherlands, NE Pacific	NE Pacific	1995	oysters	1995	oysters	Probably more widespread, but misidentified as native species (de Blauwe & Faasse 2004)
Maxillopoda	*Myicola ostreae* Hoshina & Sugiura, 1953	The Netherlands, France, Japan, Korea	NW Pacific	1972	oysters	1992	oysters	(Wolff 2005b)
	Mytilicola orientalis Mori, 1935	The Netherlands, France, British Columbia, Japan	NW Pacific	1977	oysters	1992	oysters	(Wolff 2005b)
Malacostraca	*Hemigrapsus takanoi* Asakura & Watanabe, 2005	North Sea, France, Spain, Japan to China	NW Pacific	1994	oysters, hull fouling	2000	oysters, hull fouling	first identified as *Hemigrapsus penicillatus* (Gollasch 1999)

Gastropoda	*Urosalpinx cinerea* (Say, 1822)	The Netherlands, England, NW Atlantic, Washington to California	NW Atlantic	1927	oysters	2007	mussels	(Faasse & Ligthart 2007)
	Rapana venosa (Valenciennes, 1846)	The Netherlands, Belgium, Atlantic coast France, Adriatic Sea, Aegean Sea, Black Sea, Chesapeake Bay, Rio de la Plata, Japan, China	NW Pacific	1990s	oysters	2005	oysters, natural dispersal	(Mann & Harding 2000; Kerckhof *et al.* 2006)
	Pteropurpura (*Ocinebrellus*) inornatus (Récluz, 1851)	Japan, Korea, China, Washington to California, Brittany, The Netherlands	NW Pacific	1985	oysters	2007	oysters	(Martel *et al.* 2004; Faasse & Ligthart 2009)
	Crepidula fornicata (Linnaeus, 1758)	N Atlantic, Mediterranean, NE Pacific, Uruguay, South America, South Africa, Japan, Australia, Tasmania, New Zealand	NW Atlantic	1872	oysters	1926	oysters, natural dispersal	(Gollasch *et al.* 2008)
Bivalvia	*Petricolaria pholadiformis* (Lamarck, 1818)	N Atlantic, Mediterranean, Black Sea, Gulf of Mexico, E Pacific	NW Atlantic	1890	oysters	1899	oysters, natural dispersal	(Chapter 4)
	Mercenaria mercenaria (Linnaeus, 1758)	The Netherlands, Belgium, S British Isles, France, NW Atlantic, Gulf of Mexico, NE Pacific, China	NW Atlantic	1864	deliberate introduction	1934	oysters, deliberate introduction	(Goulletquer *et al.* 2002; Wolff 2005b; Chapter 4)
Ascidiacea	*Styela clava* Herdman, 1881	North Sea, Spain, Portugal, Gulf of St Lawrence to New England, California, Sea of Okhotsk, Siberia, Japan, Korea	NW Pacific	1952	oysters, hull fouling	1974	oysters	(Lützen 1999)

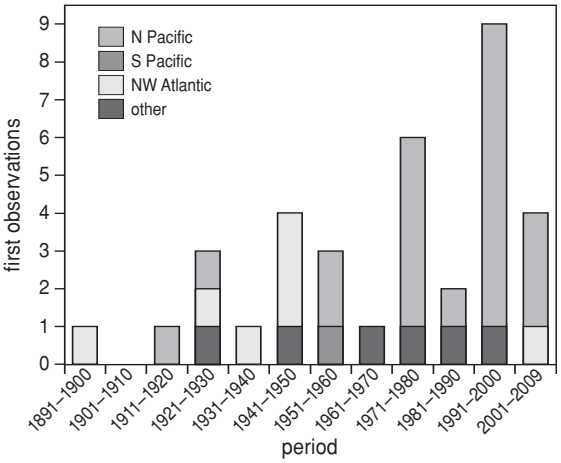

Figure 3.1 The number of established oyster-associated introductions in The Netherlands per decade from 1891 to 2009. Dates of first observations of introduced species that are now established in Dutch coastal waters were used in creating this graph. Regions of origin for the established introductions are indicated, see legend.

Oyster imports

The commercial oyster import data from 1960 to 2008, obtained from CBS, are presented in Figures 3.2 and 3.3. Quantities of imported oysters differed greatly among years, with a maximum of nearly 3,000,000 kg in 1971 and 1972, of which more than half was oyster seed. After 1982 imports of oysters into The Netherlands declined, but since 2002 we again see an increase in the amount of oysters imported. The imported oysters are categorized as 'oyster seed' or '*Ostrea* spp. smaller than 40g', and 'other oysters'. Although the definition of the categories suggests that Crass*ostrea* seed (*C. angulata*, *C. gigas* and possibly *C. virginica*) is included in the latter category, this is not certain, and Crass*ostrea* seed may have been reported as '*Ostrea* spp. smaller than 40g' as well. The 'other oysters' category includes larger live oysters of different *Ostrea* and Crass*ostrea* species. However, since these are pooled with oysters that are directly sold for consumption it is not known which proportion of this category was relaid in Dutch waters (Fig. 3.2).

The areas of origin of the imported oysters changed over time. Until the 1970s, oysters were mainly imported from France and Portugal. This includes imports of the non-indigenous Portuguese oyster *C. angulata*. After 1980, the British Isles and other North Sea countries (Denmark, Norway, Belgium and Germany) became the predominant origins for imported oysters. Quantities of oysters directly imported from the Northwest Pacific (Japan, Korea) were very small, ranging from 227 to 3,383 kg, and the reported imports occurred infrequently (1974, 1989, 1996, 1997, 1999 - 2003), but becoming more frequent since 1996. Between 1960 and 2008 reported imports of oysters from Canada occurred in 1998 and 1999, and only amounted to a total of 2,678 kg.

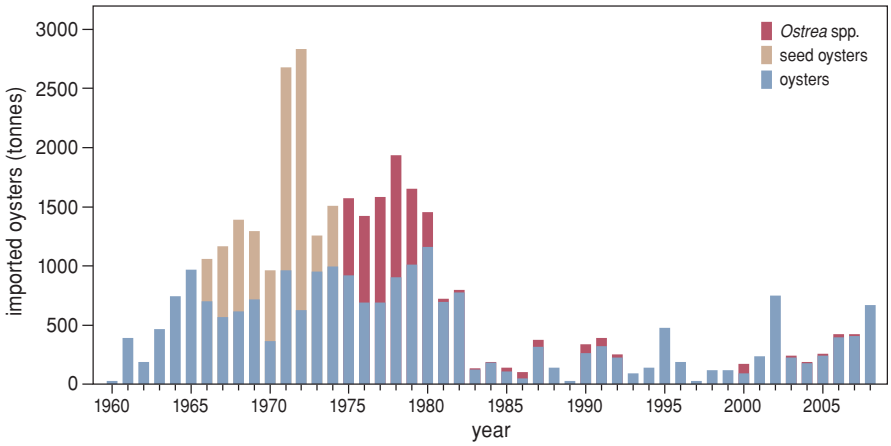

Figure 3.2 Oyster imports into The Netherlands from 1960 to 2008. Data were obtained from Statistics Netherlands (CBS). Imports were split in oysters (live, fresh, dried, pickled, frozen, larger than 40g.), seed oysters and *Ostrea* spp. smaller than 40g. The latter two categories are both intended for restocking culture plots, the "oysters" category includes oysters imported for direct sale and consumption, and oysters that are relaid in Dutch waters.

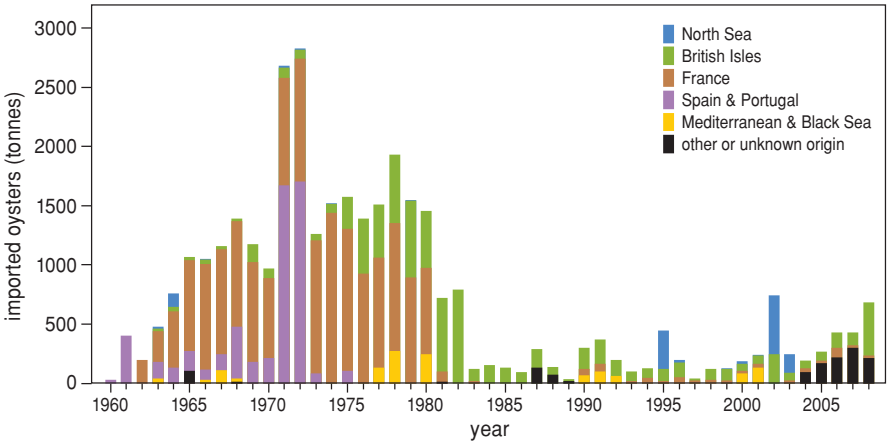

Figure 3.3 Oyster imports to The Netherlands from 1960 to 2008. Data were obtained from Statistics Netherlands (CBS). Regions of origin for imported oysters are shown, see legend. NB: The "North Sea" does not include the east coast of the British Isles.

Oyster epiflora

A total of 41 taxa of macroalgae, viz. two brown algae, ten green algae and 29 red algae, was identified from 270 analyzed oyster shells; 36 of these were identified to species level (Table 3.2). The number of macroalgal species found on a single oyster ranged from 0 to 14, with an average of 4 species per oyster.

Table 3.2 Macroalgal species recorded from Pacific oyster shells from the Oosterschelde estuary. The presence of each species on examined oysters is indicated as a percentage of the total number of oysters analyzed (n = 270). The distribution of each species was obtained from AlgaeBase (Guiry & Guiry 2009). A status was assigned to each species: native, introduced (those species that are known to have been introduced into the Northeast Atlantic), and cryptogenic (those species for which a native or introduced status can not be demonstrated without further investigation). The cryptogenic status was assigned based on association with oysters proven here and a disjunct distribution pattern based on literature. For species that occur on more than 15% of all examined oysters the percentage of oysters they occur on is printed bold, these species are included in Fig. 3.5.

species	% of oysters	distribution	status
Phaeophyceae			
Dictyota dichotoma (Hudson) J.V. Lamouroux, 1809	8	cosmopolitan	cryptogenic
Laminaria saccharina (Linnaeus) J.V. Lamouroux, 1813	2	N Atlantic, Baltic, Madeira, NE Pacific	cryptogenic
Chlorophyceae			
Codium fragile (Suringar) Hariot, 1889	4	cosmopolitan	introduced
Ulva prolifera O.F. Müller 1778	0.4	cosmopolitan	cryptogenic
Ulva clathrata (Roth) C. Agardh, 1811	1	cosmopolitan	cryptogenic
tubular *Ulva* sp.	10	n.a.	n.a.
foliose *Ulva* sp.	19	n.a.	n.a.
Cladophora rupestris (Linnaeus) Kützing, 1843	1	cosmopolitan	cryptogenic
Cladophora sp.	3	n.a.	n.a.
Rhizoclonium implexum (Dillwyn) Kützing, 1845	12	cosmopolitan	cryptogenic
Monostroma oxyspermum (Kützing) Doty, 1947	4	N Atlantic, Caribbean, NE Pacific, S Australia	cryptogenic
Prasiola stipitata Suhr ex Jessen, 1848	0.4	NE Atlantic, NW Atlantic, Chile, Australia, New Zealand	cryptogenic
Rhodophyceae			
Agardhiella subulata (C. Agardh) Kraft and M.J. Wynne, 1979	17	N and S Atlantic, Mediterranean, Indian Ocean, N Pacific	introduced
Antithamnionella spirographidis (Schiffner) E.M. Wollaston, 1968	22	NE Atlantic, Mediterranean, Adriatic, Morocco, N Pacific, S Africa, Australia	introduced
Antithamnionella ternifolia (J.D. Hooker and Harvey) Lyle, 1922	0.4	NE Atlantic Scotland S to Portugal, Chile, China, S Africa, Australia, New Zealand	introduced
Heterosiphonia japonica Yendo, 1920	54	North Sea, Atlantic coast France, Spain, Mediterranean, Alaska, California, NW Pacific	introduced
Neosiphonia harveyi (J. Bailey) M.-S. Kim, H.-G. Choi, Guiry and G.W. Saunders 2001	1	North Sea, Atlantic Europe, Newfoundland to South Carolina, California, Japan, New Zealand	introduced
Polysiphonia senticulosa Harvey, 1862	21	Netherlands, Belgium, S England, Washington State, British Columbia, Japan, New Zealand, S Australia	introduced
Polysiphonia nigra (Hudson) Batters, 1902	31	NE Atlantic Spitsbergen S to Portugal, Atlantic Islands, Namibia, New Hampshire	cryptogenic

Table 3.2 Continued

species	% of oysters	distribution	status
Polysiphonia fucoides Harvey, 1862	30	N and S Atlantic, Mediterranean	native
Polysiphonia devoniensis Maggs and Hommersand, 1993	0.4	N Atlantic	native
Polysiphonia stricta (Dillwyn) Greville, 1824	13	N and S Atlantic, Baltic, Black Sea, Mediterranean, Adriatic, N Pacific, Antarctic	cryptogenic
Polysiphonia denudata (Dillwyn) Greville ex Harvey, 1833	4	N and S Atlantic, Mediterranean, Adriatic, Black Sea, Persian Gulf, W Indian Ocean, S Australia	cryptogenic
Polysiphonia briodiae (Dillwyn) Sprengel, 1827	1	N Atlantic, Baltic, Mediterranean, W Indian Ocean, NE Pacific, Australia, New Zealand	cryptogenic
Polysiphonia sp.	11	n.a.	n.a.
Dasya baillouviana (S.G. Gmelin) Montagne, 1841	20	cosmopolitan	introduced
Erythrotrichia carnea (Dillwyn) J. Agardh, 1883	26	cosmopolitan	cryptogenic
Stylonema alsidii (Zanardini) K.M. Drew, 1956	10	cosmopolitan	cryptogenic
Colaconema daviesii (Dillwyn) Stegenga, 1985	6	cosmopolitan	introduced
Hypoglossum hypoglossoides (Steckhouse) Collins and Hurvey, 1919	7	NE Atlantic North Sea S to Senegal, Mediterranean, N Carolina to Florida, Caribbean, Australia, New Zealand	cryptogenic
Antithamnion villosum (Kützing) Athanasiadis, 1993	7	NE Atlantic, Baltic, N Carolina	cryptogenic
Antithamnion cruciatum (C. Agardh) Nägeli, 1847	1	NE Atlantic, Mediterranean, Baltic, Adriatic, Black Sea, NW Atlantic, Chile, China, S Australia	cryptogenic
Pterothamnion plumula (J. Ellis) Nägeli, 1855	16	NE Atlantic, Baltic, Atlantic Islands, Adriatic, Black Sea, Chile W Indian Ocean, Fiji, NE Pacific, Macquarie Island	cryptogenic
Aglaothamnion feldmanniae Halos, 1965	0.4	NE Atlantic, Mediterranean	native
Callithamnion pseudobyssoides P.L. Crouan and H.M. Crouan, 1867	1	NE Atlantic, Bermuda, N and S Carolina, Florida, S Australia	cryptogenic
Callithamnion corymbosum (Smith) Lyngbye, 1819	1	NE Atlantic, Baltic, , Atlantic Islands, Mediterranean, Adriatic, Black Sea, Virginia, Jamaica, Brazil, China, Korea, Japan	cryptogenic
Callithamnion tetricum (Dillwyn) S. F. Gray, 1821	1	NE Atlantic, Morocco, Azores	native
Ceramium nodulosum (Lightfoot) Ducluzeau, 1806	2	NE Atlantic, Baltic, Italy, Madeira	native
Ceramium cimbricum H.E. Petersen, 1924	3	cosmopolitan	cryptogenic
Chondrus crispus Stackhouse, 1797	1	NE Atlantic, Baltic, Atlantic islands, Mediterranean, Angola, Ghana, NW Atlantic, Alaska, Oregon, Falkland Islands, Antarctic Peninsula	cryptogenic
Lomentaria clavellosa (Turner) Gaillon, 1828	1	NE Atlantic, Madeira, Mediterranean, New Hampshire, Brazil, Falkland Islands	cryptogenic

The species accumulation curve (Fig. 3.4) shows the observed cumulative number of macroalgal species up to 270 oysters sampled, and the expected cumulative number of species up to 500 samples. The expected cumulative number of species was computed using linear regression by fitting with least squares, resulting in

$$\frac{N}{Cum.} = 0.0226 + 05332 \quad (R^2 = 0.9979).$$

The estimates of the parameters a and b were obtained from this equation, and were used to compute the expected maximum number of species (the asymptote (a/b)). The cumulative number of species observed on 270 oysters was 41; the expected maximum species richness was 44. Analyzing 230 additional oysters would have resulted in one additional species discovered, and thus we fairly well captured the species diversity present.

All identified species had already been reported from Dutch coastal waters. The majority of the identified macroalgae are considered native; nine species are known to have been introduced. In Table 3.2 we included distribution records of all species, and based on their distributions and their association with oysters and other anthropogenic vectors, we assigned a cryptogenic status to 23 species. Of the ten species that occur on more than 15% of all oysters, five are introduced (*Heterosiphonia japonica, P. senticulosa, D. baillouviana, Anthithamnionella spirographidis, Agardhiella subulata*). *H. japonica* was by far the most common species: it occurred on 54% of all examined oysters.

Samples were taken year-round and were grouped in 'Spring & Summer' and 'Fall & Winter'. Seasonality of ten algal species that occurred on more than 15% of all

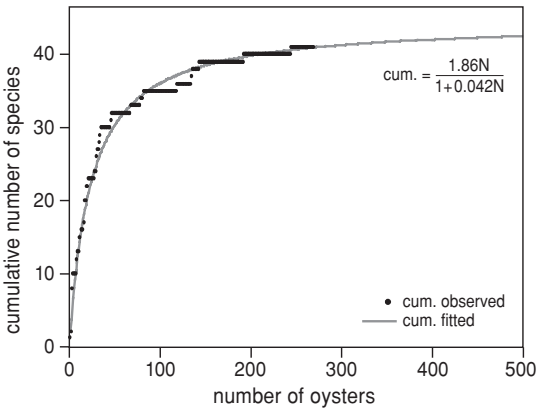

Figure 3.4 Species accumulation curve for the macroalgal taxa encountered in the epiflcora of oyster shells. The dots indicate the cumulative number of species found on randomly numbered oyster shells, the grey line is the estimated cumulative number of species up to 500 individual oysters $Cum. = \frac{1.86N}{(1 + 0.042N)}$, and the dotted line is the maximum expected number of taxa.
See text for explanation of the calculation of the fitted curve.

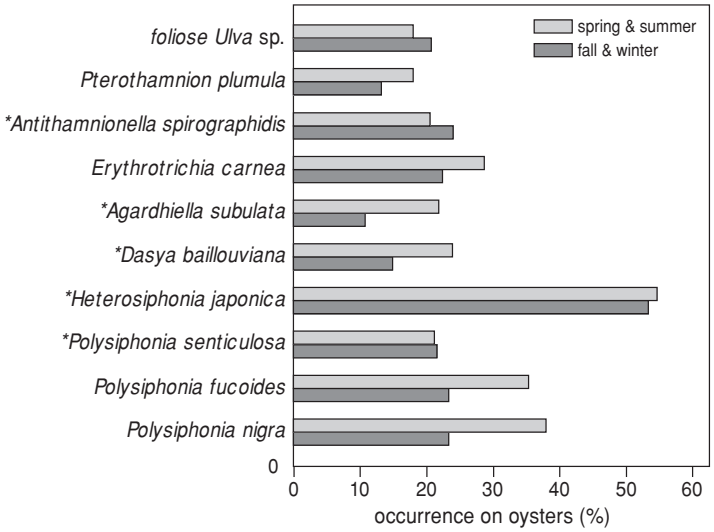

Figure 3.5 Seasonality of the most abundant identified macroalgae from oyster shells (occurring on more than 15% of all oysters examined). The frequency of occurrence of a species is indicated as a percentage of examined oysters on which this species was present. 'Fall & Winter' includes samples taken in October, November, December and February (n = 120); 'Spring & Summer' includes March, April, May, June and September (n = 150). An * indicates introduced species.

oysters is presented in Figure 3.5. The majority of species is more common in 'Spring & Summer' than in 'Fall & Winter'. Three taxa show a higher occurrence in the colder months: foliose *Ulva* sp., *A. spirographidis*, and *P. senticulosa*. However, differences between 'Spring & Summer' and 'Fall & Winter' are small. The most common species, *H. japonica*, is very abundant year round.

Culturing of the algae in seawater tanks for one month resulted in high growth of opportunistic species, particularly tubulose *Ulva* spp. However, it did not yield any species that had not been encountered previously on the 270 oysters of which the epiflora was collected and identified without culturing.

Discussion

The list of 35 oyster-associated introductions presented here is the most up-to-date list of invertebrates and algae that have been introduced to The Netherlands through imports of oysters (Table 3.1). Detailed knowledge of the impact of these introduced species on the receiving ecosystem is lacking, but some associated introductions negatively impact shellfisheries as they overgrow oysters (e.g. *H. japonica*, pers. comm. A. Cornelisse) or hamper growth (*Polydora hoplura*) (Korringa 1951; Royer *et al.* 2006) and cause mortality of oysters (*Bonamia ostrea*e in *O. edulis*) (Culloty *et al.* 1999). The oyster-

associated species were introduced with imports of *O. edulis*, *C. virginica* and *C. gigas*. *C. gigas* imports are responsible for the largest number of associated established introductions; these mostly originate from the Northwest Pacific. Natural dispersal from a site of primary introduction in Europe to Dutch coastal waters is possible for 10 of the total of 35 oyster-associated introductions, but most secondary introductions are likely to have been the result of oyster movements. Secondary spread of introduced species within Europe is accelerated by ongoing oyster transports between European culture areas.

The Rhodophyceae constitute the largest taxonomic group introduced with this vector, and they were also the most common group of macroalgae found on the shells of oysters examined for their epiflora in this study. The oyster shells provided a substrate for both introduced and native algal species; nine of the 41 macroalgal taxa are introduced. Of the most common species on the oyster shells 50% were introduced species, and the most common species, *H. japonica*, is a non-indigenous species. However, our results show that Pacific oysters not only form a habitat for introduced species, but also for native and cryptogenic flora. Movements of oysters within Europe not only accelerate secondary spread of introduced algae, but may also result in (further) regional mixing of native and cryptogenic populations.

Our sampling effort very well captured the diversity of macroalgae estimated to be present on oyster shells (Fig. 3.4). Adding more oysters to our analyses would not greatly have increased the number of species, as the estimated maximum species richness on oyster shells from the Oosterschelde estuary was 44 taxa. Investigating another 200 or 300 oysters would only have added one or two rare taxa. The relationship found also predicts that commercial oyster shipments containing thousands of oysters from the Oosterschelde estuary will contain about 44 species of macroalgae.

Other authors have also studied the occurrence of algae and invertebrates on oyster shells. Korringa (1951) made an inventory of the epifauna of oysters (*O. edulis*) from the Oosterschelde estuary. He listed 134 species from shells that were not cleaned, which may explain the high number of taxa found. Schodduyn (1931) studied epiflora as well as epifauna of oysters (*O. edulis*) transferred from the British Isles to northern France. He found 52 species of invertebrates and 14 species of macroalgae, of which nine were red algae, but unfortunately Schodduyn paid relatively little attention to the macroalgae. More recently, Mineur *et al.* (2007) investigated the epiflora on valves of Pacific oysters from the Thau Lagoon at the Mediterranean coast of France. These oyster shells were also cleaned before analysis. The number of macroalgal species recorded from our oysters (36 identified species and five higher taxa, see Table 3.2) is lower than the number of species found on Thau lagoon oysters (46 species). This can be explained by the fact that the Thau Lagoon is richer in macroalgal species than the Oosterschelde estuary (Stegenga *et al.* 1997; Verlaque 2001), and harbors an exceptionally high number of introduced macroalgae, particularly from the Northwest Pacific (Verlaque 2001). The epifloral communities were comparable regarding taxonomic groups and species composition. On the Thau Lagoon oysters the Rhodophyceae were also the dominant group and 17 species recorded on oysters from the Oosterschelde estuary were also found on Thau Lagoon oysters (Mineur *et al.* 2007).

The epiflora on oysters from the Oosterschelde estuary was present throughout the year, and occurrence of species did not differ between 'Spring & Summer and 'Fall & Winter' (Fig. 3.5). Some of the algae with the highest occurrence on oyster shells (*A. subulata, D. baillouviana, H. japonica* and *P. senticulosa*) are also reported to be the dominant macroalgal species on other substrates in parts of the Oosterschelde estuary, at the expense of native species (Stegenga *et al.* 2007). However, the seasonality of these species described by Stegenga *et al.* (2007) is not reflected in our results, possibly because we did not record size or biomass of algae. Occurrence of specimens large enough to identify to species level, of nearly all species throughout the year, demonstrates that oyster transports are a vector for macroalgal species in all seasons.

We analyzed geographical distributions of all algae identified from epiflora samples in this study. Based on disjunct or cosmopolitan distribution patterns, and the association with oysters, we assigned a cryptogenic species status to 23 of the 36 algae we identified to species level (Table 3.2). In general, the number of cryptogenic species in coastal waters is greatly underestimated, and species with a disjunct distribution pattern and association with an anthropogenic vector should be assigned to this category, unless there is proof of their native or introduced status (Carlton 1996a; 2008; Chapter 4). These cryptogenic species are potentially historically introduced either in Europe or in other regions with oyster translocations or with other anthropogenic vectors, such as ship hull-fouling, or they may in fact be different species in different regions. Historical oyster translocations go back to at least the 18th century, when *Ostrea edulis* was exchanged between European countries (Wolff & Reise 2002), and possibly even the 16th century, when the Portuguese oyster (*C. angulata*) was introduced to Europe from Asia, although it remains unknown whether this was a deliberate or an accidental introduction (Carlton 1999b; Wolff 2005b). These early relayings and introductions of oysters are likely to have been accompanied by introductions of associated non-indigenous species, and current lists of species introduced with this vector will be underestimates of the true numbers of oyster-associated introductions. Furthermore, as exchange of oysters between culture areas is still taking place within Europe, associated species are still on the move and the distribution patterns of native and cryptogenic species may become even more blurred.

There seems to be some association between the quantity of oysters imported (Fig. 3.2) and the number of associated introductions in the same period (Fig. 3.1). In 1971-72 large quantities of oysters and oyster seed were imported to The Netherlands, and in the decade 1970-79 we also see a peak in the number of associated introductions. About half of the oyster imports in those years consisted of oyster seed, which was used to restock oyster culture sites, and as the largest amount of oysters originated from France and Portugal (Fig. 3.3), the species that were imported were most likely *C. angulata* and *O. edulis*. *C. angulata* has not established in Dutch coastal waters, and we do not know of any associated introductions with this species. Imports of *O. edulis* from France did result in the introduction of associated species, some of which had great impact. The parasite *Bonamia ostrea*e was introduced to The Netherlands from France, where it probably had been introduced with oysters from California (Cigarría

& Elston 1997), and its introduction resulted in the near extirpation of the Dutch *O. edulis* stock (Wolff 2005b). However, overall we do not see a correlation between the imported quantities of oysters and introductions of associated species. The number of oyster-associated introductions has increased since the 1970s, even though oyster imports drastically decreased from 1981 onwards (Fig. 3.2). High propagule pressure therefore does not always ensure successful establishment, as would be expected (Lockwood *et al.* 2005). The reason for successful establishment of oyster-associated species even with small quantities of oysters imported might be the establishment and spread of the Pacific oyster in Dutch coastal waters (Smaal *et al.* 2009), resulting in extensive oyster reefs providing a habitat for native as well as non-indigenous epiflora and –fauna. Changing circumstances in the recipient area, such as higher temperatures and changing hydrography and community composition due to the construction of the storm-surge barrier in the Oosterschelde estuary (Nienhuis & Smaal 1994), may also promote establishment and spread of non-indigenous species, as has been shown in other disturbed systems (Occhipinti Ambrogi & Savini 2003).

In The Netherlands imports of oyster seed for restocking culture areas have nearly ceased since 1980 (Fig. 3.2). The decrease in the imports of seed oysters may also be due to the fact that the Pacific oyster has established in the Oosterschelde estuary and there is no longer a need for restocking with seed from other countries. Imports of "other oysters" are ongoing and also include live oysters. It is unclear which proportion of this category is introduced in recipient waters. Live adult oysters that are imported for consumption are often kept on the culture plots or in tanks on the shore with running sea-water from the Oosterschelde estuary for storage before sale, and introduction of non-indigenous species may thus still take place, even though the oysters concerned are not intended for restocking culture plots.

Oyster imports are not always reported to the authorities, as we did not find records of imports of Pacific oysters in the 1960s from British Columbia and Japan, although we know that they did take place and resulted in the establishment of the Pacific oyster in Dutch coastal waters (Shatkin *et al.* 1997; Drinkwaard 1999; Wolff & Reise 2002). Translocations of oysters within Europe are also suspected not always to be reported to authorities (Verlaque 2001), but secondary spread of associated non-indigenous species provides evidence of ongoing transports. A clear example is the red alga *Lomentaria hakodatensis*, which was first observed in the Thau Lagoon in the Mediterranean in 1979, after which it was found in Brittany in 1984, and from there it "jumped" to the Oosterschelde estuary in The Netherlands in 2004 (Verlaque 2001; Stegenga 2004). It has not been reported from areas in between these important oyster-culture sites, and oyster translocations are the only possible explanation for this secondary spread.

Oysters are not the only commercial bivalves that are imported and relaid in the Oosterschelde estuary. *Mytilus edulis* seed is also imported from other European countries for restocking Dutch mussel culture, and despite a risk analysis (Wijsman & De Mesel 2009), at least two non-indigenous species, the Atlantic oyster drill *Urosalpinx cinerea* and the Manila clam *Ruditapes philippinarum*, have recently been introduced by

mussel imports from the United Kingdom and Ireland (Faasse & Ligthart 2008; 2009). *U. cinerea* was indicated in the risk analysis report as a high-risk species having a large potential of negative impact. Commercial production of spat in quarantine and introduction of this spat proves to be the only way of preventing associated introductions with shellfish transports (Utting & Spencer 1992; Sindermann *et al.* 1992).

Conclusions

Oyster transports are demonstrated to have a high potential of introducing associated species. This is reflected in the high number of oyster-associated introductions in The Netherlands, and in the increase of oyster-associated introductions in recent years, despite a decrease in oyster imports. This high number of oyster-associated introductions and the discrepancy between number of introductions and amounts of oysters imported can be explained by different factors. First, not all oyster imports are reported to the authorities, and even small numbers of oysters may result in high propagule pressure of associated species due to large numbers of individuals and species that are introduced in a single event. Second, these species are introduced with their substrate, thus facilitating successful establishment. Third, the rapidly growing Pacific oyster reefs form a new habitat for associated species in The Netherlands, and are likely to facilitate the establishment of associated non-indigenous species, possibly combined with other factors such as climate change and changing circumstances in the recipient region.

Pacific oysters from the Oosterschelde estuary are a substrate for native and introduced species, and for a large number of cryptogenic species. Historical introductions with oyster transports may have resulted in cosmopolitan or disjunct distributions of species we now call native, as pre-19th century movements of non-indigenous and native species with oysters within Europe are likely to have occurred.

The ongoing shellfish movements within Europe contribute to introduction and rapid secondary spread of non-indigenous species and exchange between populations of native and cryptogenic species, thus blurring natural distributions and homogenizing diversity of algae and invertebrates in coastal waters.

PART **II** - The scale of cryptogenesis

The scale of cryptogenesis in the North Atlantic Ocean

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Summary

Cryptogenic species are species that are neither demonstrably native nor introduced, and include those species that are falsely viewed as native. One of the characteristics of cryptogenic species is a disjunct distribution pattern. Our aim was to estimate the scale of cryptogenesis in the North Atlantic Ocean by investigating disjunct amphi-Atlantic distribution patterns of marine shallow-water invertebrates. A disjunct amphi-Atlantic distribution pattern can be explained by four scenarios: natural trans-oceanic dispersal, post-glacial recolonization, cryptic species and human-mediated introductions.

We chose three taxonomic groups that differ in their natural dispersal potential: Ascidiacea, Hydrozoa and Bivalvia. Ascidiacea are poor dispersers with a short pelagic larval phase. Hydrozoa have a high dispersal potential: they have pelagic larvae, sometimes a free-swimming medusa and the polyps may be able to raft. Bivalvia have a long-lived larval phase, and they were divided in three groups: infaunal bivalves, that are able to disperse only via pelagic larvae, and epifaunal bivalves and boring bivalves, both of which may additionally be able to raft. All groups except most infaunal bivalves are epifaunal and are potentially dispersed as ship hull fouling.

We compiled extensive species lists from the literature including detailed information on habitat, distribution pattern and life-history characteristics, and assigned a species status (native, introduced, cryptogenic) and generalized distribution pattern. We reviewed literature on the four proposed scenarios and compared relative numbers of cryptogenic species among and within groups with disjunct amphi-Atlantic distributions.

Disjunct-amphi Atlantic distributions are uncommon: they occur in 10% of all listed species. Cryptic species occur in all taxonomic groups; some cryptogenic species with a disjunct distribution may in fact be distinct species. Disjunct distributions are often assumed to be caused by the Last Glacial Maximum (LGM). However, so far there are no examples of studies of strictly temperate subtidal species for which conclusive evidence has been presented of natural dispersal across the ocean after the LGM. The LGM and anthropogenic introduction can both result in either high or low levels of genetic diversity and are hard to distinguish.

Groups with high relative dispersal capacities do not more often have disjunct amphi-Atlantic distributions. Infaunal bivalves have the lowest relative number of disjunct species, and none of these are cryptogenic or have a natural disjunct distribution. Long-distance dispersal by larvae does not explain disjunct amphi-Atlantic distributions. The Hydrozoa have the highest relative number of disjunct distributions, which has often been ascribed to dispersal by rafting. However, this has not been demonstrated, and it does not rule out hull fouling as a potential agent of dispersal. Ships are more successful in dispersing coastal organisms than rafts: ships travel relatively fast, are independent of the surface currents and provide more space.

We estimated that between 1.3% and 28% of the shallow-water fauna of the North Atlantic Ocean is cryptogenic. Species that may have been present on our coasts for centuries and may be important ecological engineers that have shaped contemporary communities may falsely be viewed as native; they could be the missing introductions of historical times.

Introduction

When studying marine communities one usually finds clearly native species, clearly introduced species and a group of species for which it is unclear whether they are native or introduced. Carlton (1996) introduced the concept of cryptogenesis for such species that are neither demonstrably native, nor introduced. Estimating the scale of cryptogenesis is crucial to our understanding of modern marine community ecology and our basic assumptions about and interpretation of the natural diversity, biogeography and rate of evolution in the seas (Carlton 2003b). In this study, we use distribution patterns of selected shallow-water benthic invertebrates to investigate the scale of cryptogenesis of the North Atlantic Ocean.

The North Atlantic Ocean is a well explored part of the world's oceans. Comprehensive biological surveys of North Atlantic shores commenced as early as the mid 19th century and shallow-water benthic invertebrate communities of the North Atlantic have extensively been studied since. The distributions of individual invertebrate species in the North Atlantic are therefore relatively well known.

In general, geographic distributions of marine organisms are shaped by dispersal and vicariance events. In the North Atlantic, the Pleistocene glaciations have had a profound impact on diversity and distributions of marine biota (Hewitt 1999; 2000). After the ice-sheets retreated, organisms recolonized the shores of the North Atlantic, and geographic distributions of many shallow-water invertebrates and algae are therefore relatively recent.

Transoceanic shipping also started early in the North Atlantic. Humans have profoundly impacted the biota of the North Atlantic by shipping since at least 1000 years BP, when the Vikings first crossed the ocean, and intensive shipping across the ocean has been taking place since the 1500s. Organisms can be transported by ships in the fouling communities on and in the hull, in the cargo, in solid ballast and in ballast water. Shipping is an important anthropogenic vector for dispersal and introduction of species, and thus has greatly impacted diversity and distributions of marine biota in the North Atlantic (Carlton 2003b). Geographic distributions of shallow-water marine and estuarine benthic invertebrate species in the North Atlantic Ocean generally fall in one of four categories: 1) cold-water species - restricted to Arctic or sub-Arctic waters, 2) endemic species - present on either side of the Atlantic, 3) amphi-Atlantic species - occurring on both sides of the Atlantic, as well as in (sub-)Arctic waters, or 4) disjunct amphi-Atlantic species - present on both sides of the Atlantic, but absent from the intermediate Arctic and/or sub-Arctic region.

The deep and wide Atlantic Ocean is a geographical barrier for dispersal of coastal organisms that has to be overcome in some way for a disjunct distribution pattern to become established. Most coastal benthic species do not extend their range to deep waters and are therefore unable to reach the opposite shore. Furthermore, there are few oceanic islands in the central North Atlantic, and the stretch of open ocean that has to be crossed is about 6000 km at the widest point. There are four scenarios that can explain a disjunct amphi-Atlantic distribution: natural dispersal, post-glacial recolonization, cryptic species and human-mediated introductions.

Natural dispersal

Adaptations such as long-distance dispersal of larvae (Thorson 1950; Scheltema 1971b), and rafting of juveniles, adults or egg masses on floating substrata (Johannesson 1988; Thiel & Haye 2006), as well as dispersal by migratory vertebrates, such as birds (Frisch, *et al.* 2007) are natural mechanisms that can result in the colonization of distant shores. For larval dispersal and dispersal by rafting the direction of dispersal is largely determined by ocean surface currents. The direction of dispersal across the Atlantic Ocean in warm temperate waters is from west to east based on the current regime, and in cold temperate to Arctic waters there is a possibility of east to west dispersal (Dawson *et al.* 2005). Colonization through larval dispersal is dependant of pelagic larval duration in relation to the time needed by ocean currents to cross the ocean. Colonization through rafting is also dependant on events in the rafting community. Rafting communities change over time depending on the substrate they raft on and competition for space and resources on the raft. Furthermore, not all invertebrate groups commonly raft, and after arrival on a distant shore, success of dispersal is determined by the capability of larvae, rafting adults or juveniles to establish a new population.

Post-glacial recolonization

In the second scenario, disjunct amphi-Atlantic distribution patterns are explained by the Pleistocene glaciations, during which northernmost populations of shallow-water invertebrate species were largely or entirely eliminated on either side or both sides of the Atlantic and from the (sub-) Arctic region due to low temperatures, ice cover and lower sea-levels (Frenzel *et al.* 1992). This scenario is in line with the observation that temperature fluctuations and associated extinctions of species were more severe in the North Atlantic than in the North Pacific Ocean. The North Atlantic has a generally depauperate fauna when compared to the North Pacific (Briggs 1995). Also, the oceanic islands in the North Atlantic have a very low degree of endemism, which is another indication for the severity of the Pleistocene glaciations in this region (Briggs 1974).

After the Last Glacial Maximum (21,000 BP) both Atlantic coasts were recolonized when the ice receded. For species that have a disjunct amphi-Atlantic distribution due to the glaciations it is assumed that they recolonized both coasts from glacial refugia, from southern regions, or from across the ocean, mostly from Europe to America (Plough 1978; Hewitt 1999; Wares & Cunningham 2001; Wares 2001b; Vermeij 2005; Maggs *et al.* 2008). A relatively recent colonization of distant shores has been demonstrated in phylogeographic studies of intertidal invertebrates, e.g. the echinoderm *Asterias rubens*, the mussel *Mytilus edulis* and the isopod *Idotea balthica*, which colonized the Northwest Atlantic from the Northeast Atlantic (Wares & Cunningham 2001; Wares 2001a).

Cryptic species

Single species that appear to have a disjunct distribution may in fact be species complexes of which the individual species have not been or cannot be distinguished based on morphological characters. The identification of cryptic species has increased exponentially with the availability of DNA sequences (Bickford *et al.* 2007). In the North

Atlantic, phylogeographic studies have revealed species complexes for a variety of invertebrates and algae (e.g. van Oppen *et al.* 1995; Gómez *et al.* 2007; Caputi *et al.* 2007).

Anthropogenic introduction

In the fourth scenario disjunct amphi-Atlantic distributions are not the result of natural processes, but of historical anthropogenic activities. Ships have moved species across the North Atlantic Ocean for at least 1000 years, but only since comprehensive biological surveys began in the mid-1800s have we been able to document the appearance of novel species on either side of the North Atlantic (Carlton 1989; Carlton 2003b). The archeological record provides a possibility to detect some earlier invasions, such as the Norse movement of the clam *Mya arenaria* from the northwestern to the northeastern Atlantic (Petersen *et al.* 1992). In addition historical and cryptic introductions in the marine environment are being revealed with the help of molecular markers on a regular basis.

Cryptogenic species

It is difficult to discriminate between these four scenarios. For each widely distributed shallow-water species life-history characteristics, association with anthropogenic vectors, historical biogeography and phylogeography, and long-distance dispersal mechanisms would have to be analyzed in order to explain its distribution pattern. We often assume that species are endemic unless there is evidence that they have been introduced. However, for many species this has not been investigated and this assumption might be erroneous: these are the cryptogenic species (Carlton 1996a). Species can be assigned to the cryptogenic category on basis of several characteristics, such as association with an anthropogenic transport vector, a recent history of worldwide introductions, absence of close relatives in part of their range, and a disjunct distribution pattern. In our study, our starting point is the last characteristic: we use distribution patterns of shallow-water invertebrates to investigate the scale of cryptogenesis in the North Atlantic Ocean

Based on a literature review and consultation with taxonomic experts, North Atlantic geographic distributions are determined for three large taxonomic groups, from different phyla: the Ascidiacea, Hydrozoa and Bivalvia. These groups were chosen because of their differential life-history traits that result in differing natural dispersal potential, and they serve as model groups for other invertebrate taxa. Many species of these groups are important members of fouling communities, and are likely to have been transported historically by ships as fouling of the hull. For bivalves this generally applies only to the epifaunal and boring species (although there are some exceptions). The distribution patterns of the epifaunal and boring bivalves, the ascidians and the hydrozoans are compared with the distributions of infaunal bivalves which, with few exceptions, are not easily transported as hull fouling. Infaunal bivalves may be distributed as larvae in ballast water of ships, but since this vector has only been in use since the late 19th century, we generally know which species were introduced by this anthropogenic vector.

Aim

The aim of this study is to estimate the scale of cryptogenesis for benthic invertebrates in North Atlantic shallow waters. To this end we estimated the relative number of species with a disjunct distribution pattern across all studied groups. For these species with a disjunct distribution pattern the relative importance of natural- versus anthropogenic dispersal was evaluated and compared across groups. In doing so, we paid particular attention to the comparison of infaunal Bivalvia for which we believe to know all cases of human-aided dispersal, with epifaunal groups. Also, the possibility of cryptic speciation and post-glacial recolonization as processes responsible for disjunct distributions across the North Atlantic were reviewed.

Methods

Geographic region

Biogeographic provinces, biomes, or regions have been defined by numerous authors based on different criteria. Among the criteria used are species composition, geographical barriers, and physical conditions. Only recently have all these efforts been reviewed, and this has resulted in the Marine Ecoregions of the World (MEOW) (Spalding *et al.* 2007). The ecoregions defined by Spalding *et al.* are based on an extensive literature review, advice of a large number of independent experts and the results of an international workshop. We have adopted their classification, which for our study region also follows the classification by Briggs (1974; 1995). The geographic region considered here is the North Atlantic Ocean, bordered in the South by Cape Hatteras in the Western Atlantic, and the Strait of Gibraltar in the Eastern Atlantic (see Fig 1.1).

Taxonomic groups

The taxonomic groups concerned are Bivalvia (Phylum Mollusca), Ascidiacea (Phylum Chordata) and Hydrozoa (Phylum Cnidaria). We have selected these taxa because they span a wide variety of habitats and reproductive strategies representative of a broad array of invertebrate phyla. Furthermore, they are conspicuous and abundant components of coastal communities, are relatively well studied, there is extensive literature on each of them, and there are experts available to review the species lists. Table 4.1 presents a generalized summary of the life-history, dispersal and habitat characteristics of each taxon. Based on these characteristics, the relative natural dispersal potential and expected general distributions for each taxon were derived.

Natural dispersal potential

In sessile benthic organisms dispersal generally takes place in the pre-adult phase, and knowledge of life-cycles is therefore necessary to assess and understand dispersal potential and distribution patterns. Bivalves, ascidians and hydrozoans differ substantially in their natural dispersal capabilities.

Ascidians are hermaphrodites, and sperm is shed into the sea. Some colonial ascidians can store exogenous sperm for prolonged periods (Bishop & Ryland 1991). Most solitary species spawn their eggs into the sea, where they are fertilized and develop into tadpole larvae. Some solitary and all colonial forms brood their eggs, either in special brood chambers or in the atrium (Lambert *et al.* 1995). Eggs develop into larvae that may or may not be able to swim. Swimming of ascidian larvae functions in site selection, and probably not in dispersal, as larval duration is short, ranging from minutes up to 36 hours, although metamorphosis may be delayed for some days in some species - (Laurson 1981; Svane & Young 1989; Lambert 2005a). Spawned eggs of several *Molgula* species develop directly into a functional juvenile without going through a swimming larval stage (Berrill 1931). In general, due to the limited time larvae are free-swimming, the natural dispersal capacity of the Ascidiacea is limited, and expected distributions are narrow and continuous (Table 4.1).

In contrast, bivalves (that can either be dioecious or hermaphroditic) have a pelagic larval phase that can last days to weeks under normal conditions. There are some species that brood their offspring (e.g. some *Lasaea* species), and release them either as pelagic larvae or as benthic juveniles. Delayed metamorphosis occurs in the absence of suitable substrate for settlement and can extend the free-swimming larval phase to months (Ruppert & Barnes 1994). Byssus drifting is universal in bivalves, except in Ostreacea and Teredinidae. Growth is slowed down, shell thickening is delayed in order to keep buoyancy, and an intermediate filter-feeding mechanism is present during the byssus drifting stage (Sigurdsson *et al.* 1976). Bivalves are potentially capable of dispersing over large spatial scales, and geographic distributions are consequently expected to be wide (Table 4.1). Hence, disjunct amphi-Atlantic distributions are expected to be common in this group.

The hydrozoan life cycle is complex and consists of three phases, with varying dispersal potential. The polyp phase is generally spent attached to a biotic, abiotic, or artificial substrate and is dependent of the mobility of the substrate for its dispersal. Polyps reproduce asexually by the production of medusae via budding. Medusae can either be released as a free-swimming stage, or they can be retained, as fixed medusoids or sporosacs, which are incomplete medusae consisting only of the gonadal tissue. Free-swimming medusae have a life span of a few days up to many months. Medusae reproduce sexually and form larvae. The ciliated planula larvae spend several hours up to several days in the water column. Some species of Hydrozoa brood their larvae, which restricts the free-swimming larval phase (Ruppert & Barnes 1994). The medusa phase has the highest dispersal potential in the hydrozoan life cycle because the medusa can actively swim and has a longer lifespan than the planula larva. Hydrozoa that possess a free-swimming medusa phase are therefore expected to have relatively wide distributions and more frequently have disjunct distributions compared to Hydrozoa that retain their medusae (Table 4.1).

In all groups we only focus on shallow-water species, occurring in depths less than 100 m. Species that occur on both coasts as well as in deeper waters may in fact have continuous distributions in deep waters of the North Atlantic. We also indicated

whether species extend their range into the (sub) tropics. Larval durations in warmer waters are typically longer (Thorson 1961), the stretch of ocean that separates the continents is less wide, the surface current system may allow dispersal in both directions, and species may have survived in refugia in the tropics. Disjunct amphi-Atlantic distributions in warmer waters are therefore excluded from our analyses.

Comparing epifaunal and infaunal bivalves

Most members of the taxa we studied have a sessile adult phase that lives attached to a substrate. Long-distance dispersal by the juvenile or adult phase is possible by rafting or with anthropogenic vectors. In order to investigate whether long-distance dispersal by free-swimming larvae or by the adult phase is more important in forming disjunct distribution patterns, we need a control group within one of the studied taxa that can disperse via a long-lived pelagic phase, but is not able to attach to hard substrates such as ships or rafts.

Bivalves contain such a control group because they can be divided in infaunal and epifaunal species. Epifaunal bivalves are found on the substrate surface, infaunal bivalves burrow or bore into the substrate. Many epifaunal and boring bivalves are common components of fouling communities. They settle on, or bore in, the (wooden) hulls of ships, which can aid in dispersing these organisms.

Infaunal (burrowing) bivalves are unlikely candidates for dispersal on ship hulls. Their larvae may be transported in ballast water and introduced outside their native range. Around 1880 the use of ballast water became common practice and it is now regarded as one of the major anthropogenic vectors of introduction. Because this vector has been in effect for a relatively short period in time, we generally know which species have been introduced by it, particularly for a well-studied group such as bivalves. The distribution patterns of native infaunal bivalves before about 1880 can therefore be regarded as natural distributions that are not influenced by historical shipping (even though there are exceptions to this; see Anthropogenic dispersal section).

Table 4.1 Generalized habitat and natural dispersal characteristics of Ascidiacea, Hydrozoa and Bivalvia, and their expected distribution patterns. There are exceptions to the generalizations in this table; these are indicated in Tables 4.2, 4.3 and 4.4, and in the text.

	Bivalvia			Ascidiacea	Hydrozoa	
Habitat	infaunal	epifaunal	boring	epifaunal	epifaunal & planktonic	epifaunal
dispersal by	- long-lived larva	- long-lived larva - rafting adult	- long-lived larva - rafting adult	- short lived larva - rafting adult	- medusa - larva - rafting adult	- larva - rafting adult
Relative natural dispersal potential	high	high	high	low	high	low
Expected distribution	wide	wide	wide	limited	wide	limited

The role of Pleistocene glaciations in the creation of disjunct amphi-Atlantic distributions is assumed to be the same for all groups, and differences in relative numbers between the bivalve groups can only be explained by differing dispersal potential. Comparing the relative numbers of disjunct distributions of burrowing, boring and epifaunal bivalves will reveal whether long-distance dispersal of larvae is a strong mechanism for the creation of disjunct distribution patterns.

Data

All species of Hydrozoa, Ascidiacea and Bivalvia in North Atlantic shallow waters (minimum depth less than 100 m) were reviewed, including information on their world-wide distribution, depth range, reproduction, lifestyle, dispersal capabilities, and notable peculiarities. The completed tables were sent to taxonomic experts for review and are now up-to-date regarding systematics and nomenclature.

The complete distribution of each species was determined in detail, including the distribution outside the study area. Species were also assigned to a generalized distribution category in the North Atlantic: Northeast Atlantic, Northwest Atlantic, Arctic, amphi-Atlantic (continuous distribution), or disjunct amphi-Atlantic (not in Arctic or sub-Arctic waters).

Bivalves were divided in epifaunal, infaunal and boring species (Table 4.1). Epifaunal bivalves are all bivalves that live on a substrate and not only include attached species, but also commensal, free-swimming, nest-forming and half-buried species. Infaunal bivalves are burrowing species, boring bivalves are those that bore into hard substrates. Hydrozoa were also divided in three groups: medusa-releasing hydrozoans, medusa-retaining hydrozoans and species for which this information on the life-cycle is lacking (Table 4.1). Ascidians were not subdivided.

Rafting potential was noted if it was based on observations of animals on rafts; if rafting was inferred from the geographical distribution of a species this was added in the comment. In the evaluation of rafting potential a recent and very extensive review of rafting literature was used (Thiel & Gutow 2005). Fouling potential was only explicitly noted in the comments section if fouling of ship hulls had been documented. Fouling of other substrates was mentioned in the habitat section.

From the combined species characteristics and distribution, a species status was assigned:

- *introduced*. A species was assigned to the introduced category if it was certainly introduced on either coast by an anthropogenic vector; this had to be supported by literature.
- *cryptogenic*. Species from this category have one or more characteristics of a cryptogenic species: a disjunct distribution, history of introduction in other regions, association with an anthropogenic vector, and other life-history characteristics that facilitate introduction by humans.
- *other*. Species with an unclear or debated taxonomy ("doubtful"), species that are thought to represent a species complex, species that are known to have been wrongly identified in many places, and species for which the distribution is based

on very few records.
- *warm/deep*. This group consists of species that also occur in warm or deep waters, which may allow them to cross the barrier of the deep and wide Atlantic by natural means through deep waters or equatorial warm waters.
- *"natural"*. Species that are not allocated to one of the categories above. Their distribution is assumed to be natural.

Results

Complete lists of species of shallow-water North Atlantic Ascidiacea, Hydrozoa and Bivalvia, including distributions, depth ranges, habitat, reproduction characteristics, comments, species status and references, were created. For each group we present the number of species for each distribution category in Figure 4.1. Tables 4.2, 4.3 and 4.4 list the species with a disjunct amphi-Atlantic status and their specifics. Within each group examples of introduced and cryptogenic species are presented below. Figure 4.2 gives an overview of the species status of the disjunct-amphi Atlantic species in each group. Appendices I, II and III, which can be obtained from http://dissertations.ub.rug.nl/faculties/science/2010/, list the species of Ascidiacea, Hydrozoa and Bivalvia that do not have a disjunct amphi-Atlantic distribution.

Ascidiacea

DISTRIBUTION PATTERNS

The North Atlantic ascidian fauna consists of 184 species. In Figure 4.1A, numbers of ascidian species are presented for each distribution category. Of the total number of ascidian species, 97 (52%) occur only on the Northeast Atlantic coast. The Northeast Atlantic has more endemic species than the Northwest Atlantic, where only 9 species (5%) are endemic. An Arctic distribution was recorded for 46 species (25%), 16 species (9%) have an amphi-Atlantic distribution, and another 16 (9%) species have a disjunct amphi-Atlantic distribution.

DISJUNCT ASCIDIANS - STATUS

Of the sixteen ascidian species in the disjunct amphi-Atlantic group (Table 4.2), two species may also occur in deep waters, and for two more the distribution is unclear, with no recent records or a single record. Ten of the sixteen disjunct amphi-Atlantic ascidian species (63%) have been observed in fouling communities; three of these have also been observed on rafts (Thiel & Gutow 2005).

Eight species (50%) are known to have been introduced on either or both Atlantic coasts. None of these were introduced from the Northwest to the Northeast Atlantic; three were introduced from the Northeast to the Northwest Atlantic. *Diplosoma listerianum*, a widely distributed species in temperate and tropical waters of the Atlantic and Indo-Pacific, was introduced from Europe to North America by ships. *Botryllus schlosseri* and *Ascidiella aspersa* are common fouling species that also have a world-wide

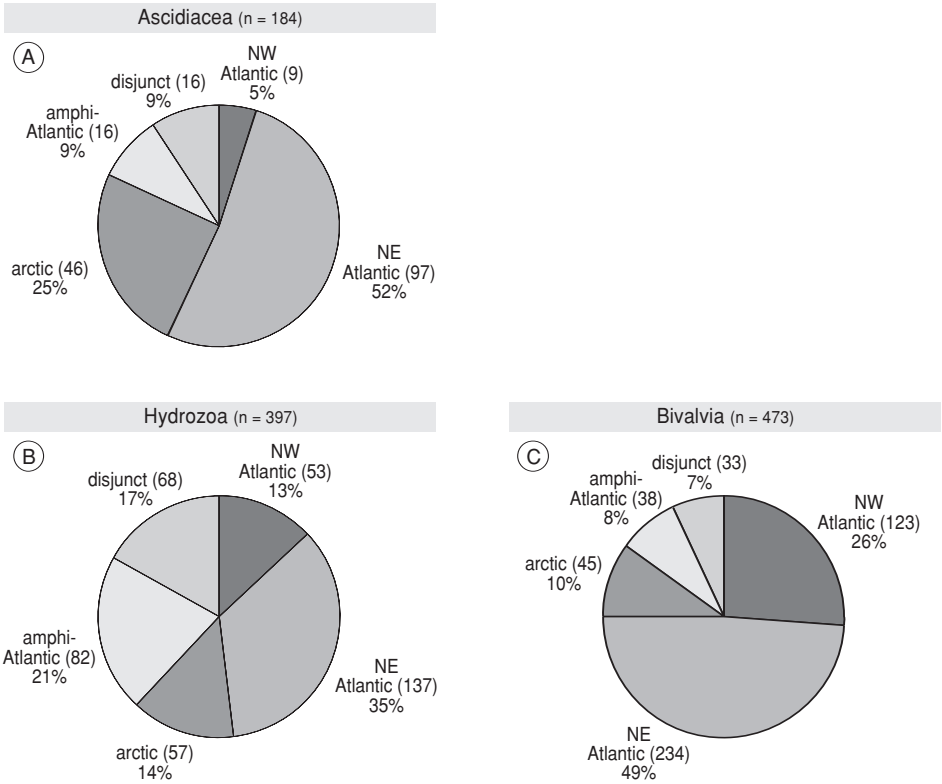

Figure 4.1 Species numbers (between brackets) and percentages of the total species number per distribution category. NE Atlantic: only occurring in the Northeast Atlantic, NW Atlantic: only occurring in the Northwest Atlantic, Arctic: restricted to Arctic waters, amphi-Atlantic: having a continuous amphi-Atlantic distribution, disjunct: having a disjunct amphi-Atlantic distribution. A: Ascidiacea. B: Hydrozoa. C: Bivalvia.

distribution and were introduced from Europe to America by shipping. *Botryllus schlosseri's* phylogeography in the North Atlantic and Mediterranean appears to be blurred because of ship-aided dispersal within this region (Lopez-Legentil, Turon, & Planes 2006; Ben-Shlomo, Paz, & Rinkevich 2006). Its origin is hypothesized to lie in the Pacific Ocean, suggesting that *B. schlosseri* was introduced by ships historically (Carlton 2005; Lopez-Legentil *et al.* 2006). The five remaining introduced disjunct amphi-Atlantic species are listed in Table 4.2. They all originate in the North Pacific.

Three species (38%) are cryptogenic: *Didemnum candidum, Molgula manhattensis* and *Perophora viridis*. The latter only occurs on the Azores in the Northeast Atlantic, and it is possible that it was introduced there by shipping (Monniot & Monniot 1983). *Didemnum candidum* has been described from many places, but many identifications are uncertain. It is also associated with shipping vectors, has been introduced to many regions and might represent a species complex (Monniot pers. comm.). *Molgula manhattensis* has been suggested to have been introduced to both the Northwest and

the Northeast Atlantic (Carlton 2003a; Wolff 2005b). It tolerates low salinities and pollution, it has not been reported rafting but is a notorious fouling organism, and it has a history of introductions in other parts of the world (Lambert 2001). It may well have been spread by humans across the Atlantic in historical times (Chapter 5).

Cnemidocarpa mollis is the only species with a "natural" disjunct amphi-Atlantic distribution for which there are no indications that it may have been introduced in historical times.

Hydrozoa

DISTRIBUTION PATTERNS

There are 397 hydrozoans in the North Atlantic Ocean. In Figure 4.1B, numbers of species are shown for each distribution category.

The Northeast Atlantic, with 137 species that occur only on this coast (35% of the total number of species), is almost three times richer in species than the Northwest Atlantic, which has only 53 endemic species (13%). There are 57 species (14%) that occur only in Arctic or sub-Arctic waters. An amphi-Atlantic distribution is noted for 82 species (21%), and 68 species (17%) have a disjunct amphi-Atlantic distribution (Table 4.3).

DISJUNCT HYDROZOANS - STATUS

The disjunct hydrozoans are a large group, and not all of them will therefore be discussed in detail. For details on all of these species we refer to Table 4.3. Of the 68 species of disjunct amphi-Atlantic Hydrozoa seven (10%) are known to have been introduced (*Bougainvillia rugosa, Nemopsis bachei, Eudendrium carneum, Cordylophora caspia, Blackfordia virginica, Gonionemus vertens, Maeotias inexpecta*). All are likely to have been introduced as hull fouling, *Gonionemus vertens* could have been introduced with oysters as well (see references in Table 4.3). Nineteen species (28%) have been observed in ship fouling communities. Four of these hull-fouling Hydrozoa have also been recorded rafting, and for four species rafting was assumed based on their disjunct distributions (Thiel & Gutow 2005).

Of the disjunct amphi-Atlantic Hydrozoa, eighteen occur in warm or deep waters and nine are "doubtful" species or possible misidentifications. For one species (*Proboscidactyla ornata*) there are morphological indications that it might represent a species complex (Calder 1970a). Six species have been reported rafting but have not been recorded from ship hulls, and their distributions could thus be natural. Two of these are obligate rafters on pelagic gastropods (*Kinetocodium danae* and *Pandea conica*). Twenty-seven species (41%) are cryptogenic (Fig. 4.2).

These cryptogenic Hydrozoa have one or more characteristics of a cryptogenic species. *Obelia dichotoma* is an example of a widely distributed hydrozoan that occurs in fouling communities and specifically on ship hulls, but it has also been observed rafting, and might represent a species complex (Woods Hole Oceanographic Institution 1952; Cornelius 1992; Govindarajan, *et al.* 2005). *Garveia franciscana* was introduced to San Francisco Bay, and could have been introduced to Atlantic North American and

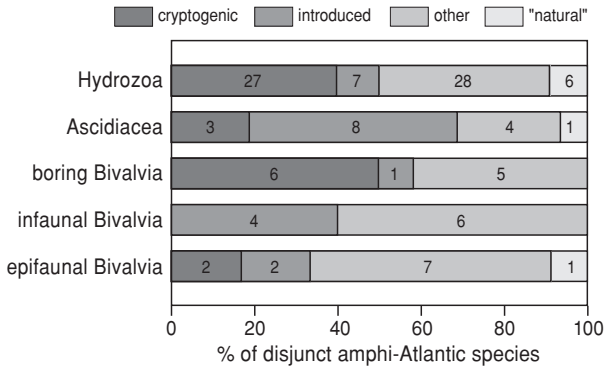

Figure 4.2 Proportions and absolute numbers of cryptogenic, introduced, other (deep water, warm water and doubtful species) and "natural" disjunct amphi-Atlantic species for Hydrozoa (n=68), Ascidiacea (n=16), boring Bivalvia (n=12), infaunal Bivalvia (n=10) and epifaunal Bivalvia (n=12). Absolute numbers per category are indicated in the bars.

European coasts as well. Its origin is unclear, but it is associated with oysters (Fraser 1944; Calder 1990; Leppäkoski & Olenin 2000). *Sarsia occulta* was described in 1978, and it has been recorded from Scotland, Massachusetts and Maine. It occurs near low water, on rocks and weeds, and it is thus not unlikely that it was introduced with rock ballast to either coast of the North Atlantic, and there is also a possibility that it is confused with other *Sarsia* species and its complete range is not known.

The Hydrozoa were divided in three groups based on their dispersal potential: the largest group (215 species) consists of hydrozoans that do not release medusae (medusa retaining) and therefore spread by means of the planula larvae. The second group (132 species) disperses additionally via a free-swimming medusa (medusa releasing), and for the third group (47 species) it is unknown whether medusae are released or retained (unknown). Three species in the genus *Candelabrum* were excluded from the analyses; they are viviparous or brood the embryos (see appendix II).

Based on their natural dispersal potential, species with a free-swimming medusa are generally expected to have a wider distribution than species that lack a free-swimming stage other than the planula larva, which has a much shorter pelagic phase. Figure 4.3 gives percentages of species of each dispersal group per distribution type. The medusa-retaining Hydrozoa are by far the largest group in each distribution category. In the disjunct amphi-Atlantic group this difference is smallest: the relative number of medusa-releasing and medusa-retaining Hydrozoa is similar (44% and 50% respectively).

In Table 4.4 the percentages for the different distribution patterns in each dispersal group are given. A large proportion of species for which it is unknown whether they retain or release their medusae occurs in the Arctic (30%) and Northwest Atlantic (34%). This may be due to the fact that these regions have been studied less than European shallow waters.

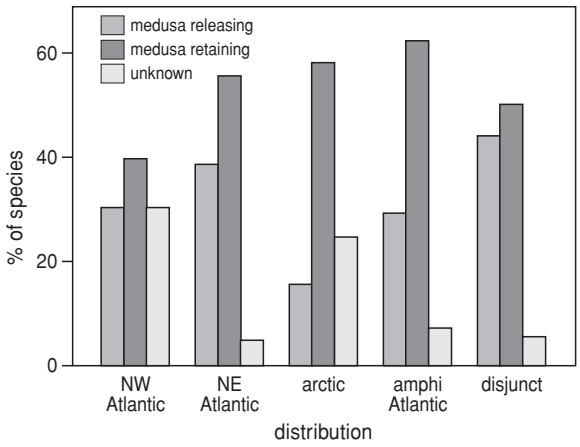

Figure 4.3 Distribution patterns in Hydrozoa. Hydrozoa were divided in three groups: medusa releasing (n=132), medusa retaining (n=215) and those species for which it is unknown whether they release a medusa (n=47). For each distribution type, the relative numbers of species from each hydrozoan group is shown.

Regarding the composition of each dispersal group, one would expect that a larger fraction of the total number of medusa-releasing Hydrozoa would have an amphi-Atlantic or disjunct amphi-Atlantic distribution, compared to the fraction of species that do not release their medusae. However, there are no notable differences between the three groups. Of the medusa-releasing Hydrozoa, 23% have a disjunct distribution; 16% of the medusa-retaining Hydrozoa have a disjunct distribution. For amphi-Atlantic distributions this is reversed, this distribution pattern is slightly more common in medusa-retaining hydrozoa (24%, in medusa-releasing Hydrozoa this is 18%). However, the differences are not very pronounced; medusa-releasing Hydrozoa do not have the wide distributions that are expected based on their life-history. In other words, widely distributed species do not more frequently have a free-swimming medusa than species with a narrow geographical distribution.

In the disjunct amphi-Atlantic group we assigned a status to all species. No differences between dispersal groups are found here: cryptogenic species are not more commonly releasing, nor retaining their medusae. Rafting as the only hypothesized long-distance dispersal mechanism is slightly more common in medusa-retaining Hydrozoa (6 species) than in medusa-releasing Hydrozoa (3 species), but the numbers are very small. We therefore combined al three groups in Figure 4.2.

Bivalvia

DISTRIBUTION PATTERNS

There are 473 species of bivalves in the North Atlantic Ocean. In Figure 4.1C, numbers of species and percentages are shown for each distribution category.

Of 473 species of bivalves in the North Atlantic Ocean, 234 (49%) occur only in the Northeast Atlantic. The Northeast Atlantic is nearly twice as rich in species as the Northwest Atlantic, which harbors 123 species (26%) that only occur on that coast. There are 45 species (10%) that occur only in Arctic or sub-Arctic waters. An amphi-Atlantic distribution was found for 38 species (8%), and 33 species (7%) have a disjunct amphi-Atlantic distribution (Table 4.4).

Comparing epifaunal and infaunal bivalves

Bivalves were subdivided in three groups: boring, epifaunal and infaunal bivalves. In Figure 4.4, the species numbers of each category of bivalves are given for each distribution type.

There are twenty-five boring bivalves, of which none have a strictly Arctic distribution and only one has an amphi-Atlantic distribution. Respectively five and seven species occur only in Northwest and Northeast Atlantic waters. Twelve boring bivalves have a disjunct amphi-Atlantic distribution.

Of the 149 epifaunal bivalves nine are strictly (sub) Arctic, 88 occur in the Northeast Atlantic and 27 occur only in the Northwest Atlantic. 13 of the epifaunal bivalves have an amphi-Atlantic distribution, and 11 have a disjunct amphi-Atlantic distribution.

The infaunal bivalves with 299 species are the largest group by far. Of these, 90 occur only in the Northwest Atlantic, 139 occur only in the Northeast Atlantic, and 36 infaunal bivalves occur only in (sub) Arctic waters. An amphi-Atlantic distribution was found for 24 species and 10 have a disjunct amphi-Atlantic distribution.

When comparing relative species numbers within boring, infaunal and epifaunal bivalves per distribution type (see Table 4.6), there are some other striking differences between groups. Just like for the other taxonomic groups we analyzed, the Northeast Atlantic is richer in bivalve species than the Northwest. However, if we compare the relative numbers of infaunal and boring bivalves with the relative number of epifaunal bivalves in these regions, we see that the difference between the Northeast and Northwest Atlantic is less pronounced for infaunal (16%) and boring (8%) bivalves than for epifaunal bivalves (41%).

Disjunct bivalves - status

All 34 disjunct amphi-Atlantic species are listed in Table 4.5. Of these, three were doubtful species, five also occur in deep waters, eight occur in warmer waters, two might represent a species complex, seven are known to have been introduced and nine are cryptogenic species. Of the 34 disjunct bivalves, 13 are recorded from fouling assemblages (38%). Of these, five are epifaunal bivalves, one is epifaunal and sometimes infaunal (*Geukensia demissa*), 6 are boring bivalves and one is infaunal (*Mya arenaria*, see below). For *Lyrodus pedicellatus*, a boring bivalve, rafting was inferred from its distribution (Thiel & Gutow 2005), although it is known to have been introduced to many places by ships (Carlton & Eldredge 2009).

Of the boring bivalves, 48% have a disjunct amphi-Atlantic distribution. This number is 8% for the epifaunal bivalves and 3% for infaunal bivalves (Fig. 4.4).

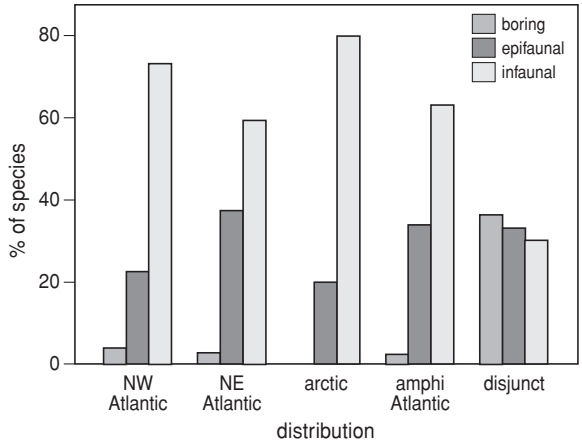

Figure 4.4 Distribution patterns in Bivalvia. Bivalvia were divided in three groups: boring (n=25), epifaunal (n = 149) and infaunal bivalves (n=299). For each distribution type, the relative number of species from each bivalve group is shown.

Table 4.5 Relative numbers of hydrozoan species for each distribution pattern. Numbers are presented for medusa-releasing Hydrozoa, medusa-retaining Hydrozoa and Hydrozoa for which knowledge of the life-cycle is incomplete.

	% of medusa-releasing Hydrozoa (n=132)	% of medusa-retaining Hydrozoa (n=215)	% of Hydrozoa with incomplete knowledge on life-cycle (n=47)
Northwest Atlantic	12	10	34
Northeast Atlantic	40	35	15
Arctic	7	15	30
amphi-Atlantic disjunct	18	24	13
amphi-Atlantic	23	16	9

Table 4.6 Relative numbers of bivalve species for each distribution pattern. Percentages are presented for boring, epifaunal and infaunal bivalves.

	% of boring Bivalvia (n=25)	% of epifaunal Bivalvia (n=149)	% of infaunal Bivalvia (n=299)
Northwest Atlantic	20	19	30
Northeast Atlantic	28	59	46
Arctic	0	6	12
amphi-Atlantic	4	9	8
disjunct amphi-Atlantic	48	7	3

Of the twelve epifaunal bivalves with a disjunct amphi-Atlantic distribution pattern two (17%) were introduced: *Mytilopsis leucophaeta* was introduced by hull fouling from warmer Northwest waters to Europe, New York and Connecticut (Nehring & Leuchs 1999; Carlton 1999b). *Ostrea edulis* was introduced for culture in the Northwest Atlantic (Carlton 1999b). Two epifaunal bivalves are cryptogenic: *Neopycnodonte cochlear* is a widespread common fouling species (Poppe & Goto 1993; Mikkelsen & Bieler 2008), *Lasaea adansoni* is a brooding bivalve that occurs in temperate and tropical Atlantic waters, as well as in the East Pacific. The phylogeography of *L. adansoni* in the North Atlantic suggests rafting and human-mediated introductions on oceanic islands (Ó Foighil & Jozefowicz 1999; Thiel & Gutow 2005). *Mytilus trossulus* and *Mytilus edulis*, sibling species in the *Mytilus* complex that hybridize in contact zones (Gosling 1992), are common foulers (Berner 1944) with disjunct distributions. The distribution of *M. edulis* is considered natural, *M. trossulus* might be a distinct species in the Baltic Sea (Rawson & Hilbish 1998). The *Mytilus* complex will be elaborated on in the discussion.

Of the ten species of infaunal bivalves with a disjunct amphi-Atlantic distribution pattern four were introduced. *Mya arenaria* was introduced from North America to Europe, probably in the late Middle Ages (Petersen *et al.* 1992), possibly as hull fouling since juvenile *Mya arenaria* can occur in fouling communities, or as a food source (Carlton, pers. comm.). *Barnea truncata* is infaunal, but is also found boring in wood, and was introduced by shipping to West Africa (Carlton, pers. comm.). *Ensis directus* was introduced to Europe from North America with ballast water (Luczak, *et al.* 1993). *Mercenaria mercenaria* was deliberately introduced for culture in French and English waters (Goulletquer *et al.* 2002). *Limopsis cristata, Limopsis minuta*, and *Axinus grandis* occur in deep waters (Abbott 1974; Turgeon *et al.* 1998; Costello *et al.* 2004; Mikkelsen & Bieler 2008) and may thus have a continuous distribution in the deep Atlantic. *Nucula delphinodonta* also occurs in deep waters, but is also present in the high Arctic, where its range is interrupted in Greenland and the East coast of Iceland (Abbott 1974; Lubinsky 1980; Turgeon *et al.* 1998). *Lyonsia* (*Lyiosiella*) *formosa*, is a warm water species and the species status is doubtful (Poppe & Goto 1993). *Thracia phaseolina* is a species complex with an undescribed species in the Northwest Atlantic that has been attributed to *T. phaseolina. T. phaseolina* is also often confused with other *Thracia* species (Mikkelsen & Bieler 2008). There are no cryptogenic infaunal bivalves, nor are there infaunal bivalves that have a disjunct distribution that can be explained by natural long-distance dispersal mechanisms, in particular pelagic larval dispersal.

Discussion

Disjunct amphi-Atlantic distributions

Amphi-Atlantic distributions and the connection between the faunas of Europe and America have been subject of several studies. The relationship of the European and the American boreal Atlantic fauna was first investigated by Lovén as early as 1846 (Briggs

1995). He found that about 8% of the purely boreal species (i.e. species not occurring in Arctic waters, the disjunct amphi-Atlantic species in our study) were shared between the two sides of the Atlantic (Lovén 1846, in Briggs 1995). For fishes the estimated number is higher: about 24% of the purely boreal Atlantic fishes occur on both sides of the ocean (Briggs 1974).

Amphi-Atlantic distributions of ascidians have been studied by several authors (Huus 1927; Plough 1978; *et al.* 1998). Plough (1978) concluded that the American ascidian fauna is largely derived from the European ascidian fauna. Huus (1927) studied amphi-Atlantic distributions in more detail, and identified four species with a disjunct amphi-Atlantic distribution: *Molgula manhattensis, Styela canopus, Cnemidocarpa mollis* and *Botryllus schlosseri*. Only for *C. mollis* does he conclude that shipping has not dispersed this species across the Atlantic, which is in accordance with our results. However, the number of disjunct amphi-Atlantic species in the present study is higher than in his report, partly because of newly introduced species that were not yet present in the early 20th century, and partly because our knowledge of ascidian distributions has increased. Huus concludes that because so few species are shared between both Atlantic coasts, the Atlantic Ocean forms an impassable barrier for ascidians. Naranjo *et al.* (1998) also show that the number of amphi-Atlantic ascidian species is low and corresponds with typically cosmopolitan species that are in general associated with shipping traffic or other anthropogenic vectors.

Molluscs have extensively been studied on both Atlantic coasts, and amphi-Atlantic relationships between the molluscan faunas have also been analyzed by various authors. However, the groups that have been studied vary and are formed based on taxonomy; species are not grouped according to their life-history characteristics. The relative numbers of amphi-Atlantic species thus also vary among studied groups. Coomans (1962) compared molluscs of the boreal regions of the Atlantic and found that about 18% of native American molluscs also occur in Europe. Of the gastropod orders Nudibranchia (33 species) and Cephalaspidea (24 species) that occur in the Northwest Atlantic, about 25–30% have a disjunct amphi-Atlantic distribution (Franz 1970). Vermeij (2005) analyzed current geographical distributions and the fossil record of all shallow-water shell bearing molluscs in cool-temperate North Atlantic waters. No distinction was made between continuous and disjunct amphi-Atlantic distributions, and together these accounted for 23% of the total number of species, compared to 15% of the North Atlantic bivalves in our study. The origin of amphi-Atlantic distributions of shell-bearing molluscs lies in the Middle Pliocene, about 3.5 million years ago. The Northeast Atlantic acted as a donor region for amphi-Atlantic shell-bearing molluscs that spread to the Northwest Atlantic coast, even though the route for this colonization remains unknown (Vermeij 2005). It is hypothesized that warm interglacial periods created the possibility of using the Arctic as a stepping stone in dispersal, which is supported by the presence of fossil European mollusc species in Greenland (Símonarson, Petersen, & Funder 1998). The uni-directionality of the invasion is certain: there are no records of amphi-Atlantic shell-bearing molluscs that spread in the other direction without the assistance of humans. Current Northwest

Atlantic populations of amphi-Atlantic shell-bearing molluscs are derived and largely isolated from Northeast Atlantic populations (Vermeij 2005).

In our study 10% of all species for all groups combined (n = 1054) has a disjunct distribution pattern. This is in the same order of magnitude as the proportions found by other authors for different taxonomic groups (see above). However, the proportion of disjunct distributions differs among groups with differing life-history characteristics. The relative number of disjunct distributions ranges from 3% for infaunal Bivalvia to 48% in the boring Bivalvia. Of the epifaunal bivalves, ascidians and hydrozoans respectively 8%, 9% and 17% have disjunct amphi-Atlantic distributions (Fig. 4.1). We will discuss differences between groups based on natural and anthropogenic dispersal potential, life-history and habitat characteristics of taxonomic groups, and the available literature and knowledge of the studied taxa.

Of all disjunct amphi-Atlantic species combined, 42% of the species with a disjunct distribution may in fact extend their distributions to deep or warm waters, and therefore do not have a strictly disjunct distribution. Anthropogenic introduction has resulted in a disjunct distribution for 19%. For 8% the disjunct distribution is natural. Seven of the species with natural disjunct distributions are hydrozoans, one is an ascidian, and one is an epifaunal bivalve. The cryptogenic species category accounts for 32% of all disjunct species, and this number varies among groups (Fig. 4.2). For these cryptogenic species, different scenarios may explain the formation of a disjunct distribution. The differences in the occurrence of cryptogenic species between and within taxonomic groups, combined with reviewed information from the literature on natural and anthropogenic dispersal and the legacy of the LGM, can tell us which of the four proposed scenarios is, or are, responsible for the formation of disjunct distributions in the North Atlantic Ocean.

Natural dispersal

Natural dispersal of shallow-water benthic invertebrates occurs mainly via pelagic propagules, or by rafting of egg masses, juveniles or adults. Dispersal of propagules of aquatic organisms by birds and by wind have been described for a variety of aquatic organisms (Bilton *et al.* 2001; Green & Figuerola 2005), but have not been documented for any of the groups involved here. We focus only on rafting and larval dispersal, which are assumed to occur frequently and maintain connectivity of populations of hydrozoan, ascidian and bivalve species across ocean basins.

Bivalves, ascidians and hydrozoans possess pelagic free swimming larvae that disperse away from the adults. The hydrozoan life cycle is more complicated and can additionally include a free swimming medusa stage, and some hydroids can also form resting stages (cysts) that may passively be transported in ocean currents as well (Calder 1990).

Larval dispersal is dependent on spawning, larval transport, survival, and settlement, and is not solely determined by currents (Cowen *et al.* 2000; Pineda *et al.* 2007). However, currents do to a great extent determine the direction of dispersal. Swimming behavior of larvae may change this direction: larvae may actively move up or down in

the water column and thus end up in different currents and change the direction of transport (Pineda *et al.* 2007), but this will not be discussed here.

Larval dispersal

The invertebrate groups in our study all potentially disperse via planktonic larvae. The planktonic larval durations of the different groups vary from minutes to hours (to days due to delayed metamorphosis) in ascidians (Svane & Young 1989; Lambert 2005a), to days to weeks in hydrozoans and weeks to months in bivalves (Thorson 1950). Crossing the Atlantic from west to east across the widest stretch of ocean is estimated to vary from four to thirteen months (Scheltema 1971b). The *average* planktonic larval duration of the ascidians, hydrozoans or bivalves in our study is not sufficient to cross the Atlantic Ocean. Besides larval transport by ocean currents, there are several other factors influencing successful dispersal of larvae, such as spawning, developmental duration and mortality, and settlement (Bilton *et al.* 2002; Pineda *et al.* 2007). Mortality rates of larvae during the planktonic phase are high, and are caused by limited food supplies, predation, and advection of larvae to deep waters where they are lost (Thorson 1950; Bilton *et al.* 2002).

Planktonic duration

Planktonic duration of larvae and dispersal distance are positively correlated; the longer the larvae spend in the plankton, the wider the dispersal scale (Jablonski 1986; Siegel *et al.* 2003; Levin 2006). Developmental duration of invertebrate larvae is variable within and between species. The time spent in the plankton until settlement depends on the larval feeding mode: non-feeding, lecitotrophic, larvae are shorter lived than feeding, planktotrophic, larvae. Species with planktotrophic larvae are expected to have wide, disjunct distributions, whereas species with lecitotrophic larvae are expected to have continuous distributions on a smaller geographic scale (Jablonski & Lutz 1983).

Developmental duration of lecitotrophic larvae is dependent of size: bigger larvae have greater nutritional reserves and can postpone settlement (Marshall & Keough 2003). Delayed metamorphosis and hence settlement in the absence of suitable settlement cues is common in invertebrates (Thorson 1950). Delay of metamorphosis up to weeks to months or even years (up to 4.5 years in a laboratory experiment, Strathmann & Strathmann 2007) is common in bivalves, which may have lecitotrophic or planktotrophic larvae. It has also been reported for ascidians, which have lecitotrophic larvae. Some ascidian species can delay metamorphosis with days (e.g. up to six days in *Ciona intestinalis*) (Svane & Young 1989). For Hydrozoa delayed metamorphosis has not been demonstrated, but the resting stages of Hydrozoa can stay dormant for long periods (Calder 1990). Delaying metamorphosis by cessation of growth (Pechenik *et al.* 1984) increases the time spent in the plankton, and thus increases dispersal potential. The increase of dispersal potential is especially pronounced in bivalves. Bivalves have another way of increasing the planktonic duration, which is byssus drifting during the early post-larval stage (Sigurdsson *et al.* 1976). Byssus drifting is common in many marine bivalves and gastropods, regardless of their mode of development. Species

with long larval durations, but also brooding species show post-metamorphic byssus drifting (Martel & Chia 1991). Byssus drifting is expected to be of importance in final site-selection of bivalves before settlement and does not take place for a long period of time. Delayed metamorphosis may also come at a cost, it may result in a decrease in settlement success and post-settlement growth and survival (Hunt & Scheibling 1997).

Dispersal scale

Dispersal scales in the ocean are generally larger than on land due to the fact that many marine organisms spend part of their life cycle in the water column and can then be transported in currents in this three-dimensional fluid environment (Kinlan & Gaines 2003). Marine populations are viewed as open systems, with propagules dispersing outside the populations and maintaining connectivity of metacommunities (Caley *et al.* 1996). However, this paradigm has recently changed: marine populations are less open than they were thought to be and retention of larvae in coastal waters is not uncommon (Levin 2006).

Dispersal distances are very variable between species, ranging from meters to hundreds of kilometers (Kinlan & Gaines 2003; Gaines *et al.* 2007; Bradbury *et al.* 2008). The largest invertebrate dispersal scales, of bivalves, are still not enough to cross the Atlantic Ocean on a regular basis and maintain connectivity between disjunct populations. However, it is hypothesized that a small proportion of larvae may indeed make it across by long-distance dispersal due to rare events, such as extreme weather conditions (hurricanes, tornadoes, typhoons) (Cowen *et al.* 2000; Nathan 2006; Pineda *et al.* 2007).

Long-distance dispersal is generally seen as a rare, stochastic event that can nevertheless have huge consequences for distribution patterns of terrestrial and marine organisms. Proof for long-distance dispersal comes from ecological and biogeographical studies. Multiple colonizations of remote islands, such as the Hawaiian islands (Visher 1925), and intercontinental disjunctions, for example of plants across the Atlantic Ocean, may not only be caused by vicariance, but may also be the result of long-distance dispersal (Queiroz 2005; Nathan 2006).

Larvae cannot individually be tracked to follow their dispersal pathway, and our knowledge of dispersal scales is therefore mostly indirect, and comes from genetic studies, studies of rates of spread of non-indigenous species, and biophysical modelling (Gaines *et al.* 2007). Indications of the ability of larvae to cross the Atlantic Ocean are observations and estimates of extended larval durations, postponement of metamorphosis and byssus drifting (see previous section), and the presence of planktotrophic larvae of coastal polychaetes, gastropods and bivalves in plankton samples from the central Atlantic Ocean (Scheltema 1971a; 1971b; 1986; 1995). However, these are all *indications* of long-distance dispersal across the Atlantic, but they do not demonstrate actual dispersal and gene flow between populations of both sides of the Atlantic.

Looking at the larval composition of plankton samples from the central Atlantic, the number of species and the number of individual larvae diminishes from west to east in the Gulf Stream towards the European coast (Scheltema 1971a; Laurson 1981).

Furthermore, the presence of larvae is not necessarily accompanied by presence of adults of the same species, as has been shown for gastropods (Laurson 1981) and polychaetes (Bhaud 1998). Larvae typically cover an area larger than adults, and do not spread as far as might be expected when modelling dispersal taking only ocean currents and advection into account (Cowen *et al.* 2000). The numbers of species on which Scheltema's conclusions of teleplanic larvae being a mechanism for maintaining contact across the Atlantic Ocean are based are few, and identifications of species are doubted by other authors (Laurson 1981). Regarding the taxonomic groups considered here, there are no records of presence of larvae of Ascidiacea and Hydrozoa in ocean currents. Although Scheltema did collect bivalve larvae from the open ocean, he did not identify them to species level (Scheltema 1995), and we can therefore not use these data to see whether they can explain disjunct distributions of particular species. The studies by Scheltema stand out in the larval dispersal literature, and in a recent review of dispersal scales the estimates from Scheltema's studies were excluded from analyses because they differ too much from other dispersal estimates (Bradbury *et al.* 2008).

Wide distributions and long larval durations

Dispersal potential and the resulting expected distribution are different between and within taxonomic groups in our study (Table 4.1). The Hydrozoa were divided in two groups: those that release medusae and those that retain the medusa and for which the dispersive stage is therefore the larva. The species that have a free swimming medusa stage were expected to have wide distributions. However, this is also not shown in our results: proportions of disjunct distributions are similar in medusa-releasing and medusa-retaining hydrozoa. A long pelagic phase in the life cycle apparently does not necessarily result in a wide (disjunct) distribution. Absence of this relationship may be due to the fact that the longevity of the free-swimming medusae is not known and may differ per species. Furthermore, Hydrozoa possess other mechanisms that increase their dispersal potential and that occur in medusa-releasing and medusa-retaining groups. Many Hydrozoa form resting stages in the form of dormant cysts (Rees 1957). Furthermore, reverse development has been demonstrated in the hydrozoan *Turritopsis nutricula*: medusae transform back into colonial hydroids, directly or through a resting period, thus achieving potential immortality and high dispersal potential (Piraino *et al.* 1996). Dormant tissue in the hydroid stems and stolons provides another means for survival of unfavorable conditions. Cessation of growth, followed by redifferentiation and resorption of hydranths, was observed in 13 hydrozoan species (Calder 1990). Detached, floating polyp colonies of *Ectopleura crocea* in the plankton can also reattach to substrates by new rhizomal growth (Carlton, pers. comm.) These mechanisms occur in both medusa-releasing and medusa-retaining Hydrozoa, and this may explain the absence of differing patterns in distributions of medusa-releasing and medusa-retaining Hydrozoa. These characteristics of Hydrozoa greatly increase their dispersal potential (Calder & Burrell 1969), be it by natural or anthropogenic dispersal agents, and it may thus also explain the relatively high proportion of hydrozoan species with a disjunct distribution pattern.

The proportion of infaunal bivalves with a disjunct distribution gives an indication of the importance of long-distance dispersal by larvae in creating and maintaining disjunct amphi-Atlantic distributions, because infaunal bivalves have no other natural means by which to disperse across the ocean. The relative number of disjunct distributions in this control group is low (3%) compared to that for epifaunal and boring bivalves (8% and 48% respectively). More importantly, for all disjunct infaunal bivalves the distribution can be explained by occurrence in deep or warm waters, by anthropogenic introduction, by misidentifications or cryptic speciation (see Results). There are no infaunal bivalves with a disjunct distribution that can only be explained by long-distance dispersal of larvae. Presence of a long-lived pelagic larval stage in the life-cycle apparently does not guarantee widespread occurrence of a species.

In general, there is no positive relationship between dispersal ability and range size on large geographic scales, such as ocean basins, as was shown in a review of empirical studies of dispersal scales (Lester *et al.* 2007). A study of gastropods (cowries) in the Pacific Ocean, where distances are even larger than in the Atlantic, shows that larval duration does not correlate with species range, nor is dispersal capacity negatively correlated with species diversity in tribes or families (Paulay & Meyer 2006). For Hydrozoa, cosmopolitanism is more common in species with fixed gonophores than in those that release medusae (Jackson 1986; Cornelius 1992).

The group with the highest proportion of disjunct distributions in our study is the group of the boring bivalves. Boring bivalves do not extend their distribution to cold waters, possibly because of the absence of drift wood. The Teredinidae (shipworms) are wood-boring bivalves that may or may not brood their larvae, and teredinid larvae have been described from central Atlantic waters (Scheltema 1971a), but these may also be the larvae of oceanic teredinids and not coastal species (Carlton pers. comm.). Of the twelve teredinid bivalves in the North Atlantic, eleven have a disjunct distribution (see Table 4.4). In this bivalve group, the species that lack a long pelagic larval phase but instead brood their larvae, are more successful invaders and are often more widely dispersed (Hoagland & Turner 1980).

This pattern has been demonstrated in other taxa as well. Comparing species with long larval durations with direct developers, brooding species have wider distributions than related taxa with planktotrophic larvae, contrary to what would be expected based on their larval dispersal potential (Johannesson 1988; Ó Foighil 1989). The brooding bivalve *Lasaea adansoni* in the North Atlantic has a disjunct amphi-Atlantic distribution (see Table 4.4) that can not be explained by larval dispersal (Ó Foighil & Jozefowicz 1999). Two gastropod sister-species, *Littorina littorea* and *Littorina saxatilis*, of which the former releases planktotrophic larvae that are free swimming for four weeks, and the latter broods the embryos, have contrasting distribution patterns in the North Atlantic. Brooding *L. saxatilis* is more widespread than its sister species, inhabiting even the remotest islands in the North Atlantic, where *L. littorea* is absent (Johannesson 1988). Remote islands have high relative numbers of direct developing invertebrate species (Johannesson 1988), which can be explained by dispersal of the adult or juvenile stages on floating substrata, such as algal or other rafts, or attached to

ships, rather than by dispersal via planktotrophic larvae and subsequent loss of a pelagic larval stage (Ó Foighil & Jozefowicz 1999). *L. saxatilis* occurs on floating *Ascophyllum* and *Fucus* in the Gulf of Maine and off Iceland, but whether individuals can hang on for a full ocean crossing is not known.

In summary, our results and the literature show that a life cycle with a long-lived pelagic larval stage does not guarantee settlement success and presence of adults on shores distant from the source populations of the larvae. Larvae found in the open ocean may be lost to their populations and are not a common mechanism in maintaining gene flow over such large distances as the width of the temperate North Atlantic Ocean. The larvae found in the open ocean may not be of coastal species. The occasional long-distance dispersal of larvae that settle and form new populations has not directly been proven, and long larval durations do not guarantee wide or disjunct distributions. In fact, brooding species are often more widely dispersed than their relatives with planktotrophic larvae. Long-distance dispersal of larvae does not explain the disjunct amphi-Atlantic distributions of invertebrates and algae, and other modes of transport, such as rafting and shipping, are more likely candidates for dispersal of organisms across the Atlantic Ocean.

Rafting

The literature on rafting in the marine environment has recently been reviewed (Thiel & Gutow 2004; Thiel & Gutow 2005; Thiel & Haye 2006). The authors compiled extensive lists of rafting marine and terrestrial species from all phyla, discussed the substrates they raft on, and reviewed ecological and evolutionary consequences of rafting for different taxonomic groups. The results of these studies were used to indicate rafting for individual species in our study (see Tables 4.2-4.4). Here we review some key characteristics of rafting and its potential for creating disjunct amphi-Atlantic distributions in our study groups.

A successful long distance rafting event, resulting in establishment of a founder population on a distant shore, is dependent of the ability of organisms to hold on to the substrate, establish and compete successfully, and survive the voyage and settle or recruit at arrival (Thiel & Gutow 2005). The dynamics of a rafting community are dependent of the rafting substrate and changes in species compositions. Competition for space and resources, colonization by new species, predation, detachment of species, and loss of the substrate (e.g. by grazing on macroalgal rafts) are factors that affect species composition and abundance of organisms on rafts (Thiel & Gutow 2005). Rafts may become too heavy to remain floating by the increasing mass of the rafting community. Rafting communities are different from communities on the same substrate while it is still attached. Many mobile organisms that live as epibiota on macroalgae abandon their substrate when it is detached (Ingólfsson 1995), and thus a rafting community is not necessarily a subset of the coastal community from the area where it originates. During the voyage species are added and lost, some of them being facultative rafters and others obligate rafters that colonize the substrate on the way. Abiotic substrata are mostly colonized by pelagic organisms (Thiel & Gutow 2005) and

therefore play a minor role in dispersal of benthic biota such as the groups dealt with here.

A total of 1205 terrestrial and marine species that are known or inferred to raft have been listed, of which 41 are obligate rafters. Almost all rafting species have internal fertilization and many incubate their offspring or deposit eggs on rafts (Thiel & Gutow 2004; 2005). The most common rafters are Amphipoda (108 species) and Hydrozoa (102 species); 51 species of Bivalvia and 11 species of Ascidiacea are known or inferred to raft (Thiel & Gutow 2005).

Rafting may be an especially successful mechanism for the dispersal of clonal invertebrates. Aclonal invertebrates are dependent of sexual reproduction for settlement in a new range, whereas clonal invertebrates have the advantage of being simultaneous hermaphrodites and being capable of asexual reproduction, e.g. by budding or fragmentation (Jackson 1986). Dispersal by rafting is potentially more successful than dispersal by planktonic larvae. Not only can clonal organisms settle easily by asexual reproduction, those species that do reproduce sexually might also be more successful in colonizing new regions: chances of successful colonization of a brooding female are larger than those of a single larva. Maintaining a population after dispersal on rafts is easier for species that have short-lived larvae: larvae are not lost and mates are easily found because the founding population is spread over a small spatial scale (Johannesson 1988).

Ascidians, in particular compound ascidians, have all the characteristics needed for successful dispersal by rafting: they have short-lived larvae that are sometimes brooded, and some are clonal, capable of asexual reproduction or self-fertilization. These characteristics enhance the chance of establishment of a population after arrival. Of the eleven ascidians listed as rafting species by Thiel and Gutow (2005), four are solitary and the others are compound ascidians. Compound ascidians are apparently more often observed rafting, which is expected for clonal organisms. Of the four solitary species, only two have actually been observed on rafts, and for the other two rafting was inferred from circumstantial evidence. In our study, compound ascidians would be expected to be more widespread than compound species. However, of the sixteen ascidian species with a disjunct amphi-Atlantic distribution, only four are compound species. All three compound ascidians with a disjunct distribution pattern that have been observed rafting (*Diplosoma listerianum, Botrylloides violaceus* and *Botryllus schlosseri*) happen to have been introduced by humans to either or both Atlantic coasts (see Table 4.2). None of the solitary ascidians with a disjunct amphi-Atlantic distribution have been reported rafting.

The same pattern is seen for Hydrozoa, representatives of which are often found on rafts. Rafting of the hydroid stage is often assumed to be the most important dispersive mechanism for this group of organisms (Jackson 1986; Cornelius 1992). Of the 68 hydrozoan species with a disjunct amphi-Atlantic distribution pattern, rafting has been reported for 21 species (see Table 4.3). Two species are obligate rafters, and for eight species rafting appears to be a dispersal mechanism, besides dispersal of the medusa stage. Most of these also occur in warmer waters. For five species rafting was inferred

from their disjunct distributions, and seven species were not only reported rafting, but have also been observed on ship hulls, which would provide another possibility for long-distance dispersal. An example is *Laomedea calceolifera*, which has a disjunct distribution and has been observed rafting, but was introduced to Cape Town, South Africa, probably on ships (Millard 1959). *Obelia bidentata*, *O. dichotoma* and *Plumularia setacea* are near cosmopolitan species that have been observed rafting, but are also common fouling species.

Epifaunal and boring bivalves are also likely candidates for dispersal by rafting. However, of the 33 disjunct bivalve species, of which 23 are epifaunal and boring, only 14 have been observed rafting (see Table 4.4). Of these 14 rafting bivalves, 11 are shipworm species, which bore in wood and can thus be dispersed by drifting wood. Most disjunct distributions of shipworm species are based on single records on either side of the ocean, where established populations are absent. Shipworms often occur in warmer waters. For example, *Bankia carinata* has a circumglobal distribution in (sub)tropical waters and is occasionally carried to Western European waters in drift wood. Another obvious dispersal mechanism for this group of bivalves is shipping. The notorious shipworm *Teredo navalis* that was first described from Europe as early as the 18th century might be of Pacific origin, and is likely to have been brought back to Europe by trading vessels (Wolff 2005b, Chapter 2). Besides the shipworms, three bivalve species have been observed rafting. *Geukensia demissa* was introduced to the Northeast Atlantic from the Northwest Atlantic. *Lasaea adansoni*, which was also mentioned in the larval dispersal section, is a brooding bivalve that occurs on remote islands in the North Atlantic Ocean, which it probably reached by both rafting and anthropogenic dispersal (Ó Foighil & Jozefowicz 1999). *Mytilus edulis* has not only been observed rafting, but it is also a common fouling species. The North Atlantic phylogeography of *Mytilus* will be further elaborated on below.

All examples above of observations of rafting organisms come from observations of these organisms on coastal rafts. Descriptions of rafting communities on offshore rafts are missing, and therefore disjunct distributions are often used as an indication for the ability of an organism to raft. In the review by Thiel and Gutow (2005), for 18% of the ascidians, 32% of the hydrozoans, and 35% of the bivalves, inclusion in their species lists was based on "circumstantial evidence" of rafting, meaning their disjunct distribution patterns. The underlying assumption was that the original authors of the papers included in the review by Thiel and Gutow (2005) had considered all possible explanations and concluded that rafting was the only possible dispersal mechanism resulting in a disjunct distribution pattern. However, to prove that rafting is the only means by which a disjunct distribution pattern can be created, genetic, biogeographic, ocean circulation modelling and often even historical or paleontological evidence have to be combined.

This was elegantly performed in the study of the trans-Pacific range extension of the oyster *Ostrea chilensis* (Ó Foighil *et al.* 1999). *O. chilensis* lacks a long pelagic larval phase and has a disjunct distribution pattern with populations in New Zealand and Chile that are separated by a 7000 km open-ocean barrier. Fossils and molecular phylo-

genetic analyses were used to test different dispersal theories of vicariance, anthropogenic introduction, larval dispersal by ancestral lineages with long larval durations, and rafting. Dispersal by rafting from New Zealand to Chile was according to the authors by far the most likely explanation for this disjunct distribution pattern. Rejection of anthropogenic dispersal was based on radiocarbon age estimates of specimens from Chile that predated human settlement in New Zealand (Ó Foighil *et al.* 1999). However, the date of human settlement of New Zealand has recently been pushed back to 2000 BP instead of 800 BP (Sutton *et al.* 2008), meaning that *Ostrea chilensis* could also have been transported as fouling on Polynesian ship hulls and the human dispersal hypothesis cannot be discarded.

Other studies use only one type of data; in the case of the southern hemisphere coastal sea-star *Patiriella exigua* rafting was inferred from mtDNA haplotype analyses. The authors rejected an anthropogenic introduction hypothesis based on the absence of shared haplotypes on different continents. However, their sample size was small, with a total of 43 individuals sequenced and between 1 and 6 sequences per location (Waters & Roy 2004).

Rafting is not as important as a long-distance dispersal mechanism as is commonly assumed, not even for organismal groups with low larval dispersal capacity and the life-history characteristics that ensure successful rafting, such as asexual reproduction and brooding. Rafting is no doubt an important means of maintaining population connectivity on smaller spatial scales, e.g. along coastlines. However, the ability to raft alone can not explain the disjunct distributions patterns of ascidians, hydrozoans and bivalves in the North Atlantic Ocean, as all records of rafting organisms come from coastal rafts and not from the open ocean. Presence of species on coastal rafts does not demonstrate their ability to disperse across the ocean.

Post-glacial recolonization

The most recent events in geological history with huge impact on the biogeography of North Atlantic benthic marine organisms are the Pleistocene Glaciations, 25,000 – 18,000 BP, culminating in the Last Glacial Maximum (LGM), ~21,000 BP. Species ranges contracted and expanded during that period due to ice cover and drops and rises in sea level. Temperate species ranges were contracted southwards in glacial refugia. From these refugia in southern regions benthic invertebrates and algae recolonized part of their former range in northern waters.

Glacial refugia

Phylogeographic studies on a variety of benthic marine invertebrates and algae have resulted in the definition of several glacial refugia in the North Atlantic, most of them in southern regions. So far, seven refugia have been identified in the East Atlantic, and two in the West Atlantic. Only recently has it been demonstrated that northern periglacial refugia also existed, in the Northeast and Northwest Atlantic. Identification of refugia was based on haplotype diversities and distributions of particular haplotypes (Maggs *et al.* 2008).

In the Northwest Atlantic, the main glacial refugia were located in northern Canada, and south of Cape Hatteras, where hard substrates are sparse (Wares & Cunningham 2001; Wares 2002; Maggs *et al.* 2008, see Fig. 1.4). In our results, for all studied groups the Northwest Atlantic is poorer in species than the Northeast Atlantic (by a factor of 2.5). Missing endemics on the American rocky shores have been attributed to the absence of rocky habitat south of Long Island Sound, the steep temperature gradient, and input of glacial melt water after the LGM (Bousfield & Thomas 1975; Vermeij 1978). Remarkably, this difference in species richness is less pronounced for the infaunal bivalves: the Northeast Atlantic is a factor 1.5 richer in species of infaunal bivalves, compared to factors 11 for ascidians, 2.6 for hydrozoans, and 2.9 for epifaunal bivalves. The reason for this could be that shallow-water infaunal bivalves suffered less from the Pleistocene glaciations, i.e. they may be less susceptible to low temperatures and ice-scouring because they are protected by a substrate cover.

For epifaunal hard-substrate species that occur only in temperate waters, and not in Arctic waters, it is difficult to explain disjunct amphi-Atlantic distributions with trans-oceanic dispersal after the LGM. On the Northwest Atlantic coast, species that are obligatory hard substrate species would have had to either retreat to a northern refuge, meaning that they were capable of surviving low temperatures and would now be likely to occur in the Arctic as well, or they would have had to survive in a southern refugium, where hard substrates are rare. The recolonization from refugia is assumed to have taken place across the Atlantic Ocean as well, mostly from Europe to America (Briggs 1974; Vermeij 2005). In the North Atlantic, the Gulf Stream, originating in the Gulf of Mexico, follows the North American coast north to Newfoundland, where it crosses the ocean. It splits in two, forming the North Atlantic current, which continues northwards along the Northern European coast, and the Canary Current, which runs south to the Iberian Peninsula and Western Africa. The North Equatorial current is directed back to the West Atlantic (Tomczak & Godfrey 2003). The predominant current direction in the North Atlantic Ocean is thus from west to east. However, the direction of migration is thought to be from east to west, as the European coast harbors a higher number of species and species on the American Atlantic coast are thought to originate in Europe (Vermeij 2005). The reason for the uni-directionality of invasion remains unknown, since the Gulf Stream would have been able to carry larvae across to Europe and dispersal in this direction would have been more likely (Vermeij *et al.* 2008). Modelling has shown that there might be an occasional route of dispersal from east to west in Arctic waters (Dawson *et al.* 2005), but low temperatures are likely to inhibit larval dispersal and settlement of temperate species.

There is no evidence that currents were at the time of the LGM different from the modern current regime, making it difficult to explain how extensive populations with high diversity could have established through natural dispersal across the open ocean. Relict populations on both coasts should show some level of genetic divergence and a signature of a long evolutionary history. This has been shown for the bivalve *Mytilus edulis*. The shell bearing molluscs are a group of invertebrates that is well-studied and

that provides a reliable fossil record for comparison of current and past ranges and dating of population divergence. *Mytilus* is perhaps the most extensively studied bivalve genus; it has been used as a model organism for studying evolution in the sea. *Mytilus edulis* has a disjunct distribution in the North Atlantic Ocean, but fossil *M. edulis* specimens from the Pliocene were found in northern Greenland where it does not occur now (Símonarson *et al.* 1998). It recently reappeared in Svalbard after having been absent for 1000 years; unusually high mass transport of warm waters resulted in supply of larvae and elevated sea-surface temperatures which enabled settlement of larvae (Berge *et al.* 2005). *Mytilus edulis* apparently became extinct in Arctic waters when temperatures dropped, and its disjunct distribution is thus a result of the Last Glacial Maximum, after which populations remained on both Atlantic coasts. The direction of gene flow in the northern North Atlantic is from west to east (Riginos & Henzler 2008), which is in accordance with the current regime. There are no examples of studies of strictly temperate species for which conclusive evidence was presented of natural dispersal across the ocean after the LGM.

Genetic diversity

Newly colonized ranges were long thought to be characterized by low haplotype diversities and high frequencies of alleles originating in a glacial refugium (Hewitt 1996; 1999; 2000). The low diversity is caused by population bottlenecks due to small founding populations. However, not all populations on recolonized coasts have low genetic diversity. Newly colonized ranges can even harbor higher genetic diversity than refugia, because of admixture after invasion from more than one refugium (Maggs *et al.* 2008). The signature left by recolonization after retraction in a glacial refugium can thus be very different across species, depending on the location of refugia and whether recolonization occurred from one or more refugia. The only way to distinguish between different scenarios of post-glacial colonization is by extensive sampling throughout the range of a species, investigation of distribution patterns of individual haplotypes and the structure of haplotype networks (Maggs *et al.* 2008).

To complicate things further, low genetic diversity due to population bottlenecks can also be the result of anthropogenic dispersal (Geller *et al.* 1994; Holland 2000; Dlugosch & Parker 2008). Similar to natural populations that are shaped by the LGM, introduced populations do not always exhibit low genetic diversity; multiple introductions can result in high diversity. Genetic diversity may even be higher than in the native range, because between-population diversity of different sources accumulates in the introduced range and results in high within population diversity (Roman 2006; Roman & Darling 2007), and diversity levels increase over time (Dlugosch & Parker 2008).

As genetic diversity of introduced populations may either be higher or lower than that of native populations, and the result of the LGM may also be low or high diversity, it is hard to discriminate between the two patterns. This is evident from the debate about the status of *Littorina littorea* in North America, which went on for a century. Arguments in favor of and against anthropogenic introduction of *L. littorea* were based

on genetic data, sudden rapid range expansion in the 19th century and dating of subfossils (Carlton 1999b; Wares *et al.* 2002; Chapman *et al.* 2007). Molecular data were at the same time interpreted to prove and disprove the anthropogenic introduction hypothesis, depending on the analyses used (Wares *et al.* 2002; Chapman *et al.* 2007; Cunningham 2008). The debate has finally been resolved by using genetic data of *L. littorea*'s host-specific trematode parasite in combination with extensive sampling of *L. littorea* throughout its range (Blakeslee *et al.* 2008). Multiple lines of evidence are often necessary to distinguish between natural patterns of diversity and human-mediated genetic diversity (Chapter 5).

Cryptic species

Another possible explanation for a disjunct amphi-Atlantic distribution pattern is that the species in question is in fact not a single species, but a species complex. Cosmopolitan species are common among marine invertebrate taxa, especially among the less known and less conspicuous taxa. The 'everything is everywhere' hypothesis states that small organisms (bacteria, protists, small invertebrates), due to their high dispersal potential, have cosmopolitan distributions and do not show biogeographical patterns. This hypothesis has been challenged in the past decades by molecular genetic studies, which have resulted in the discovery of cryptic species complexes across all taxonomic groups and in all habitats. The marine realm harbors an especially high number of cryptic species because of the high species richness and complex interspecific interactions (Bickford *et al.* 2007). Phylogeographic studies have revealed cryptic diversity for many marine taxa, e.g. polychaetes (Westheide & Schmidt 2002), sponges (Klautau *et al.* 1999; Nichols & Barnes 2005), bryozoa (Mackie *et al.* 2006; Gómez *et al.* 2007; Nikulina *et al.* 2007), jellyfish (Dawson *et al.* 2005) and even the smallest eukaryotes (Slapeta *et al.* 2006). Everything is not everywhere, meaning that either environmental conditions do not allow settlement of propagules that do make it everywhere (we have discussed this aspect in the natural dispersal section), or that the traditional method of distinguishing species based on morphological characteristics is not sufficient.

Many cosmopolitan species appear not to be truly cosmopolitan when analyzed with molecular methods. *Ciona intestinalis* is a well-known cosmopolitan ascidian that has an amphi-Atlantic distribution. It is a model organism of which the genome has been sequenced to study chordate evolution. *Ciona intestinalis* appears to be a species complex, consisting of two species (species A and species B). While the two species appear to have some pigmentation differences, they do not differ distinctly in morphology, although they do have distinct geographic distributions (Suzuki *et al.* 2005; Iannelli *et al.* 2007; Caputi *et al.* 2007; Nydam & Harrison 2007). *Ciona intestinalis* species B occurs on both coasts of the North Atlantic. Species A has a near cosmopolitan distribution, but in the North Atlantic only occurs south of the English Channel, where it co-occurs with species B. The two species hybridize, but the hybrids are infertile (Caputi *et al.* 2007). The genetic structure of species A is homogeneous, indicating recent spread. This species has been introduced by shipping to many areas, possibly

also to the English Channel. Species B is genetically structured with fixed haplotypes, indicating an ancient origin (Caputi *et al.* 2007). Species B has a continuous amphi-Atlantic distribution, whereas species A has a patchy distribution. The origin of species A is unknown: it is a cryptogenic species. We have assigned the *C. intestinalis* species complex, which might even consist of more than two subspecies (Nydam & Harrison 2007), to the amphi-Atlantic category, because the exact distributions of the two species in this complex are not yet known in detail (see appendix I).

Even in a well-studied group such as the bivalves species boundaries are not clear and cryptic speciation is not uncommon (Luttikhuizen *et al.* 2003). The *Mytilus* complex consists of three species: *Mytilus edulis, Mytilus galloprovincialis* and *Mytilus trossulus*. In Europe, the three species hybridize in zones of contact (Skibinski *et al.* 1978; Gosling 1992), and differentiation of species is debated, depending on the genetic marker used (Riginos & Henzler 2008). *M. trossulus* has a circumpolar distribution, extending into boreal waters in the North Pacific, and also occurring in the Baltic Sea in Europe. The Baltic population is genetically different from the North Pacific populations, but is regarded as the same species with introgression of *M. edulis* mtDNA (Rawson & Hilbish 1998). *M. galloprovincialis* is native in Europe and has been introduced world-wide (Carlton 1999b; Gérard *et al.* 2008). Its introduction in California went unnoticed as it was mistaken for the native *M. trossulus*, whose decline in abundance was masked by the invasion of *M. galloprovincialis* (Geller *et al.* 1994; Geller 2002).

Compared to bivalves, the Hydrozoa are little studied. They are less conspicuous and identification of species is tedious and requires a high level of expertise. Species boundaries are not always clear, and medusa and polyp phases have not always been coupled. The taxonomic status of many species is debated, even in species that are well-known and widely occurring. For example, the 120 species of *Obelia* were synonymized in 4 species (Cornelius 1990). However, based on genetic data, populations of the cosmopolitan hydrozoan *Obelia geniculata* from the North Atlantic, North Pacific and South Pacific may be cryptic species (Govindarajan *et al.* 2005). *O. geniculata* has a disjunct distribution in the North Atlantic Ocean, which is hypothesized to be a result of post-glacial recolonization from refugia in New Brunswick and Iceland. However, the number of samples analyzed from Northwest Europe is low (four individuals from one population), and this conclusion is therefore premature. An anthropogenic introduction of *O. geniculata* in the North Atlantic Ocean was also demonstrated; a population in Massachusetts being a recent human-mediated introduction (Govindarajan *et al.* 2005).

Cryptic speciation is common across all taxonomic groups. Especially in less conspicuous taxa, species complexes remain to be discovered with the use of molecular tools. It is thus not unlikely that species with disjunct amphi-Atlantic distributions are in fact two or more species.

Anthropogenic dispersal

Humans have been moving across the Atlantic Ocean since at least 1000 BP when the Vikings first reached the Atlantic coast of North America. This first discovery of

America was not followed up by permanent settlement and exploitation, but contact between the coastal biotas of the Northeast and Northwest Atlantic was thus established. The next "discovery" of America by Columbus in 1492 was followed by intensive trade and shipping across the Atlantic, and in addition whaling and fishing fleets were sailing across the ocean.

Vectors

The major vectors for introduction of non-indigenous coastal organisms with historical shipping were the solid ballast they carried for stability, the shipments that were transported, in particular live oysters that were relaid in recipient waters, and the fouling and boring communities on and in the hull. Ballast rocks or sand were collected mostly from the intertidal, and associated introductions are thus intertidal organisms, such as the rockweed *Fucus serratus*, the snail *Littorina littorea* and the European shore crab *Carcinus maenas* that were introduced to America from Europe in the 19th century (Carlton & Cohen 2003; Blakeslee *et al.* 2008; Brawley *et al.* 2009). Rock ballast was replaced by ballast water around 1880. Ballast water potentially contains species from all phyla, all stages in the life cycle and from all aquatic habitats, and is a very potent vector in modern times. Live American oysters, *Crassostrea virginica*, have repeatedly been introduced to Europe from America from 1870 to 1939 and in 1948 (Carlton & Mann 1996; Wolff & Reise 2002), and there are many examples of associated introductions, such as the slipper limpet *Crepidula fornicata* (Blanchard 1997, Chapter 2) and the boring bivalve *Petricolaria pholadiformis* (Carlton 1999b) that were introduced to Europe in the late 19th century. Because ballast water use and oyster shipments occur since the late 19th century, we generally know which species were introduced by these vectors. However, ships have been moving around with hull fouling and boring communities for centuries before that, and this shipping vector was therefore the most important vector of introduction for subtidal species between the 11th and the 19th century.

Hull fouling

Historically, ships were made of wood, they traveled at slow speeds, and had long port residence times, allowing rich hull fouling communities to develop and increasing the chance of successful introduction and establishment of a permanent population (Allen 1953). Early anti-fouling treatments were the use of lead sheathing (since ancient times) and a variety of tar, oils and other substances the hull was impregnated with. These treatments mainly aimed at preventing shipworm settlement, which was a big hazard for ships underway, and did not effectively prevent fouling of the hull. The ships periodically had to be careened and scraped or sailed into freshwater rivers, to remove fouling and thus to reduce drag, and in the mean time carried a rich fouling community. A wooden sailing vessel around 1750 could theoretically carry an approximated total number of 156 species of invertebrates, algae and plants on and in its hull, on the anchor, and in its sand and rock ballast (Carlton 1999a). It additionally carried various life-stages of invertebrate species and algae in the bilge water (Carlton, pers. comm.).

A common hull fouling community consists mainly of barnacles, tube worms, bryozoans, hydrozoans, and oysters. Mussels, anemones, solitary and colonial ascidians and saddle oysters have all been recorded. If settlement of organisms of these groups has been sufficiently abundant, the hull-fouling community offers shelter to errant species, such as nereid worms, gastropods, and crustaceans (Chilton 1910; Pyefinch 1950; Woods Hole Oceanographic Institution 1952; Gollasch 1999). Hull fouling communities are not simply a subset of the epifaunal species present in a certain area, and fouling communities on moving substrates are different from those on static panels (Berntsson & Jonsson 2003). In Tables 4.2-4.4, we included references on hull fouling for individual species.

In the disjunct amphi-Atlantic category, 28% of the Hydrozoa, 35% of the Bivalvia and 63% of the Ascidiacea are known to occur in ship-fouling communities. This number is especially high for Ascidiacea, and in addition, most of the hull-fouling Ascidiacea have been introduced to either or both Atlantic coasts. The Ascidiacea are thus likely candidates for ship-aided dispersal, which has also been acknowledged by other authors (Huus 1927; Monniot & Monniot 1983; Monniot *et al.* 1985; Naranjo *et al.* 1998). The extent to which shipping has influenced current ranges of Ascidiacea is illustrated by the ascidian fauna of the Azores, a remote North Atlantic island archipelago with a relatively low ascidian species richness. Remarkably, species richness on islands with a port is highest, supporting an anthropogenic origin of a substantial proportion of the ascidian fauna (Monniot & Monniot 1983). Hydrozoa are also common ship-fouling species, and the role of shipping in creating cosmopolitan distributions of Hydrozoa has also been noted by other authors (Millard 1959). Of the disjunct amphi-Atlantic Hydrozoa 31% have been observed or inferred to disperse on rafts, but these observations are not from rafts on the open ocean and rafting is therefore not demonstrated to result in successful dispersal across ocean basins. Of these Hydrozoa that are inferred to raft across the ocean, 30% have also been recorded from ship hulls, which are known to travel across ocean basins. Although rafting may be a possible mechanism for dispersal of Hydrozoa on a regional scale, shipping is a more likely trans-oceanic dispersal agent.

Those species that are capable of attaching to rafts are also likely to be able to attach to ships, which more effectively disperse biota to distant shores than rafts. The time required for fouling organisms of temperate waters to reach maturity varies from 11 to 120 days (with some exceptions that mature after 1 year, e.g. *Mytilus edulis*) (Crisp 1965). This means that organisms can settle in one region and be able to reproduce fairly soon at arrival in shallow waters of the recipient region. This was witnessed on the hull of a ship in Pearl Harbor, where spawning of *Mytilus galloprovincialis* specimens in the hull fouling assemblage of the ship was followed by ephemeral recruitment of this mussel in the harbor (Apte *et al.* 2000).

Dispersal by ships is particularly important for species that are sedentary and brood their larvae. The larvae may be ready to metamorphose and settle when the ship arrives in a new harbor (Thorson 1961). An example of a species that has characteristics that promote dispersal by ships is the ascidian *Diplosoma listerianum*, which can store

exogenous sperm for at least a month (Bishop & Ryland 1991), enabling it to release fully developed embryos upon arrival in a harbor. This can explain the success of *D. listerianum* as an invader; it has successfully colonized many regions in tropical and temperate waters (Lambert 2001). *D. listerianum* was also introduced from Europe to America by shipping.

Circumstances in a port of call (e.g. temperature, salinity) can trigger spawning or release of embryos. However, successful colonization does not necessarily have to occur in port areas, but during a journey exotic species may also be able to release propagules that may settle on nearby open coasts (Carlton & Hodder 1995). Studies on en route survival and composition of hull fouling communities across ocean basins are lacking and are a gap in our knowledge.

Examples of historical ship-mediated introductions are numerous. The Portuguese oyster, *Crassostrea angulata*, was introduced to Portugal from Taiwan (Boudry *et al.* 1998; Ó Foighil *et al.* 1998; Huvet *et al.* 2000), possibly in hull fouling of Portuguese merchant vessels. The ascidian *Botryllus schlosseri* was introduced from Europe to America in hull fouling, and it may also have been introduced from the Pacific to Europe (Carlton 2005; Lopez-Legentil *et al.* 2006). The Hydrozoa *Bougainvillia rugosa* and *Nemopsis bachei* were introduced as ship fouling from North America to Europe (Leppäkoski & Olenin 2000; Wolff 2005b; Schuchert 2007). In other taxonomic groups repeated cryptic invasions have also been demonstrated, for example in the cosmopolitan bryozoan *Bugula neritina* (Mackie *et al.* 2006). Bryozoans are a group for which fouling ability is strongly correlated with range, more so than larval development, environmental tolerance, species abundance and the ability to raft, which suggests that transport in hull fouling is a very important dispersal mechanism for bryozoans (Watts *et al.* 1997).

In summary, dispersal of epifaunal invertebrates on ship hulls is fast, efficient and independent of the ocean current regime. Upon arrival in a harbor release of propagules may be triggered by the conditions that are met. Long-distance dispersal by ships is not rare or uncommon: the great numbers of ships that have been sailing across the North Atlantic for centuries have provided a regular dispersal mechanism for a variety of invertebrate species, and have profoundly influenced species ranges, as has been demonstrated for various taxonomic groups (Allen 1953; Monniot & Monniot 1983; Watts *et al.* 1997; Carlton 2003b).

Conclusions: The scale of cryptogenesis

Disjunct amphi-Atlantic distributions are not common: 10% of all shallow-water species of Ascidiacea, Bivalvia and Hydrozoa have a disjunct distribution pattern. About half of these are cryptogenic and introduced species. The proportion of disjunct distributions differs among groups with differing life-history characteristics and relative natural dispersal potential, and ranges from 3% to 48%. This includes species that also occur in deep or warm waters, species complexes and disjunct distributions that

are based on single records on one coast, and are therefore not truly disjunct amphi-Atlantic species. The relative number of cryptogenic and introduced species within the disjunct amphi-Atlantic category ranges from 1.3% to 28% per group. The difference in relative numbers is caused by differing life-history characteristics of the studied groups, which were selected because they represented a range in natural dispersal potential. Generalizing this to the North Atlantic Ocean, this means that between 1.3% and 28% of the shallow-water invertebrate fauna is cryptogenic and may have been introduced; these could be hundreds or thousands of overlooked invasions. This is a conservative estimate: we have only considered species with a disjunct amphi-Atlantic distribution, although species occurring on either Atlantic coast or with an amphi-Atlantic distribution may also be cryptogenic or introduced.

Natural dispersal by either planktotrophic larvae or rafting on various substrates does not explain disjunct amphi-Atlantic distribution patterns of individual species of Hydrozoa, Bivalvia and Ascidiacea. Dispersal by these mechanisms is highly unlikely and has not been proven for any organism in the North Atlantic Ocean. Additionally, based on the surface current regime the direction of transport would have to be from west to east, which is in conflict with other studies of migration and invasions in the North Atlantic Ocean.

Infaunal bivalves have the lowest proportion of disjunct amphi-Atlantic distributions. There are no cryptogenic infaunal disjunct amphi-Atlantic bivalves, nor are there infaunal bivalves that have a disjunct amphi-Atlantic distribution that can be explained by pelagic larval dispersal. The disjunct distributions of infaunal bivalves are either explained by known anthropogenic introduction, or by other factors, such as occurrence in deep or warm waters. In Hydrozoa, a free-swimming medusa does not guarantee long-distance dispersal and a wide, possibly disjunct, distribution. Although rafting may be a possible mechanism for regional dispersal of Hydrozoa, this does not rule out shipping as a dispersal agent, because those species that are able to raft are also likely to be able to attach to ships. Dispersal on ships is more effective than dispersal on rafts; ships travel fast, are independent of surface currents and provide more space.

Cryptic species are common in all taxonomic groups and could explain some of the disjunct amphi-Atlantic distributions, as has previously been shown for several invertebrate species.

Although the role of Pleistocene glaciations in the creation of disjunct amphi-Atlantic distributions is assumed to be the same for all groups, there are no infaunal bivalves with a natural disjunct distribution pattern, nor are there cryptogenic infaunal bivalves. Additionally, for none of the groups do studies exist that give conclusive evidence for a disjunct amphi-Atlantic distribution pattern that is the result of post-glacial recolonization across the North Atlantic. *M. edulis* is the only species that has been demonstrated to have a natural distribution that is caused by the LGM. However, it now occurs in Arctic waters as well, meaning that it may actually have a continuous distribution. Gene flow of *M. edulis* is in the west-to-east direction, which is consistent with the surface-current regime. It is difficult to distinguish between genetic diversity

patterns caused by the LGM and by an anthropogenic invasion, as both processes can result in either high or low levels of genetic diversity.

Of the disjunct amphi-Atlantic species 42% do not have a strictly disjunct distribution; they may extend their range into deep or warm waters. A "natural" disjunct distribution was found for 8% of all disjunct species. Known anthropogenic introduction has resulted in a disjunct distribution for 19%, the remaining 32% are cryptogenic species. There are no examples of species with a natural disjunct amphi-Atlantic distribution in the North Atlantic Ocean that have been demonstrated using a combination of phylogeographic, palaeobiological and historical data. The cryptogenic species from this study would be excellent candidates for this type of research, which could provide conclusive evidence on the status of these species.

In marine ecology historical invasions are generally ignored, assuming that the 19th century coastal biota was natural or indigenous (Carlton 1989; 2003b). The assumption of limited influence of historical shipping on dispersal potential of coastal organisms has great implications for understanding modern-day distributions of species in North Atlantic waters. The estimated 1.3 – 28% of species that may have been introduced is not limited to inconspicuous species, rare species or species playing a minor role in coastal communities. Key species of coastal communities may have been introduced by ships centuries before the onset of biological surveys, but are viewed falsely as native (Carlton 2003b). This is an example of the "shifting baseline syndrome" (Pauly 1995) in marine ecology. The perception of the natural state of the system has shifted: historical introductions are regarded as natural components of the studied community. Assigning species the cryptogenic status creates possibility for further studying the scale of historical invasions. A multidisciplinary approach, combining palaeoecological, archaeological and historical resources, and molecular techniques is essential in order to gain insight into the scale and consequences of marine invasions.

Table 4.2 Disjunct amphi-Atlantic Ascidiacea.

species	distribution	depth	habitat	reproduction	comments	status	references
Enterogona							
Aplousobranchia							
Clavelinidae							
Archidistoma aggregatum Garstang, 1891	NEA: Plymouth, France NWA: North Carolina	0-30m		larvae in atrium	only one record in W Atlantic	doubtful	1, 2
Didemnidae							
Didemnum candidum Savigny, 1816	NEA: The Netherlands NWA: Maine to Brazil Other: Mediterranean, Red Sea, Mozambique, New Zealand, Australia, Philippines, Galapagos, Hawaii, and many more	shallow	shells, stones, etc.		- often misidentified, experts do not agree on neotype created - possibly species complex - possibly introduced to many regions - fouling	cryptogenic	1, 3, 4, 5, 28, 26
Didemnum vexillum Kott, 2002	NEA: France, The Netherlands, Ireland NWA: New England Other: New Zealand, Japan	shallow			- introduced, origin uncertain - mistakenly described as an endemic, species for the NW Atlantic coast by 6	introduced	6, 7, 27
Diplosoma listerianum (Milne-Edwards, 1841)	NEA: N Norway, W Europe, Azores NWA: New Hampshire to Connecticut Other: Mediterranean, South Africa, Brazil, Hawaii, widely distributed	0-80m	ascidians, algae, rocks, piers, hulls of ships in harbors	- stores exogenous sperm for several weeks, - incubates larvae until hatched	- introduced from Europe to South Africa, Atlantic coast N America, Pacific, Indian oceans - possibly native to Pacific - most widely distributed species in the world, in both temperate and tropical regions - rafting on macroalgae in Sargasso Sea - fouling	introduced	1, 5, 2, 8, 9, 10, 11, 12 13, 14, 15
Phlebobranchia							
Ascidiidae							
Ascidiella aspersa (Müller, 1776)	NEA: W Norway to North Sea NWA: New England Other: Mediterranean, Australia, New Zealand, South Africa, Argentina	shallow-80m	shells, algae, rocks, piers etc.	eggs with large follicle cells that float	- introduced to New Zealand, Australia, NW Atlantic, South Africa, Argentina from NE Atlantic - fouling	introduced	2, 5, 8, 10, 11, 16, 17
Perophoridae							
Perophora viridis Verrill, 1871	NEA: Azores NWA: Massachusetts to West Indies, Bermuda	very shallow 25m	hydroids, ascidians, rocks, piles		- W Atlantic species, extending to Azores - solitary or colonies	cryptogenic	1, 18

Pleurogona							
Stolidobranchia							
Molgulidae							
Molgula manhattensis (De Kay, 1843)	NEA: N Norway to Portugal NWA: Massachusetts to Gulf of Mexico Other: White Sea, Pacific coast USA, Japan, Australia	0-90m	algae, rocks, piers. Mostly attached, sometimes free. In harbors and estuaries	oviparous	- introduced to Japan, Australia, California to Washington, Russia - patchy distribution in Europe - fouling	cryptogenic	1, 2, 3, 5, 8, 6, 17, 19, 26
Pyuridae							
Cratostigma singularis (Van Name, 1912)	NEA: off Azores? NWA: Cape Cod to Long Island Sound	0m – deep?			- European distribution unclear - probably deep water species	deep waters	1, 3, 4
Styelidae							
Botrylloides violaceus Oka, 1927	NEA: Netherlands, Channel, NWA:Canadian Maritimes to Long Island Sound, Chesapeake Bay, Other: Venice Lagoon, Pacific NW to California, Australia, Japan	shallow	shells, ascidians, algae, rocks, etc.	-	introduced from Japan, in New England initially identified as *Botrylloides diegensis* - rafting on macroalgae - fouling	introduced	5, 10, 15, 20, 21, 22, 26, 28
Botryllus schlosseri (Pallas, 1766)	NEA: N Norway to Mediterranean NWA: Maine to Florida Other: Black Sea, South Africa, Japan, Australia, New Zealand	0-100m	ascidians, algae, rocks, boats, floats, etc.	larvae in atrium, eggs develop in brood sacs	- introduced to W Atlantic, South Africa, Australia, New Zealand, from Europe - native to Pacific Ocean? - rafting - fouling	introduced	1, 2, 3, 5, 8, 10, 11, 15, 16, 17
Cnemidocarpa mollis (Stimpson, 1852)	NEA: Scotland, Orkneys, Shetlands NWA: Hudson Bay to New York	0-100m	buried in sand or mud, loosely attached	oviparous			1, 2, 3, 9, 17
Styela atlantica Van Name, 1912	NEA: Faeroes, Bay of Biscay NWA: 38-40 degrees	shallow deep			- also in deep waters on American coast	deep waters	28-
Styela canopus (Stimpson, 1852)	NEA: S England to Mediterranean NWA: Massachusetts S to Florida, W Indies, Bermuda Other: Red Sea, W Africa, S Africa, Indian Ocean, Korea, Japan	shallow-30m	rocks, artificial oviparous substrates		- introduced from the Pacific - fouling	introduced	1, 2, 3, 5, 10, 11, 16, 26

Table 4.2 Continued

species	distribution	depth	habitat	reproduction	comments	status	references
Styela clava Herdman, 1882	NEA: Denmark to Channel, Channel, NW Spain, W Portugal NWA: Gulf of St Lawrence (Prince Edward Island) to New England Other: Sea of Okhotsk, Siberia, Japan, Korea, California	shallow	shells, rocks, artificial substrates		- introduced from Korean waters - patchy distribution in Europe - fouling	introduced	2, 17, 23, 24, 25
Styela plicata (Lesueur, 1829)	Cosmopolitan, warmer parts Atlantic ocean NWA: Florida, W Indies Other: San Diego, Asia, Philippines, Australia	shallow			- introduced from Pacific - fouling	introduced	1, 5, 28
Dendrodoa carnea (Rathke, 1806)	NEA: Europe? NWA: Newfoundland to Long Island Sound	shallow	shells, rocks, etc.		- according to 4 also in Europe - no distribution records found for Europe	doubtful	1, 3, 4, 9
Enterogona							
Aplousobranchia							
Clavelinidae							
Archidistoma aggregatum Garstang, 1891	NEA: Plymouth, France NWA: North Carolina	0-30m		larvae in atrium	- only one record in W Atlantic	doubtful	1, 2
Didemnidae							
Didemnum candidum Savigny, 1816	NEA: The Netherlands NWA: Maine to Brazil Other: Mediterranean, Red Sea, Mozambique, New Zealand, Australia, Philippines, Galapagos, Hawaii, and many more	shallow	shells, stones, etc.		- often misidentified, experts do not agree on neotype created - possibly species complex - possibly introduced to many regions - fouling	cryptogenic	1, 3, 4, 5, 28, 26
Didemnum vexillum Kott, 2002	NEA: France, The Netherlands, Ireland NWA: New England Other: New Zealand, Japan	shallow			- introduced, origin uncertain - mistakenly described as an endemic, species for the NW Atlantic coast by 6	introduced	6, 7, 27
Diplosoma listerianum (Milne-Edwards, 1841)	NEA: N Norway, W Europe, Azores NWA: New Hampshire to Connecticut Other: Mediterranean,	0-80m	ascidians, algae, rocks, piers, hulls of ships in harbors	- stores exogenous sperm for several weeks,	- introduced from Europe to South Africa, Atlantic coast N America, Pacific, Indian oceans - possibly native to Pacific - most widely distributed	introduced	1, 5, 2, 8, 9, 10, 11, 12, 13, 14, 15

	South Africa, Brazil, Hawaii, widely distributed			- incubates larvae until hatched	species in the world, in both temperate and tropical regions - rafting on macroalgae in Sargasso Sea - fouling		
Phlebobranchia							
Ascidiidae							
Ascidiella aspersa (Müller, 1776)	NEA: W Norway to North Sea NWA: New England Other: Mediterranean, Australia, New Zealand, South Africa, Argentina	shallow-80m	shells, algae, rocks, piers etc.	eggs with large follicle cells that float	- introduced to New Zealand, Australia, NW Atlantic, South Africa, Argentina from NE Atlantic - fouling	introduced	2, 5, 8, 10, 11, 16, 17
Perophoridae							
Perophora viridis Verrill, 1871	NEA: Azores NWA: Massachusetts to West Indies, Bermuda	very shallow-25m	hydroids, ascidians, rocks, piles		- W Atlantic species, extending to Azores - solitary or colonies	cryptogenic	1, 18
Pleurogona							
Stolidobranchia							
Molgulidae							
Molgula manhattensis (De Kay, 1843)	NEA: N Norway to Portugal NWA: Massachusetts to Gulf of Mexico Other: White Sea, Pacific coast USA, Japan, Australia	0-90m	algae, rocks, piers. Mostly attached, sometimes free. In harbors and estuaries	oviparous	- introduced to Japan, Australia, California to Washington, Russia - patchy distribution in Europe - fouling	cryptogenic	1, 2, 3, 5, 8, 6, 17, 19, 26
Pyuridae							
Cratostigma singularis (Van Name, 1912)	NEA: off Azores? NWA: Cape Cod to Long Island Sound	0m – deep?			- European distribution unclear - probably deep water species	deep waters	1, 3, 4
Styelidae							
Botrylloides violaceus Oka, 1927	NEA: Netherlands, Channel, NWA:Canadian Maritimes to Long Island Sound, Chesapeake Bay, Other: Venice Lagoon, Pacific NW to California, Australia, Japan	shallow	shells, ascidians, algae, rocks, etc.		- introduced from Japan, in New England initially identified as *Botrylloides diegensis* - rafting on macroalgae - fouling	introduced	5, 10, 15, 20, 21, 22, 26, 28

Table 4.2 Continued

species	distribution	depth	habitat	reproduction	comments	status	references
Botryllus schlosseri (Pallas, 1766)	NEA: N Norway to Mediterranean NWA: Maine to Florida Other: Black Sea, South Africa, Japan, Australia, New Zealand	0-100m	ascidians, algae, rocks, boats, floats, etc.	larvae in atrium, eggs develop in brood sacs	- introduced to W Atlantic, South Africa, Australia, New Zealand, from Europe - native to Pacific Ocean? - rafting - fouling	introduced	1, 2, 3, 5, 8, 10, 11, 15, 16, 17
Cnemidocarpa mollis (Stimpson, 1852)	NEA: Scotland, Orkneys, Shetlands NWA: Hudson Bay to New York	0-100m	buried in sand or mud, loosely attached	oviparous			1, 2, 3, 9, 17
Styela atlantica Van Name, 1912	NEA: Faeroes, Bay of Biscay NWA: 38-40 degrees	shallow-deep			- also in deep waters on American coast	deep waters	28
Styela canopus (Stimpson, 1852)	NEA: S England to Mediterranean NWA: Massachusetts S to Florida, W Indies, Bermuda Other: Red Sea, W Africa, S Africa, Indian Ocean, Korea, Japan	shallow-30m	rocks, artificial substrates	oviparous	- introduced from the Pacific - fouling	introduced	1, 2, 3, 5, 10, 11, 16, 26
Styela clava Herdman, 1882	NEA: Denmark to Channel, Channel, NW Spain, W Portugal NWA: Gulf of St Lawrence (Prince Edward Island) to New England Other: Sea of Okhotsk, Siberia, Japan, Korea, California	shallow	shells, rocks, artificial substrates		- introduced from Korean waters - patchy distribution in Europe - fouling	introduced	2, 17, 23, 24, 25
Styela plicata (Lesueur, 1829)	Cosmopolitan, warmer parts Atlantic ocean NWA: Florida, W Indies Other: San Diego, Asia, Philippines, Australia	shallow			- introduced from Pacific - fouling	introduced	1, 5, 28
Dendrodoa carnea (Rathke, 1806)	NEA: Europe? NWA: Newfoundland to Long Island Sound	shallow	shells, rocks, etc.		- according to 4 also in Europe - no distribution records found for Europe	doubtful	1, 3, 4, 9

References: 1: (Van Name 1945), 2: (Millar 1970), 3: (Plough 1978), 4: (Costello *et al.* 2004), 5: (Woods Hole Oceanographic Institution 1952), 6: (Kott 2004), 7: (Lambert 2005b), 8: (Hayward & Ryland 1990b), 9: (Huntsman 1912), 10: (Lambert 2001), 11: (Monniot et al, 2001), 12: (Lafargue & Wahl 1987), 13: (Vázquez 1993), 14: (Monniot 1974b), 15: (Thiel & Gutow 2005), 16: (Lafargue 1970), 17: (Millar 1966), 18: (Monniot 1974a), 19: (Monniot 1969b), 20: (Wolff 2005b), 21: (Stachowicz *et al.* 1999), 22: (Berman *et al.* 1992), 23: (Lützen 1999), 24: (Lambert & Lambert 2003), 25: (Davis & Davis 2005), 26: Carlton pers. comm., 27: (Coutts & Forrest 2007), 28: Monniot pers. comm.

Table 4.3 Disjunct amphi-Atlantic Hydrozoa.

species	distribution	depth	habitat	reproduction	comments	status	references
Anthoathecata							
Bougainvilliidae							
Bimeria vestita Wright, 1859	Near cosmopolitan NEA: British Isles, Belgium to Mediterranean, NWA: W Atlantic Other: Black Sea, W&S Africa, S Atlantic, Indian Ocean, Pacific	0-200m	hydroids, pebbles, hard substrates, fouling	fixed P gonophore	- cosmopolitan - not in cold waters, in W Atlantic S of Cape Hatteras - fouling	cryptogenic	1, 2, 3, 4, 5
Bougainvillia muscus (Allman, 1863)	Near cosmopolitan, circumtropical NEA: Norway to Morocco Other: Mediterranean, Bermuda, Australia, Puget Sound	0-100m	variety of substrata, ship fouling In estuaries	medusa M	- cosmopolitan	cryptogenic	1, 2, 5, 6, 7, 8, 10
Bougainvillia platygaster (Haeckel, 1879)	(sub)tropical Atlantic and Indian Ocean NWA: Gulf of Maine	0-500m	hydroid not known	medusa M	Maine record (in 5) exception, warm water species	warm water	2, 5
Bougainvillia rugosa Clarke, 1882	NEA: Baltic Sea, Kattegat NWA: Chesapeake Bay to Caribbean	shallow	ship fouling	medusa M	- introduced to Europe from North America by shipping - seasonal dormancy - ship fouling	introduced to Europe	2, 4, 10, 11, 12
Garveia franciscana (Torrey, 1902)	NEA: North Sea, Baltic Sea, NWA: Virginia to Brazil Other: Mediterranean, W Africa, E & W India, Australia, California	very shallow	in estuaries	planula P	- seasonal dormancy - introduced to San Francisco Bay - possibly also introduced to Europe, and Atlantic American coast - possible vector: oysters - origin unclear, Indian Ocean?	cryptogenic	1, 5, 11, 13, 14, 15
Nemopsis bachei Agassiz, 1849	NEA: Norway to Bay of Biscay NWA: Woods Hole to Florida, Gulf of Mexico,	shallow	fouling In estuaries	medusa M	- ship fouling	introduced to Europe	5, 14, 15
Cladocorynidae							
Cladocoryne floccosa Rotch, 1871	Circumglobal temperate to tropical waters NEA: NW Ireland, Channel, Iberian Peninsula NWA: Cape Cod to Cape Hatteras Other: Mediterranean, Indian Ocean, W & E Pacific	0-50m	hydroids, sponges, Sargassum, other algae,	fixed P gonophore rocks	rafting on Sargassum, also in warm waters	rafter, warm waters	1, 2, 4, 16, 17

Table 4.3 Continued.

species	distribution	depth	habitat	reproduction	comments	status	references
Cladonematidae							
Cladonema radiatum Dujardin, 1843	NEA: Norway, British Isles to Iberian Peninsula NWA: Bermuda Other: Mediterranean, Black Sea, Brazil, Indian Ocean, New Zealand, Japan	intertidal – shallow	algae, eelgrass, rocks	medusa M	- possibly introduced in Japan - probably species complex - easy to culture, a colony kept for 30 years in aquarium in Berlin	cryptogenic	1, 2, 6, 16, 18
Eleutheria dichotoma Quatrefages, 1842	NEA: All European coasts, N to Bergen. NWA: Caribbean Other: Mediterranean, Black Sea, Madeira, Belize?, Australia	intertidal - 20m	polyp rarely found Medusa attached to algae: *Ulva, Cystoseira, Gelidium, Sargassum*	crawling medusa M	recently introduced to Australia. Belize record doubtful		18, 19
Corynidae							
Dipurena halterata (Forbes, 1846)	NEA: British Isles, France NWA: Florida Other: Mediterranean, W&S Africa	0-5m	on and in sponges	medusa M		warm waters	18, 20
Sarsia occulta Edwards, 1978	NEA: Scotland NWA: Massachusetts, Maine	intertidal	algae, rocks	medusa, but sometimes retained M	- might have been introduced to either coast with rock ballast - inconspicuous, late description	cryptogenic	2, 18, 20
Eudendriidae							
Eudendrium album Nutting, 1896	NEA: Plymouth, Roscoff, off Belgium, NW Spain NWA: Canada to Florida	10-40m		planula P	- warm water species according to 11, Boreal according to 1 - seasonal dormancy - Eudendrium spp. recorded from ship fouling	cryptogenic	1, 2, 4, 6, 10, 11
Eudendrium capillare Alder, 1856	near cosmopolitan NEA: Europe, Iberian Peninsula, NWA: Greenland to Florida Other: Mediterranean, Bermuda, South Africa	0-100m, sometimes deeper	hydroids, other invertebrates, shells, algae, rocks, other substrates	planula P	- near cosmopolitan, but many identifications not reliable	misidentifications	1, 2, 4, 6, 7, 21, 22
Eudendrium carneum Clarke, 1882	NEA: Mediterranean NWA: Massachusetts to Caribbean Other: Brazil, Sydney harbor	shallow	docks, piles, fouling		- widespread, introduced - seasonal dormancy - fouling	introduced	4, 11, 23, 24

Hydractiniidae								
Hydractinia borealis (Mayer, 1900)	NEA: S Norway, S Iceland to Belgium NWA: Cobscook Bay	shallow-100m	barnacles, large crustaceans, hermit crab-occupied snail shells, hydroids	medusa	M	- Synonym: *Podocoryna borealis* - Podocoryna spp. reported from ship fouling	cryptogenic	2, 6, 7, 10, 21
Oceaniidae								
Cordylophora caspia (Pallas, 1771)	circumglobal in temperate and subtropical regions NEA: Europe, British Isles NWA: Quebec to Caribbean Other: Mediterranean, SF Bay	0-85m	fouling of various substrata, ships, often in shade In estuaries, river deltas, lagoons, also fresh water	planula	P	- introduced from Caspian Sea to Europe (canals) and America. - brackish to nearly fresh water, seasonal dormancy - species complex - ship fouling	introduced	2, 4, 6, 10, 11, 25, 36, 40
Merona cornucopiae (Norman, 1864)	NEA: Norway, Faroes, British Isles, Channel, Iberian Peninsula, NWA: New England Other: Mediterranean, South Africa, Seychelles, NE Pacific	10-274m	mollusc shells	fixed gonophore	P	widespread, on molluscs	cryptogenic	1, 2, 4, 25, 26
Pandeidae								
Amphinema dinema (Péron & Lesueur, 1810)	Circumtropical NEA: Shetlands to W Africa NWA: Cape Cod to Florida Other: Mediterranean, Brazil, Indian Ocean, tropical Pacific, New Zealand	hydroid: 0.5-20m; medusa: shallow	bryozoans, sponges, barnacles, algae	medusa	M		warm waters	1, 2, 5, 11
Amphinema rugosum (Mayer, 1900)	Circumglobal NEA: Shetlands to Azores NWA: New England to Florida Other: Mediterranean, Puerto Rico, Brazil, New Zealand	hydroid: 0.5-20m; medusa: 0-deep	hydroids, bryozoans, sponges, polychaete tubes, algae	medusa	M		warm waters	2, 5
Kinetocodium danae Kramp, 1921	NEA: Portugal, Azores NWA: W Indies Other: Mid Atlantic, Madagascar		pelagic gastropod	medusa	M	pelagic	obligate rafter on pelagic gastropod	5

Table 4.3 Continued.

species	distribution	depth	habitat	reproduction	comments	status	references
Pandea conica (Quoy & Gaimard, 1827)	widely distributed world-wide NEA & NWA: Scotland to Bermuda	hydroid: shallow; medusa: 45-300m	pelagic gastropod *Clio cuspidate*	medusa M	pelagic	obligate rafter on pelagic gastropod	5
Proboscidactylidae							
Proboscidactyla ornata (McCrady, 1859)	NEA: warm waters NWA: Massachusetts to Caribbean Other: Mediterranean, warm waters	shallow	sabellid polychaetes	medusa M	morphological and developmental differences between Mediterranean and American specimens, but no sufficient basis for separation	species complex?	2, 11, 27
Protohydridae							
Protohydra leuckarti Greeff, 1869	circumglobal temperate northern hemisphere NEA: Baltic, S Norway, German North Sea, Belgium, S England, Brittany, Bay of Biscay NWA: E coast N America Other: Mediterranean, Black Sea, Aral Sea, NE Pacific, NW Pacific	0-20m	on fine sand or mud with much detritus, rarely on algae, able to creep and burrow In estuaries, lagoons	planula P	type locality: Ostende, in mud among oyster cultures. Reaches very high densities	cryptogenic	2, 16, 18
Rathkeidae							
Podocorynoides minima (Trinci, 1903)	NEA: Channel NWA: North Carolina Other: Mediterranean, Red Sea, New Zealand, Papua New Guinea, NW Pacific	medusa: 0-70m		medusa M	hydroid unknown	not well known	5
Tubulariidae							
Ectopleura crocea (Agassiz, 1862)	widely distributed NEA: Atlantic France, off Iberian Peninsula NWA: Canada to Florida Other: Mediterranean, Gulf of Alaska to California, S Australia	shallow – deep	fouling man-made substrates In estuaries	fixed gonophore P	- Synonyms: Tubularia crocea, *Pinauay crocea* - also in deep waters - seasonal dormancy - detached floating colonies can reattach to substrates - ship fouling - rafting inferred from distribution	cryptogenic	1, 2, 4, 10, 11, 13, 17, 18, 21, 42, 44

Ectopleura dumortierii (Van Beneden, 1844)	Circumglobal temperate and tropical waters NEA: S Scotland to Iberian Peninsula, NWA: Massachusetts to Caribbean. Other: Mediterranean	shallow	bryozoan *Flustra*, crabs, hydroids, wood	medusa	M	- seasonal dormancy - cryptogenic	cryptogenic	1, 2, 4, 6, 11, 13, 15, 18,
Zancleidae								
Zanclea costata Gegenbaur, 1856	NEA: Barents Sea, Norwegian Sea, North Sea, Belgium, Britain, Bay of Biscay NWA: Cobscook Bay, Cape Cod to Florida Other: Mediterranean	shallow	bivalve shells, *Sargassum*	medusa	M	- rafting on *Sargassum* - records outside Mediterranean are not confirmed	doubtful	1, 2, 4, 17, 18, 21, 43
Leptothecata								
Aequoreidae								
Aequorea forskalea Péron and Lesueur, 1810	Circumglobal temperate to tropical waters NEA: N to Norwegian Sea	shallow		medusa	M	- very wide-spread and common species, also in warm waters - disjunct distribution - also offshore	warm waters	1, 28
Aequorea macrodactyla (Brandt, 1834)	near cosmopolitan in warm waters NEA: N to SW Ireland	shallow		medusa	M		warm waters	28
Aequorea pensilis (Haeckel, 1879)	Near cosmopolitan in warm waters NEA: N to S British Isles	shallow		medusa	M		warm waters	28
Aglaopheniidae								
Aglaophenia apocarpa Allman, 1877	NEA: Iberian Peninsula NWA: warm waters Other: Mediterranean	shallow		fixed gonophore	P	- disjunct distribution in warmer waters - Synonym: *A. lophocarpa*	warm waters	1, 2, 9
Aglaophenia latecarinata Allman, 1877	NEA: Europe (warm waters?) NWA: Cape Cod to Caribbean	shallow -270m	*Sargassum*	planula brooded in corbula	P	- rafting on *Sargassum*	rafter	2, 4, 17
Cladocarpus sigma (Allman, 1877)	NEA: Europe NWA: Cape Cod to Gulf of Mexico	40-1574m			P		mostly deep waters	2, 4
Blackfordiidae								
Blackfordia virginica Mayer, 1910	Worldwide in estuaries NEA: Europe NWA: Chesapeake Bay Other: Black Sea, San Francisco Bay, Chile	shallow	in estuaries	medusa	M	- native in the Black Sea, introduced to many estuaries worldwide by shipping	introduced	2, 9, 29, 30

Table 4.3 Continued.

species	distribution	depth	habitat	reproduction	comments	status	references
Campanulariidae							
Campanularia hincksii Alder, 1856	near cosmopolitan NEA: Lofoten, S and E Iceland to Iberian Peninsula, Azores NWA: New Brunswick to Florida Other: Mediterranean, W Africa, S Africa, Mexico, California	10-200m, 580m	hydroids, algae, rocks, seagrasses, floating substrates	planula P brooded in gonotheca	- near cosmopolitan - little studied - rafting	cryptogenic	1,2, 4, 6, 7, 17, 28, 31
Clytia paulensis (Vanhöffen, 1910)	widespread in warm waters NEA: S British Isles, France, Iberian Peninsula NWA: Chesapeake Bay to Caribbean Other: Mediterranean	intertidal - 384m	hydroids, bryozoans, shells, crustaceans, echinoderms, algae	medusa M	- tolerates low salinity - not reported rafting	cryptogenic	1, 2, 6, 11, 28, 31
Hartlaubella gelatinosa (Pallas, 1766)	NEA: Baltic Sea, Sweden to Morocco NWA: Labrador to Chesapeake Bay Other: Mediterranean, Black Sea, Pacific coast USA, Patagonia, New Zealand	intertidal - 15m	mollusc shells, rocks, sand, mud, ships in estuaries	planula P brooded in gonotheca	- tolerant of silt and brackish water, in estuaries - unreliable identifications - not reported rafting - ship fouling	cryptogenic	1, 2, 4, 6, 11, 28, 31, 32, 33
Laomedea calceolifera (Hincks, 1871)	Widespread in warm waters NEA: Germany, S England, NW France, Iberian Peninsula, Azores NWA: Bay of Fundy to Cape Hatteras Other: Mediterranean, Black Sea, Cape Town, Pacific coast USA, Brazil	intertidal - 100m, possibly deeper	polychaete tubes, crabs, ascidians, coralline algae, seagrasses, rocks, pebbles	planula P	- also at slightly reduced salinity - rafting inferred from distribution - introduced to Cape Town, probably by ships	cryptogenic	1, 2, 4, 17, 28, 31, 34
Obelia bidentata Clark, 1875	Circumglobal temperate to warm waters NEA: Netherlands, Belgium, France NWA: Maine to Caribbean Iberian Peninsula, Other: Mediterranean, not in Black Sea	intertidal - 681m	hermit crab shells, isopods, shrimps, algae, sea grasses. piles, ships, wrecks	medusa M	- global temperate to warm water - tolerates brackish water - rafting - ship fouling	cryptogenic	1, 2, 4, 6, 10, 11, 15, 17, 28, 31

Obelia dichotoma (Linnaeus, 1758)	near cosmopolitan NEA: Iceland to Iberian Peninsula NWA: Quebec to Caribbean Other: Mediterranean	medusa: shallow hydroid: intertidal - 100m, - 300m, - 521m	sharks, turtles, sea horses, fishes, parasitic copepods on fish, crabs sponges, algae, seagrasses, man-made hard substrates, ships	medusa	M	- near cosmopolitan, tolerant of reduced salinity - possibly confused with other *Obelia* species - possibly species complex - rafting - ship fouling	cryptogenic	1, 2, 4, 6, 7, 10, 11, 15, 17, 21, 28, 31, 35
Campanulinidae								
Calycella gracilis Hartlaub, 1897	NEA: Helgoland and N Norfolk (North Sea) NWA: Hudson Bay	shallow	bryozoan	sporosac	P	uncertain validity, poorly known species	doubtful	2, 6, 28, 32
Campanulina pumila (Clark, 1875)	NEA: British Isles, The Netherlands (former Zuiderzee), Baltic Sea NWA: New Brunswick to Chesapeake Bay	intertidal ? - shallow	hydroids, algae, floating docks, wharf piles	planula	P	- Synonym: *Opercularella pumila* - tolerates reduced salinity - fouling	cryptogenic	4, 7, 11, 28
Cuspidella grandis Hincks, 1868	NEA: Bay of Biscay NWA: Canadian Arctic	shallow		fixed gonophore	P	doubtful species, is probably *Cosmetira pilosella*	doubtful	1, 2, 4, 32
Eirenidae								
Eutima gegenbauri Haeckel, 1864	NEA: Sweden, Scotland to Mediterranean, Canaries, Morocco NWA: N Carolina Other: twice from China	shallow	shells, rocks	medusa	M	hydroid not well known	not well known	28
Haleciidae								
Halecium articulosum Clark, 1875	NEA: British Isles, Belgium, NWA: Arctic to Cape Hatteras	shallow-deep			P	- also in deep waters	also deep waters	2, 4, 21
Halecium lankesterii (Bourne, 1890)	NEA: British Isles to S Africa, NWA: Cape Cod to Cape Hatteras Other: Mediterranean, Mozambique	intertidal - 100m or more	hydroids, crabs, bryozoans, algae, seagrasses, rocks	planula	P	- probably more widely distributed, overlooked because of small size - Rafting inferred	not well known	1, 2, 4, 6, 17, 28, 31
Halecium nanum Alder, 1859	NEA: Channel, Bay of Biscay NWA: Cape Cod to Caribbean Other: Mediterranean	15m	hydroids, algae: *Sargassum, Halimeda*	planula	P	- rafting - warm waters	warm waters	1, 2, 4, 17, 28, 31

Table 4.3 Continued.

species	distribution	depth	habitat	reproduction	comments	status	references
Halecium tenellum Hincks, 1861	Cosmopolitan in tropical to temperate waters	shallow -500m	hydroids, bryozoans	planula P	- unreliable identifications - rafting inferred	cryptogenic	1, 2, 4, 6, 7, 17, 28, 31, 37
Halopteriddidae							
Antennella secundaria (Gmelin, 1791)	Near cosmopolitan in tropical to temperate waters NEA: British Isles southwards NWA: Sargasso Sea Other: Mediterranean	shallow -550m	algae, ships, variety of substrata	planula P brooded in gonotheca	- near cosmopolitan - might be a species complex - introduced to tropical Australia - ship fouling - rafting on algae	cryptogenic	1, 2, 6, 8, 17, 28
Halopteris catharina (Johnston, 1833)	Widely distributed in temperate waters NEA: Iceland, Faroes, Norwegian Sea to Morocco NWA: S to Straits of Magellan Other: Mediterranean	intertidal - 412m	bryozoans, hydroids, floating *Sargassum*, seagrass, rocks	planula P brooded in gonotheca	- rafting, one record in NW Atlantic on drifting Sargassum	rafter	1, 2, 6, 7, 17, 28, 31
Monostaechas quadridens (McCrady, 1859)	circumtropical? NEA: Europe NWA: Cape Cod to Caribbean	20-500m	sponge		warm waters	warm waters	2, 4
Kirchenpaueriidae							
Ventromma halecioides (Alder, 1859)	cosmopolitan NEA: Scotland to Iberian Peninsula, Canaries, Cape Verdes, Azores NWA: Florida and Caribbean Other: Mediterranean, Black Sea, Suez Canal, Red Sea, Kuwait, India, Japan, Enewetak Atoll, NE Australia	intertidal - 20m	hydroids, algae, eel grass, rocks, ships	planula P brooded in gonotheca	- near cosmopolitan boreal to tropical - rafting inferred - ship fouling	cryptogenic	1, 2, 6, 10, 17, 28, 31
Lafoeidae							
Acryptolaria conferta (Allman, 1877)	Cosmopolitan? NEA: Iberian Peninsula NWA: Cape Cod to Florida Other: Mediterranean	shallow -deep		fixed P gonophore	subspecies *A. conferta australis* (valid?) occurs at 4400m	also deep waters	1, 2, 4, 37
Lovenellidae							
Lovenella clausa (Lovén, 1836)	NEA: Sweden, Denmark to Morocco. Not in Belgium, Netherlands, Norway NWA: maybe N Carolina, but uncertain Other: Mediterranean	medusa: shallow, hydroid: intertidal - 50m	molluscs, other animals, algae	medusa M	- sparsely recorded, possibly overlooked - not reported rafting - patchy distribution	cryptogenic	1, 2, 6, 28, 31

Lovenella gracilis Clarke, 1882	NWA: Europe NWA: Cape Cod to Cape Hatteras	shallow	clam shells	medusa	M	European distribution unclear, only found in ERMS	cryptogenic	2, 4, 11, 38
Melicertidae								
Melicertum octocostatum (Sars, 1835)	NEA: Arctic to British Isles, Baltic Sea NWA: Cobscook Bay to Washington State Other: Japan, Papua New Guinea	medusa: shallow, 30km from shore; hydroid: shallow - 90m	mussel *Modiolus modiolus*	medusa	M	only in Cobscook Bay on American East coast, curious distribution	cryptogenic	21, 28
Plumulariidae								
Monotheca margaretta Nutting, 1900	NEA: Europe (warm waters?) NWA: Cape Cod to Caribbean	shallow	*Sargassum*, other algae	fixed gonophore	P	rafting on Sargassum, northern records rafting on algae	rafter	2, 4, 17, 39
Plumularia filicula Allman, 1877	NEA: Gibraltar NWA: Cape Cod to Caribbean Other: Tropical Atlantic	mode-rately deep		fixed gonophore	P	warm water species, usually moderately deep	warm waters	1, 2, 4, 9
Plumularia setacea (Linnaeus, 1758)	near cosmopolitan tropical to temperate waters NEA: N to Iceland (rare, one locality), Norwegian Sea, absent from Faroes NWA: Bay of Fundy to Caribbean	shallow - 90m, 604m	hydroids, sponges, ascidians, molluscs, algae, seagrasses pebbles, rocks, ships	planula brooded in gonotheca	P	- near cosmopolitan - intolerant of reduced salinity - rafting inferred from distribution - ship fouling	cryptogenic	1, 2, 4, 6, 7, 17, 28, 31, 37
Sertulariidae								
Diphasia alata (Hincks, 1855)	NEA: mid-Norway, British Isles to Azores, Morocco, NWA: W Atlantic, Nova Scotia	5-580m	worm tubes once, substrata unknown	planula	P	mostly in deep waters, but temperature may be more important than depth. Should be named *D. pinaster* (Cuvier, 1830). See 7	also deep waters	2, 4, 6, 28
Dynamena disticha (Bosc, 1802)	cosmopolitan? NEA: Iberian Peninsula NWA: Massachusetts to Caribbean Other: Mediterranean	shallow	*Sargassum*, other algae, seagrass *Zostera*	fixed gonophore	P	- seasonal dormancy according to Carlton - rafting on algae	cryptogenic rafter	1, 2, 4, 11, 15, 17
Sertularella distans Lamouroux, 1816	NEA: British Isles, Netherlands, Belgium, Roscoff, Iberian Peninsula NWA: Massachusetts to Caribbean Other: Mediterranean, warm Atlantic and Pacific waters	intertidal - 60m, 150-826m	hydroids, mollusc shells, algae, rocks	planula brooded in acrocyst	P	- rafting on algae - warm waters	rafter, warm waters	1, 2, 4, 6, 11, 17, 28, 31

Table 4.3 Continued.

species	distribution	depth	habitat	reproduction	comments	status	references
Sertularella gayi (Lamouroux, 1821)	NEA: Spitsbergen to tropics NWA: Bay of Fundy to tropics Other: Mediterranean, S Atlantic Islands, S Africa?, New Zealand	shallow -edge of shelf, 550m, 890m		planula P brooded in acrocyst - deep waters	- taxonomy uncertain - maybe present but unrecorded in high Arctic	doubtful	1, 2, 4, 6, 7, 28, 37
Sertularia (*Tridentata*) *marginata* (Kirchenpauer, 1864)	circumtropical? NEA: Iberian Peninsula NWA: Cape Cod to Caribbean Other: Mediterranean	shallow	*Sargassum*, ships	fixed P gonophore	- rafting on Sargassum - hull fouling	cryptogenic	1, 2, 4, 10, 17, 37
Tridentata distans (Lamouroux, 1816)	Circumtropical NEA: British Isles NWA: NW Atlantic Other: Hawaii (introduced?)	shallow	algae		warm water species	warm waters	4, 9, 28
Limnomedusae							
Olindiidae							
Gonionemus vertens Agassiz, 1862	NEA: North Sea, British Isles, Brittany, Portugal, NWA: NW Atlantic Other: San Francisco Bay, NW Pacific	shallow	shells, fouling	medusa M	- introduced as hull fouling or with oysters in the 19th century from probably the W Pacific - ballast water also possible vector for secondary introductions and spread	introduced	14, 15, 21, 40
Maeotias marginata (Modeer, 1791)	NEA: Europe NWA: Chesapeake Bay Other: Mediterranean, Black Sea, San Francisco Bay	shallow	in estuaries, also in fresh water		- synonym: *Maeotias inexspectata* - ship fouling - native in Black Sea, introduced to Chesapeake Bay by ships	introduced	2, 41, 44
Anthoathecata							
Corymorphidae							
Corymorpha nutans Sars, 1835	NEA: Iceland, N Norway to Mediterranean, not on Atlantic coast Iberian Peninsula NWA: Cobscook Bay Other: Black Sea, San Francisco Bay?	0-100m or more	anchored in silt or gravel	medusa M	- only record in W Atlantic from Cobscook Bay, doubtful - introduced to San Francisco Bay?	doubtful	1, 2, 6, 7, 18, 21, 40

M= medusa-releasing, P= medusa-retaining, dispersal by planula larva. X= incomplete knowledge of life-cycle. References:1: (Medel & López-González 1996), 2: (Costello *et al.* 2004), 3: (Rajagopal *et al.* 1997), 4: (Fraser 1944), 5: (Schuchert 2007), 6: (Hayward & Ryland 1990a), 7: (Dahlgren *et al.* 2000), 8: (Hewitt 2002), 9: Calder pers. comm., 10: (Woods Hole Oceanographic Institution 1952), 11: (Calder 1990), 12: (Leppäkoski & Olenin 2000), 13: (Watling & Maurer 1972), 14: (Wolff 2005b), 15: (Carlton 2003a), 16: (Schuchert 2006), 17: (Thiel & Gutow 2005), 18: (Hansson 1998), 19: (Fraser *et al.* 2006), 20: (Schuchert 2001), 21: (Trott 2004), 22: (Calder 1972), 23: (Marques *et al.* 2000), 24: (Australian Museum Business Services (AMBS) 2002), 25: (Schuchert 2004), 26: (Rees 1956), 27: (Calder 1970a), 28: (Cornelius 1995), 29: (Streftaris *et al.* 2005), 30: (Ruiz *et al.* 2000), 31: (Cornelius 1992), 32: (Calder 1970b), 33: (Reverter-Gil & Fernandez-Pulpeiro 1999), 34: (Millard 1975), 35: (Govindarajan *et al.* 2005), 36: (Carlton 2003a), 37: (Vervoort 1966), 38: (Calder 1975), 39: (Leclère *et al.* 2007), 40: (Carlton 1979), 41: (Calder & Burrell 1969), 42: Carlton pers. comm., 43: (Boero *et al.* 2000), 44: (Mills *et al.* 2007)

Table 4.4 Disjunct amphi-Atlantic Bivalvia.

species	distribution	depth	habitat		comments	status	references
Dreissenidae							
Mytilopsis leucophaeata (Conrad, 1831)	NEA: Europe NWA: Chesapeake Bay to Florida Other: Texas, Mexico	shallow	attached to rocks and twigs in clumps brackish to fresh water near rivers	EPI	- fouling - introduced into the Hudson River, New York, as early as 1937 and later to the lower Charles River, Massachusetts - introduced to Europe	introduced	1, 2, 3 , 4, 5
Gryphaeidae							
Neopycnodonte cochlear (Poli, 1795)	NEA: Iceland to Angola, Azores, Madeira, NWA: N Carolina to Florida, Bermuda, W Indies Other: Mediterranean, Red Sea, Madagascar, Japan, Philippines, Hawaii	45-250m	secondary hard substrates (e.g. shells) on muddy gravel	EPI	- fouling - very widespread - possibly species complex	cryptogenic	3, 5, 6
Lasaeidae							
Entovalva perrieri (Malard, 1903)	NEA: Europe NWA: Cape Cod	very shallow	attached to holothurian *Leptosynapta inhaerens*	EPI	- *L. inhaerens* on American and European shores suggested to be distinct species, unkown if symbiont is the same species	species complex?	2, 3
Lasaea adansoni (Gmelin, 1791)	NEA: Norway to Mediterranean, Madeira, Canaries NWA: Florida, Bermuda, Bahamas Other: Caribbean to Argentina, E Pacific	intertidal	nestled among barnacle/turf colonies, in algal holdfasts, among mussel byssal threads	EPI	- hermaphroditic, polyploid clones which brood their young in pallial cavity. Synonym: *L. rubra* - *Lasaea* phylogeography suggests rafting and human mediated introductions in Oceanic islands in the North Atlantic	cryptogenic	5, 6, 7, 8, 9, 10
Limidae							
Limaria hians (Gmelin, 1791)	NEA: Iceland, Lofoten to Mediterranean, Canaries, Azores NWA: Caribbean	0-100m	nest forming, free living coarse sand, gravel, sea grass Posidonia.	EPI	- also in warmer waters	warm waters	6, 7
Limaria inflata Link, 1807	NEA: Portugal, Azores, Canaries NWA: Caribbean Other: Mediterranean	shallow	nest forming. all types of bottom	EPI	- Anti-Lessepsian. Validity uncertain, could be *Limaria tuberculata*, occurring in Europe, W and S Africa	warm water, doubtful	6
Limatula confusa (Smith, 1885)	NEA: NE Atlantic to Azores NWA: N Carolina to Florida, W Indies Other: Brazil	shallow -deep	sand, mud or gravel	EPI	- not in 8 - description in 5 based on original drawing	deep, doubtful	3, 5

Table 4.4 Continued.

species	distribution	depth	habitat		comments	status	references
Limopsidae							
Limopsis cristata Jeffreys, 1876	NEA: W Europe NWA: Cape Cod to Florida, W Indies Other: Gulf of Mexico	offshore, deep	soft sediments	IN?		deep	2, 3, 5
Limopsis minuta Philippi, 1836	NEA: off Spain to Senegal, Cape Verdes NWA: Newfoundland to Brazi Other: W Mediterranean	80-1353m	mud	IN?		deep	2, 3, 5, 6
Lyonsiidae							
Lyonsia(*iella*) *formosa* Jeffreys, 1882	Amphiatlantic NEA: Azores Other: Mediterranean			IN	- valid? not in 3 - tropical W Atlantic, warm water species?	warm water, doubtful	3, 6
Myidae							
Mya arenaria Linnaeus, 1758	NEA: Barents Sea to France NWA: Labrador to S Carolina Not in Greenland Other: Alaska to N California, Kamchatka to Japan, Black Sea, not in Mediterranean	0-70m, offshore	sand, mud, sandy mud/gravel, also fouling In estuaries	IN + EPI	- also in fouling - introduced to Pacific coast North America and Europe	introduced	2, 3,6, 7, 8, 11, 12, 13, 14
Mytilidae							
Geukensia demissa (Dillwyn, 1817)	NEA: Europe NWA: Gulf of St Lawrence to NE Florida Other: California	intertidal	salt marsh, at baes of rock retaining walls in estuaries, occasional in ship fouling	EPI + IN	- introduced to Europe and California - ship fouling - reported rafting, substratum unknown	introduced	2, 3, 9
Mytilus edulis Linnaeus, 1758	NEA: Greenland, Iceland, Spitsbergen to Iberian Peninsula NWA: Atlantic American coast Other: Kerguelen Islands, Argentina, Chile	intertidal - 40m	attached to hard substrates	EPI	- Northern limit unclear. not in high Arctic, reappeared on Spitsbergen after 1000yrs - used to be present in N Greenland, used to have a continuous distribution in the Arctic - fouling - reported rafting on macroalgae, plastics; rafting also inferred from distribution - hybridizes with other *Mytilus* species	cryptogenic	1, 2, 3, 9, 14 1, 16, 17, 21

Mytilus trossulus Gould, 1850	NEA: Baltic Sea NWA: NE Canada to Nova Scotia Other: Pacific coast America	shallow	attached to hard substrates	EPI	- hybridizes with other *Mytilus* species - fouling	cryptogenic	3, 14, 18, 19
Nuculidae							
Nucula delphinodonta Mighels and Adams, 1842	NEA: Murmansk, Barents Sea, Norway NWA: W Greenland, Labrador to North Carolina, Florida Keys	shallow –deep	sand, mud or gravel	IN	- not present in E Greenland and Iceland	deep	2, 3, 12
Ostreidae							
Ostrea edulis Linnaeus, 1758	NEA: Norway to Spain NWA: Maine to Rhode Island, occasional records Connecticut Other: Mediterranean, Black Sea	shallow -90m	all substrate types	EPI	- introduced for culture in W Atlantic - fouling	introduced	1, 6
Pectinidae							
Palliolum striatum (Muller, 1776)	NEA: Iceland, N Norway to Spain NWA: Newfoundland to off Massachusetts Other: Mediterranean	4-800m	all substrate types	EPI	also deep, especially in southern part of range - northern distribution limit?	deep, Arctic?	2, 3, 6
Pholadidae							
Barnea truncata (Say, 1822)	NEA: W Africa NWA: E Canada to Florida Other: Gulf of Mexico to Brazil	shallow	bores in clay, mud or peat, sometimes in wood	IN + B	- also found boring in wood - introduced to W Africa	introduced	2, 3, 5, 11, 23
Solenidae							
Ensis directus (*americanus*) Conrad, 1843	NEA: Germany, Netherlands, Belgium NWA: Labrador to S Carolina	shallow	sand flats	IN	- introduced from N America with ballast water in Europe	introduced	1, 2, 3, 6, 11, 2
Teredinidae							
Bankia carinata (Gray, 1827)	NEA: Europe NWA: N Carolina to Florida, W Indies Other: Gulf of Mexico, Brazil, Indo Pacific	shallow	bores in wood	B	- warm water species - carried to Europe in drift wood or ships	warm waters	2, 3, 5
Bankia fimbriatula Moll and Roch, 1931	NEA: British Isles NWA: N Carolina to Florida, W Indies Other: Gulf of Mexico, Colombia, Brazil, Pacific Panama	shallow	bores in wood	B	- warm water species - carried to Europe in drift wood - single European record dates from 1860 - native in W Atlantic	warm waters	3, 5, 6, 8

Table 4.4 Continued.

species	distribution	depth	habitat		comments	status	references
Lyrodus bipartitus (Jeffreys, 1860)	Atlantic NEA: Guernsey, England Other: Australasia, Indo-Pacific	shallow	bores in wood	B	- European single record from drift wood from he Gulf Stream at Guernsey, England - cryptogenic in Atlantic	cryptogenic	3, 8, 6, 20
Lyrodus pedicellatus (Quatrefages, 1849)	NEA: W Europe, NWA: Massachusetts to Florida, W Indies Other: S Africa, Gulf of Mexico, Caribbean, Brazil, E Pacific, Hawaii, Australia, Indian Ocean, Indo Pacific	shallow	bores in wood	B	- introduced to many places by ships - probably originates in southern hemisphere many synonyms - rafting inferred from distribution	cryptogenic	2, 3, 5, 6, 8, 9
Nausitora fusticulus (Jeffreys, 1860)	Atlantic NEA: Scotland, drift wood	shallow	bores in wood	B	- warm water species - occasionally carried to Europe in drift wood or ships	warm waters	6, 8, 20
Nototeredo norvagica (Spengler, 1792)	N Atlantic Other: W Africa, Azores, Mediterranean	shallow	bores in wood	B	- common coldwater borer - reported rafting in wood - in ship hulls	cryptogenic	2, 3, 6, 7, 8, 9
Psiloteredo megotara (Hanley, 1848)	N Atlantic NEA: British Isles, Azores NWA: New England Other: Mediterranean	shallow	floating wood and lobster pots	B	reported rafting in wood from the Azores	cryptogenic	2, 3, 6, 8
Spathoteredo spatha (Jeffreys, 1860)	Caribbean NEA: England, drift wood NWA: Florida	shallow	bores in wood	B	warm water species occasionally carried to Europe in ships or drift wood in the Gulf Stream	warm waters	3, 6, 8, 20
Teredo bartschi Clapp, 1923	NEA: W Europe NWA: N Carolina to Florida, Bermuda Other: Gulf of Mexico, Brazil to Uruguay, Hawaii, Indian Ocean, Australia, introduced to numerous locations in tropical and temperate N Pacific	shallow	bores in wood	B	warm water species might be native to Pacific Ocean	cryptogenic	3, 5, 23
Teredo navalis Linnaeus, 1758	Cosmopolitan, temperate seas	shallow	bores in wood	B	possibly native to Pacific Synonyms: *T. beachi, T. beaufortana, T. borealis, T. morsei, T. novangliae, T. teredo*	cryptogenic	2, 3, 6, 22

Teredora malleolus (Turton, 1822)	Tropical and subtropical Atlantic Ocean Other: Mediterranean, Gulf of Mexico	shallow	bores in wood	B	warm water species, carried to Europe in drift wood or ships synonym: *T. thomsonii*	warm waters	2, 3, 5, 6, 8, 20
Thraciidae							
Thracia phaseolina (Lamarck, 1818)	NEA: N Norway to Morocco, Madeira NWA: W Atlantic Other: Mediterranean, Black Sea	0-55m	mixed sand	IN	Records from W Atlantic are undescribed species, not true *T. phaseolina* (5). geographic range uncertain, confusion with *T. villosiuscula*	species complex	5, 7, 13
Thyasiridae							
Axinus grandis (Verrill & Smith, 1885)	N Atlantic NEA: Bay of Biscay, Azores, Cape Verdes NWA: Virginia, New Jersey, N Carolina to Florida Keys, W Indies Other: Gulf of Mexico, Caribbean	shallow -deep?	deeply infaunal in sand, mud	IN	deep water?	deep waters?	5, 8
Veneridae							
Mercenaria mercenaria (Linnaeus, 1758)	NEA: S & SE Britain, France, The Netherlands, Belgium NWA: Gulf of St Lawrence to Florida, Bermuda Other: Gulf of Mexico, Pacific coast USA, China	intertidal – 10m	mud or sandy mud	IN	deliberately introduced to French and English waters, also introduced to American Pacific Coast and China	introduced	1, 5, 6, 7, 11, 13, 23
Petricolaria pholadiformis (Lamarck, 1818)	NEA: S Norway to Congo NWA: Gulf of St Lawrence to Florida, W Indies Other: Mediterranean, Black Sea, Gulf of Mexico, E Pacific	0-8m	bores in hard clay, limestone, chalk	B	introduced in NW Europe and Pacific N America probably with oysters	introduced	1, 2, 3, 6, 8

EPI= epifaunal, IN=infaunal, B=boring. References: 1: (Carlton 1999b), 2: (Abbott 1974), 3: (Turgeon et al. 1998), 4: (Nehring & Leuchs 1999), 5: (Mikkelsen & Bieler 2008), 6: (Poppe & Goto 1993), 7: (Hayward & Ryland 1990b), 8: (Costello et al. 2004), 9: (Thiel & Gutow 2005), 10: (Ó Foighil & Jozefowicz 1999), 11: (Emerson & Jacobson 1976), 12: (Lubinsky 1980), 13: (Tebble 1976), 14: (Woods Hole Oceanographic Institution 1952), 15: (Dudas et al. 2005), 16: (Berge et al. 2005), 17: (Riginos & Henzler 2008), 18: (Riginos et al. 2004), 19: (Geller 2002), 20: (Turner 1966), 21: (Símonarson et al. 1998), 22: (Wolff 2005b), 23: Carlton pers. comm., 24: (Luczak et al. 1993), 25: (Carlton & Eldredge 2009).

Introduced or glacial relict - Phylogeography of the cryptogenic ascidian *Molgula manhattensis* De Kay, 1843 (Ascidiacea, Pleurogona)

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Summary

Biological invasions are still generally assumed to be the result of 20th century transport while ignoring the fact that ships have been moving species across the oceans for centuries. The unrecorded invasions of those times are today's cryptogenic species: species that are neither demonstrably native, nor introduced. The ascidian *Molgula manhattensis* has a disjunct amphi-Atlantic distribution pattern and a recent history of world-wide introductions. Its natural dispersal capacities are limited, and its disjunct amphi-Atlantic distribution can either be the result of post-glacial recolonization or dispersal by an anthropogenic vector. To determine whether the North Atlantic distribution of *M. manhattensis* is natural or human-induced, we analyzed mtDNA COI sequence variation in individuals collected from the cryptogenic and introduced ranges.

We collected samples from North Atlantic Europe and America, the Bulgarian coast of the Black Sea, San Francisco Bay, USA and Osaka Bay, Japan, and analyzed mt DNA COI sequences.

mtDNA haplotype diversity was nearly three times as high with deeper relationships among haplotypes on the Northeast American coast as compared with Europe. Latitudinal south to north attenuation was present in American but not in European populations. In areas of known introductions, *M. manhattensis* showed variably high or low levels of haplotype diversity. Medium-to-high frequency haplotypes originating from the Northwest Atlantic were present in two locations of known introduction, but not in Europe as would be expected. Private haplotypes were found on both sides of the Atlantic.

Our results demonstrate that *M. manhattensis* is native in Northeast America and that known introductions to San Francisco and Osaka Bays, originally stemmed from Northwest Atlantic sources. However, whether *M. manhattensis* was introduced or is native to Europe remains equivocal due to contrasting diversity patterns between the Northeast and Northwest Atlantic in combination with extreme differences in propagule pressure among suspected and confirmed locations of invasion.

Introduction

Biological invasions are an important component of global change (Carlton 2000; Occhipinti Ambrogi & Savini 2003; Harley *et al.* 2006) and globalization (Sax & Gaines 2003; Ehrenfeld 2005), and are considered to be a major threat to coastal marine biodiversity (Chapin *et al.* 2000; Bax *et al.* 2003). The growing magnitude of global trade and associated transport vectors, and the multiplied number of acting vectors (Carlton & Cohen 2003) are greatly accelerating the rate of non-indigenous species introductions in coastal communities (Ruiz *et al.* 1997; Leppäkoski *et al.* 2002; Hewitt *et al.* 2004).

The general view is that the impact of biological invasions on coastal communities increases with the rate of introductions and that biological invasions are primarily a phenomenon of the 20th century. However, seminal work by Carlton has shown that modern invasions are only the tip of the invasion iceberg (Carlton 1979; 1989; 1996). In the North Atlantic humans have been moving coastal species across the open ocean for the past thousand years starting with the Viking explorations of the North American coast. From the 16th century onwards, opportunities for introductions in both directions increased exponentially with the onset of intensive shipping and emigration. Carlton (1999) estimated that a single 17th century ship carried >150 species in fouling and boring communities on and in its wooden hull, on the anchor and in its sand or rock ballast. Most significantly, these events occurred long before the first coastal surveys were carried out by naturalists in the mid-1800s (Carlton 2003b). Two well-studied examples are the soft shelled clam, *Mya arenaria*, which was introduced to Europe from America by the Vikings in the late Middle Ages (Petersen *et al.* 1992) and the periwinkle snail, *Littorina littorea*, which was introduced to America from Europe probably in the 18th century with rock ballast (Blakeslee *et al.* 2008; Brawley *et al.* 2009). The number of unrecognized introductions is only now being discovered.

Cryptogenic species

The "candidate species" for studying unrecorded historical introductions are today's cryptogenic species, i.e. species that are neither demonstrably native, nor demonstrably introduced (Carlton 1996a). Assigning species to the "cryptogenic" category can be based on one or more of the following characteristics: (1) interaction with an anthropogenic transport mechanism, now or in the past, (2) absence of close relatives in the current range, (3) a disjunct distribution pattern that cannot be explained by natural dispersal mechanisms, and (4) a recent history of invasions elsewhere (Carlton 1996a; 2008). The total number of cryptogenic species is greatly underestimated, as we assume that species are native, unless they are proven to be introduced (Carlton 2008). Studying the phylogeography of cryptogenic species can aid in estimating the impact of anthropogenic vectors on the dispersal and biogeography of coastal biota.

Phylogeography in the North Atlantic

The distributional ranges of coastal organisms across the North Atlantic Ocean have been significantly shaped by the Last Glacial Maximum (LGM) (~21,000 years ago)

(Hewitt 1996). Phylogeographic studies have revealed a general pattern of post-glacial expansion into northern regions from predominantly southern, peri-glacial refugia along both European and North American coasts (reviewed in Maggs *et al.* 2008). Because the effects of the LGM were generally more severe along Northwest Atlantic shores due to the compression of sea-surface isotherms and the absence of hard substrates south of Cape Hatteras, many species became locally extinct (Wares & Cunningham 2001; Vermeij 2005). Northwest Atlantic shores were only subsequently recolonized from either small regional refugia, or from the Northeast Atlantic at the end of the LGM, with mid-Atlantic islands such as Iceland and Greenland typically recolonized from Europe and acting as stepping stones for recolonization of the Northwest Atlantic, resulting in amphi-Atlantic species distributions.

Amphi-Atlantic distributions can be categorized as continuous or disjunct. Species with an amphi-Atlantic distribution occur on European and American coasts of the North Atlantic, including northern Norway, Iceland, southern Greenland and Northeast Canada. In contrast, a disjunct amphi-Atlantic distribution pattern is characterized by an absence of the species in Arctic and sub-Arctic waters, while occurring on European and American coasts. A disjunct amphi-Atlantic distribution pattern is dependent on long-distance dispersal (LDD) capacities of the organism for crossing the ocean barrier. Natural LDD can occur through larval transport on ocean currents and/or rafting of egg-masses, juveniles and adults. However, if life-history traits preclude LDD (as is the case with many shallow-water invertebrates) and anthropogenic vectors have been at work (such as shipping and translocation of shellfish), then the disjunct distribution is mostly likely human mediated (Chapter 4).

Molgula manhattensis: a cryptogenic ascidian

The Ascidiacea (commonly referred to as sea squirts) are a Class of tunicates with low natural dispersal potential. Of the 184 shallow-water ascidian species in the North Atlantic Ocean 16 have a disjunct amphi-Atlantic distribution. Five of these extend their distribution to warm or deep waters, eight are known to have been introduced on either or both Atlantic coasts, and three are cryptogenic species (Chapter 4). One of these is the sea grape, *Molgula manhattensis* (Pleurogona, Ascidiacea), a marine tunicate first described from New York harbor by De Kay in 1843. Its Northwest Atlantic distribution extends almost continuously from Cape Cod, MA to southern Louisiana (interrupted by the Florida peninsula) (Van Name 1945). Although its Northeast Atlantic distribution extends from Norway to Portugal, the European distribution is discontinuous. Ascidian taxonomists and invasion biologists have inferred human aided transport for both European and North American *M. manhattensis* populations because of its occurrence in fouling communities, its seasonally high local densities, and its patchy European distribution (Huus 1927; Van Name 1945; Monniot 1969; Carlton 2003a; Wolff 2005b).

Moreover, *M. manhattensis* also has a recent history of world-wide introductions having been reported from the Mediterranean (Sigean Lagoon, southern France), Adriatic (Venice Lagoon) (Monniot 1969), northern Aegean (Aslan, pers. comm.) and Black Seas (Micu, pers. comm.). In the Eastern Pacific it was first recorded in Tomales

Bay, California in 1949 (Cohen & Carlton 1996) and subsequently spread northwards along the Pacific coast as far as Puget Sound in Washington Sate (Lambert 2001). In 1972 *M. manhattensis* was first recorded from Japanese waters (Tokioka & Kado 1972), and in 1999 in the harbor of Vladivostok in Peter the Great Bay, where it is now common (Zvyagintsev 2003; Zvyagintsev *et al.* 2003). *M. manhattensis* also occurs in southeast Australian waters, after it was first found in two rivers in Victoria and Queensland in 1967 (Kott 1976; 1985; 2005). The inferred anthropogenic vectors for these introductions are hull fouling and oyster translocations (Tokioka & Kado 1972; Cohen & Carlton 1996; Lambert 2001), and possibly ballast water (Hewitt *et al.* 2004).

Habitat and dispersal potential

Molgula manhattensis occurs on hard substrates, ranging from shells in an otherwise muddy environment to rocky shores. It commonly occurs on American oyster (*Crassostrea virginica*) shells, and it can seasonally be the most abundant oyster-fouling organism (Galtsoff 1964). It is tolerant of high turbidity, organic content and polluted waters. It is reported from pontoons, dikes, buoys, and ship hulls (Visscher 1928; Woods Hole Oceanographic Institution 1952).

Ascidians are simultaneous hermaphrodites, sperm is released in the water column and eggs are fertilized internally. Self fertilization, which is an advantage in long-distance dispersal (because a single individual can found a population), occurs in several ascidian species (Lambert 2005a) including *M. manhattensis*. Artificial self-fertilization has been documented in the lab (Morgan 1904; 1942). However, there is no information on the incidence and frequency of self-fertilization in the field. Larval duration is short, ranging from minutes to several hours (Laurson, 1981; Svane & Young, 1989; Lambert, 2005a). *M. manhattensis* larvae have urodele development (i.e., tadpole larvae that can actively swim), whereas other molgulids have anural (tailless, non-swimming larvae) development (Berrill 1931; Hadfield *et al.* 1995; Huber *et al.* 2000). Rafting of eggs, juveniles or adults has not been reported (Thiel & Gutow 2005). These life-history traits make natural LDD unlikely, whereas LDD as hull-fouling and with oyster translocations has been demonstrated (see above).

Here we ask whether the disjunct amphi-Atlantic distribution of *M. manhattensis* is natural or is the result of anthropogenic introduction. In order to address this question, we: 1) establish the identification and monophyly of *M. manhattensis* with its sister species; and 2) reconstruct its phylogeographic history by comparing individuals from both coasts of the North Atlantic, as well as samples collected from known introductions in other parts of the world.

Methods

Sampling

Molgula manhattensis was sampled from 12 locations across its distributional range including putatively natural and non-natural locations (Table 5.1). Samples were

collected from ropes and floating dock surfaces. Individuals were collected at least a few meters apart and placed immediately in 95% ethanol. A piece of the gonadal tissue was used for DNA extraction.

In European waters, *M. manhattensis* is often confused with the morphologically similar and closely related *Molgula socialis* (Arenas *et al.* 2006). Both species occur in the same habitat, although *M. manhattensis* seems to be more euryhaline. In order to compare levels of intra- and inter-specific genetic diversity two populations of *M. socialis* were included in this study (Table 5.1). In order to further verify the taxonomic identification of earlier, 19th century collection records from both sides of the Atlantic, the DNA of a formalin-preserved specimen of *M. manhattensis* (National Museum of Natural History Naturalis, The Netherlands; Invertebrate Collection, accession number 336, under *M. macrosiphonica*) collected in 1878 from the former Zuiderzee (now Lake IJssel) in The Netherlands, and a single individual of *M. provisionalis* from Hudson Bay, Canada was also extracted.

DNA extraction, amplification and sequencing

DNA extraction was performed with a CTAB protocol according to (Hoarau *et al.* 2002).

In order to confirm species identities and to clarify inter-specific relationships we sequenced a 1-kb section of the 18S rDNA gene for four *M. socialis* individuals, nine *M. manhattensis* individuals and one *M. provisionalis* individual from Hudson Bay. These individuals were selected to represent different mt-COI haplotypes (see below) and different locations. The fragment was amplified using the primers 18SA 5'-AGCAGCCGCGGTAATTCCAGCTC-3' and 18SB 5'-AAAGGGCAGGGACGTAATCAACG-3' (Wada *et al.* 1992).

All PCR reaction consisted of 25-µL reaction volumes containing 2-20 ng DNA, 1X reaction buffer (5PRIME), 0.2 mM of each dNTP, 0.5 U HotMaster Taq DNA polymerase (5PRIME), and 0 .5 µM of each primer. PCR was performed in a Veriti Thermal Cycler (Applied Biosystems). The reaction profile was 2 min at 94ºC followed by 40 cycles of: 20 s at 94ºC, 20 s at 66ºC, 2 min at 65ºC; and 65ºC for 10 min. PCR products were cleaned using ExoSapIt (USB Corporation) enzyme following the provider's instructions. Both strands were cycle-sequenced using the dGTP Big Dye Terminator Kit (Applied Biosystems), purified on a Sephadex G-50 fine Column and run on an ABI 3730 automatic sequencer. Sequences were aligned manually using BIOEDIT v.7.0.5 (Hall 1999).

For the phylogeographic analyses, we analyzed variation in the mitochondrial cytochrome *c* oxidase subunit I (COI) subregion for 244 *M .manhattensis* and 41 *M. socialis* individuals. The COI subregion is highly polymorphic in most ascidian species investigated so far, and has been a successful tool in the identification of previously unrecognized or cryptic ascidian invasions (Tarjuelo *et al.* 2001; Castilla *et al.* 2002; Turon *et al.* 2003; Tarjuelo *et al.* 2004; López-Legentil *et al.* 2006; López-Legentil & Turon 2006). The universal primers HCO2198 and LCO1490 (Folmer *et al.* 1994) were initially used to amplify a segment of the mitochondrial COI gene, and based on these

sequences specific primers were developed for *M. manhattensis*: MMCO1F 5′-TCCGCTTTGAGTGGAGTTTT-3′ and MMCO1R 5′-AGATTGGATCTCCCCCTCCT-3′, and for *M. socialis*: MSCO1F 5′-TGGTACGATAGCAGCGCTTA-3′ and MSCO1R 5′-TAGGATCTCTCCCTCCAGCA-3′. All PCR reaction consisted of 50-µL reaction volumes containing 2-20 ng DNA, 1X reaction buffer (Promega), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.25 U Taq DNA polymerase (Promega), 0.15 µM of each primer and 0.1 $mg \cdot mL^{-1}$ Bovine Serum Albumin (BSA). PCR was performed in either a Gene-Amp-System 9700 (Perkin-Elmer) or a MyCycler (BioRad). The reaction profile was 2 min at 94ºC followed by 40 cycles of: 20 s at 94ºC, 30 s at 5 ºC, and 90 s at 7 ºC; and 72ºC for 7 min. PCR products were cleaned using ExoSapIt (USB Corporation) enzyme following the provider's instructions. Both strands were cycle-sequenced using the dGTP Big Dye Terminator Kit (Applied Biosystems), purified on a Sephadex Column and run on an ABI 377 automatic sequencer. Sequences were aligned manually using BIOEDIT v.7.0.5 (Hall 1999), there were no gaps in the sequences. The final fragment length was 550 bp for *M. manhattensis* and 583 bp for *M. socialis*, the extra 33 bp for *M. socialis* were at the 3′ and 5′ end of the sequence.

Data analyses

Aligned 18S rDNA sequences, with a final alignment length of 965 bp, were analyzed with Bayesian maximum likelihood using MRBAYES 3.1 (Ronquist & Huelsenbeck 2003). Sequences of *Molgula* spp. available on GenBank were included in the alignment. The optimal model of sequence evolution for the Bayesian analysis was determined using MODELTEST (Posada and Crandall, 1998). The following parameters were used: model of sequence evolution = GTR + Γ, generations =3,000,000, burn-in = 1,000,000. Trees were rooted using *M. bleizi, M. citrina, M. complanata* and *M. echinosiphonica* (Hadfield *et al.* 1995).

For the COI sequences, estimates of haplotype (*h*) and nucleotide diversities (π) were performed with the DNASP software v 4.10.9 (Rozas & Rozas 1995). In order to compare haplotype diversities across sampling locations, rarefaction was used to correct for unequal sample sizes (n = 20) using the software HPRARE (Kalinowski 2005). Statistical testing was done with the software FSTAT v 2.9.3.2 (Goudet 1995). Haplotype richness estimates were performed using ESTIMATES software v 8.0.0 (Colwell 2006). ESTIMATES calculates a non-parametric estimator, Chao2, that can be used to predict the eventual asymptote in haplotype diversity for a certain number of samples in a region. The Chao2 estimator includes the effects of private or rare haplotypes on the total haplotype diversity. The greater the number of rare haplotypes, the more likely it is that haplotypes that are in fact present, were not sampled (Gotelli & Colwell 2001). Estimated haplotype richness (Chao 2) was plotted against the number of samples for pooled North American and European populations. Haplotype networks were inferred using statistical parsimony in the software TCS v. 1.13 (Clement *et al.* 2000).

Table 5.1 Sampling locations, haplotypes per site and diversity measures for samples of *Molgula manhattensis* and *Molgula socialis*. N = number of individuals per location. n_h = number of haplotypes, n_{hc} = number of haplotypes after rarefaction to 20 individuals, n_p = number of private haplotypes, n_{pc} = number of private haplotypes after rarefaction to 20 individuals, h = haplotype diversity, N_p = number of polymorphic sites, π = nucleotide diversity. Location names given in bold are used to indicate the populations in the figures.

region	location	latitude-longitude	N	haplotype codes	n_h	n_{hc}	n_p	n_{pc}	h	N_p	π
Molgula manhattensis											
Europe	**Sylt**, Germany	55° 01′ N - 8° 43′ E	21	H1, H18	2	2.00	1	1.00	0.095	2	0.00035
	Delfzijl, The Netherlands	53° 32′ N - 6° 92′ E	22	H1, H6, H16	3	2.99	2	1.99	0.385	3	0.00090
	Grevelingen, The Netherlands	51° 74′ N – 3° 89′ E	21	H1	1	1.00	0	0.00	0.000	0	0.00000
	Oostende, Belgium	51° 22′ N - 2° 94′ E	21	H1, H3, H14, H15	4	4.00	2	2.00	0.471	3	0.00094
	Le Havre, France	49° 48′ N - 0° 12′ E	20	H1, H3, H17	3	3.00	1	1.00	0.279	2	0.00053
North America	**Woods Hole**, Massachusetts	41° 55′ N - 70° 54′ W	22	H2, H12	2	1.99	0	0.02	0.091	1	0.00017
	Mystic River, Connecticut	41° 35′ N - 71° 96′ W	21	H1, H2, H5, H31	4	4.00	2	2.00	0.586	7	0.00483
	Long Island Sound Bridgeport, Connecticut	41° 17′ N - 73° 18′ W	24	H1, H2, H3, H10, H11, H12, H30	7	6.93	2	1.99	0.768	6	0.00238
	Chesapeake Bay James River, Virginia	36° 94′ N - 76° 33′ W	28	H1, H3, H4, H8, H9, H21, H22, H23, H24, H25, H26, H27, H28, H29	14	13.21	11	10.29	0.817	15	0.00266
Introduced range	**Black Sea**, Bulgaria	44° 16′ N - 28° 64′ E	6	H1, H19	2	-	1	-	0.333	1	0.00061
	Osaka Bay, Japan	34° 68′ N – 135° 38′ E	16	H1, H4	2	-	0	-	0.125	1	0.00023
	San Francisco Bay, California, USA	37° 85′ N – 122° 48′ W	22	H1, H3, H4, H7, H10, H13, H20	7	6.99	3	3.02	0.840	6	0.00290
Molgula socialis											
Europe	**Oosterschelde**, The Netherlands	51° 67′ N - 3° 75′ E	21	HA, HB	2	-	0	-	0.181	3	0.00093
	Oléron Island, France	46° 03′ N - 1° 37′ W	20	HA, HB, HC	3	-	1	-	0.195	4	0.00069

Results

Phylogeny

Phylogenetic analysis of 23 sequences of the 18S gene resolved the three species of interest for this study (*M. provisionalis*, *M. manhattensis* and *M. socialis*). There was no evidence for misidentifications or for the presence of cryptic species (Fig. 5.1).

Haplotype diversity and private haplotypes

Among 550 sites, 34 were polymorphic. Nucleotide diversities were an order of magnitude higher in the southern populations on the Atlantic coast of North America and in San Francisco Bay as compared with all Europe. All mutations except one (in a sequence from an individual from Long Island Sound) were at the third codon position.

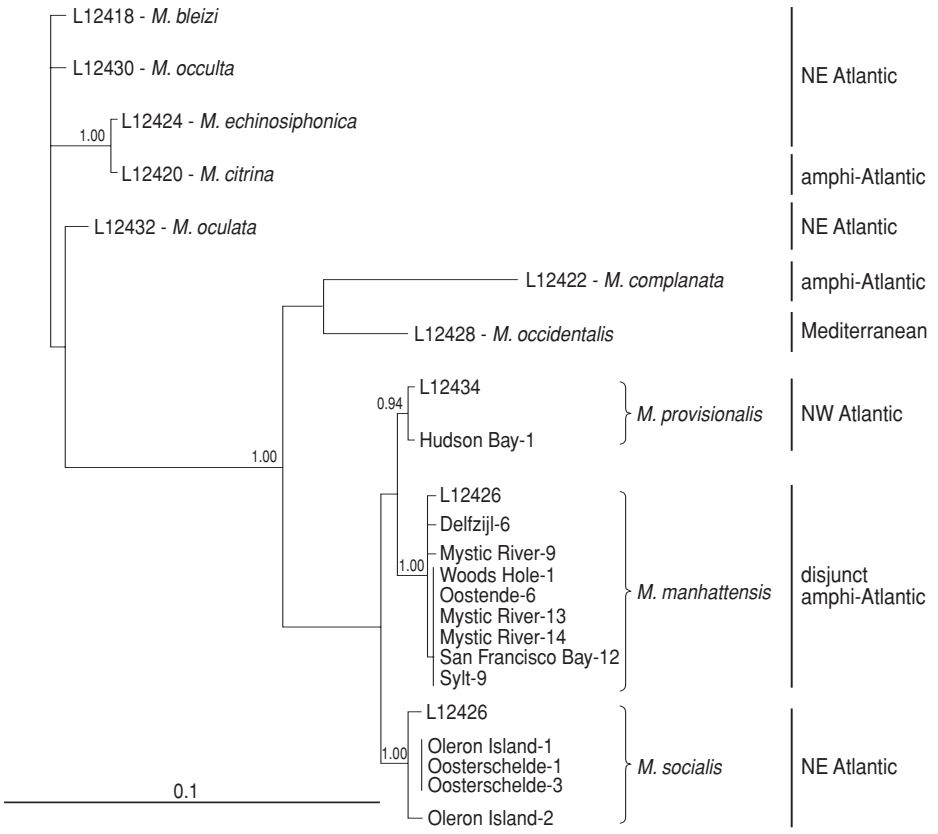

Figure 5.1 18S DNA Bayesian tree of *Molgula* spp. Species names and Genbank accession numbers are given for the reference sequences (Hadfield *et al.* 1995). Locations and individual numbers are given for sequences obtained in this study. Numbers on branches are Bayesian posterior probabilities (≥ 90%). The tree is rooted with *M. citrina, M. echinosiphonica, M. occulta* and *M. bleizi*. The scale bar represents the number of expected changes per site. For each species or species group, the generalized distribution in the North Atlantic Ocean is indicated.

The mtDNA diversity was moderately high with 31 haplotypes recovered from the 244 *M. manhattensis* individuals sequenced (Table 5.1, Fig. 5.2). In Europe, the total number of haplotypes was 8 (corrected haplotype richness = 7); the total number of haplotypes in America was 21 (corrected haplotype richness = 20). This included 2 shared haplotypes (Table 5.1).

Haplotype richness (n_h) was higher than the average (average n_h = 4.25; corrected average n_{hc} = 4.61) for populations at Long Island Sound, Chesapeake Bay and San Francisco Bay; all other locations had a lower than average number of haplotypes (Fig. 5.3, Table 5.1). Haplotype diversity decreased from South to North along the Atlantic coast of North America; in Europe no such pattern was found. Of the three sampled populations which are known introductions, two exhibited low haplotype richness, and one (San Francisco Bay) had high haplotype richness (Fig. 5.3, Table 5.1).

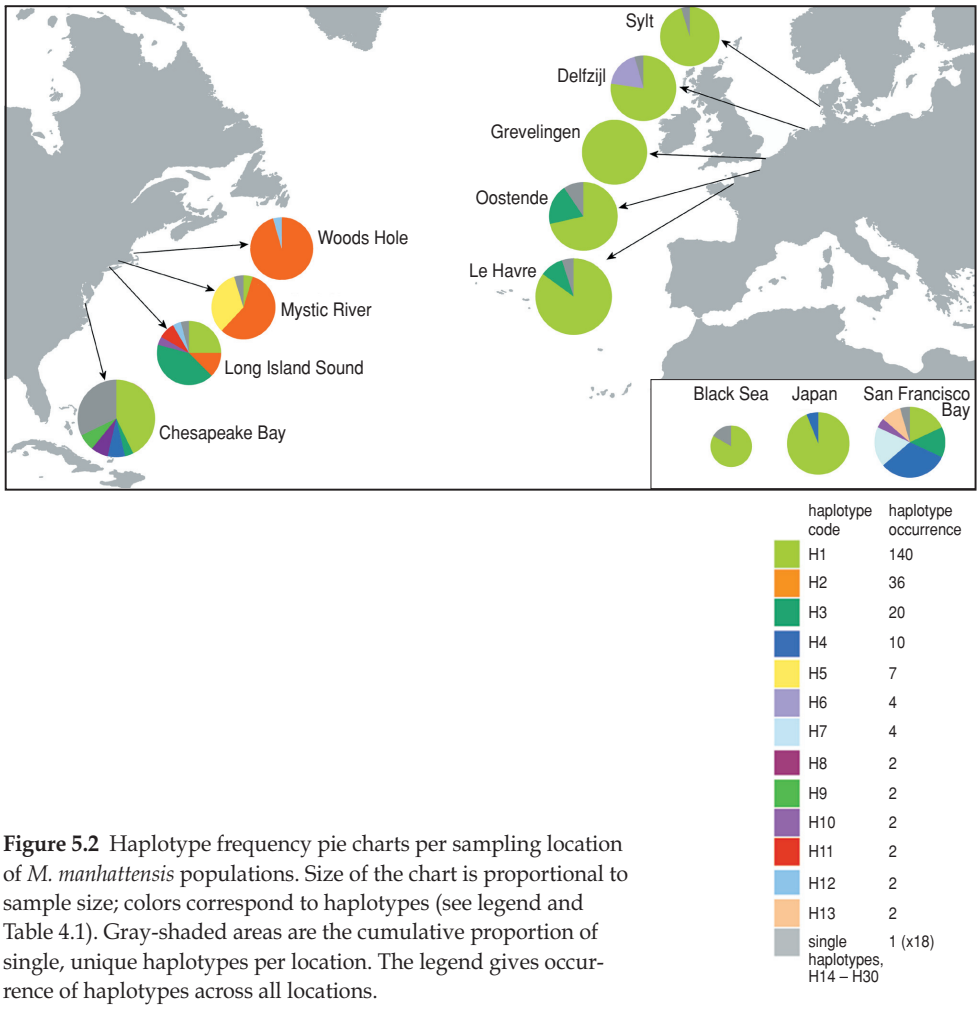

Figure 5.2 Haplotype frequency pie charts per sampling location of *M. manhattensis* populations. Size of the chart is proportional to sample size; colors correspond to haplotypes (see legend and Table 4.1). Gray-shaded areas are the cumulative proportion of single, unique haplotypes per location. The legend gives occurrence of haplotypes across all locations.

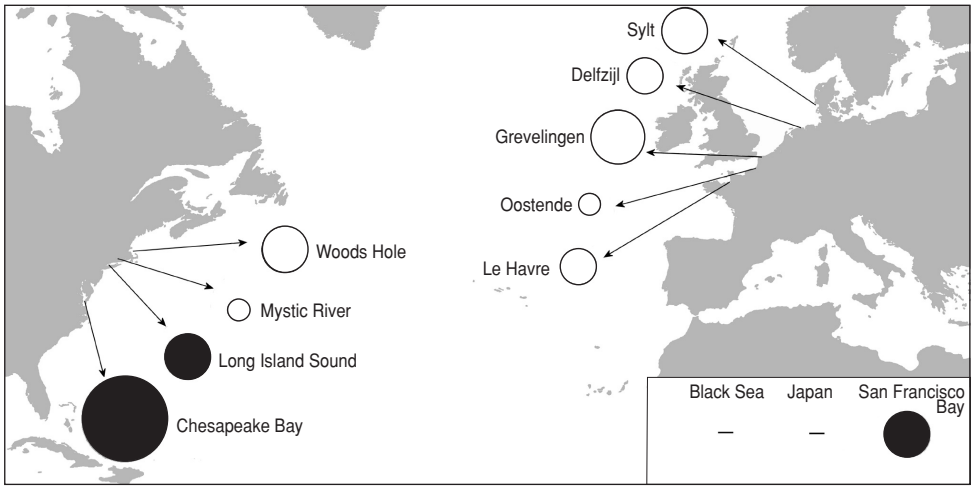

Figure 5.3 Haplotype richness at the sampling locations after rarefaction using HP-RARE (Kalinowski 2005). Circle sizes are proportional to the deviation from the mean for all populations (= 4.61); filled circles indicate richness above the mean and open circles indicate richness below the mean. The least diverse sample is represented by the largest open circle and the most diverse sample by the largest filled circle. Estimated haplotype richness is not given for the Black Sea and Japan as rarefaction could not be performed due to the smaller sample sizes of these populations.

Private haplotypes were present in all geographic regions. The corrected number of private haplotypes in the Northeast American range was 16, whereas the number of private haplotypes in the European range was six. In the introduced range, four private haplotypes were found. The number of private haplotypes did not differ significantly between Europe and America (Mann-Whitney, not significant).

Diversity against sampling effort was compared for both sides of the Atlantic (Fig. 5.4). In Europe the mean expected haplotype richness for 5 sampled populations was 20, compared to the 8 haplotypes observed. In Northeast America, 93 haplotypes were predicted for 4 sampled populations compared to the 21 that were observed. The expected vs. observed haplotype richness indicates that the sampling effort did not capture the actual diversity—especially on the American side. Nevertheless, the observed haplotype richness in Northeast America was still 2.6 times greater than in Europe, and the expected haplotype richness in Northeast America was 4.6 times greater than in Europe. This relationship would not be expected to change even with greater sampling.

The COI sequence of the formalin preserved *M. manhattensis* museum specimen from The Netherlands confirmed that it was indeed *M. manhattensis*. Because of degradation of the DNA the sequence was not used in phylogeographic analyses. The COI sequence of the single *M. provisionalis* individual from Hudson Bay corresponded to haplotype H1.

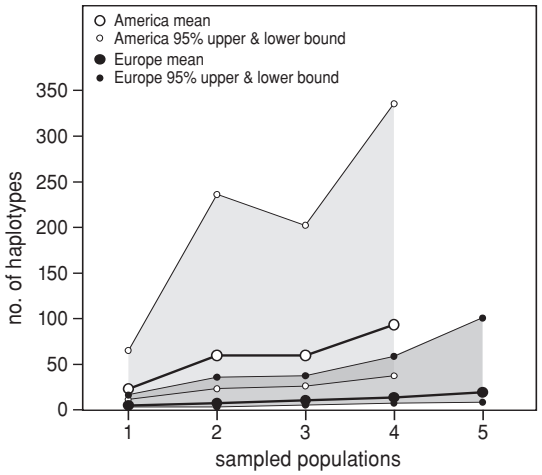

Figure 5.4 Haplotype estimation curves for European and Northeast American populations of *M. manhattensis* using ESTIMATES (Colwell 2006). The European Chao2 estimator suggests a maximum expected number of haplotypes of 20 (95% Confidence interval: 100-9), the Northeast American maximum expected number of haplotypes is 93 (95% Confidence interval: 38-334).

Haplotype networks

The central haplotype (H1) in Fig. 5.5A accounts for 55% of the total number of *M. manhattensis* individuals sampled, for 86% of the individuals from Europe, and for 20% of the Northeast American individuals. H1 was present at all locations except Woods Hole, MA. All European haplotypes were within one or two point-mutations from the central haplotype, whereas the Northeast American haplotypes ranged from one to five steps from H1. The relationships of Northeast American haplotypes are, therefore, deeper and older. High frequency nested Northeast American haplotypes were present in the introduced range, but were absent in European populations.

In the haplotype network for *M. socialis* (Fig. 5.5B), the 42 sequences yielded only three observed haplotypes (HA-HC) and low diversity (Table 5.1). The depth of the haplotype network was comparable to the European *M. manhattensis* haplotypes; the maximum distance from the central haplotype HA was three point mutations (Fig. 5.5B).

Discussion

The North Atlantic Ocean is the center of molgulid diversity, with 31 described shallow-water species. Four of these have a distribution that is restricted to the Northwest Atlantic, whereas 14 species have been ascribed to Northeast Atlantic coasts. Ten species of *Molgula* occur in Arctic waters and two have an amphi-Atlantic distribution. *M. manhattensis* is the only *Molgula* species that has a disjunct amphi-Atlantic distribution.

Phylogeny

The 18S phylogeny (Fig. 5.1) confirms that our study was not affected by misidentifications of species or specimens and that the classically described *Molgula* species form

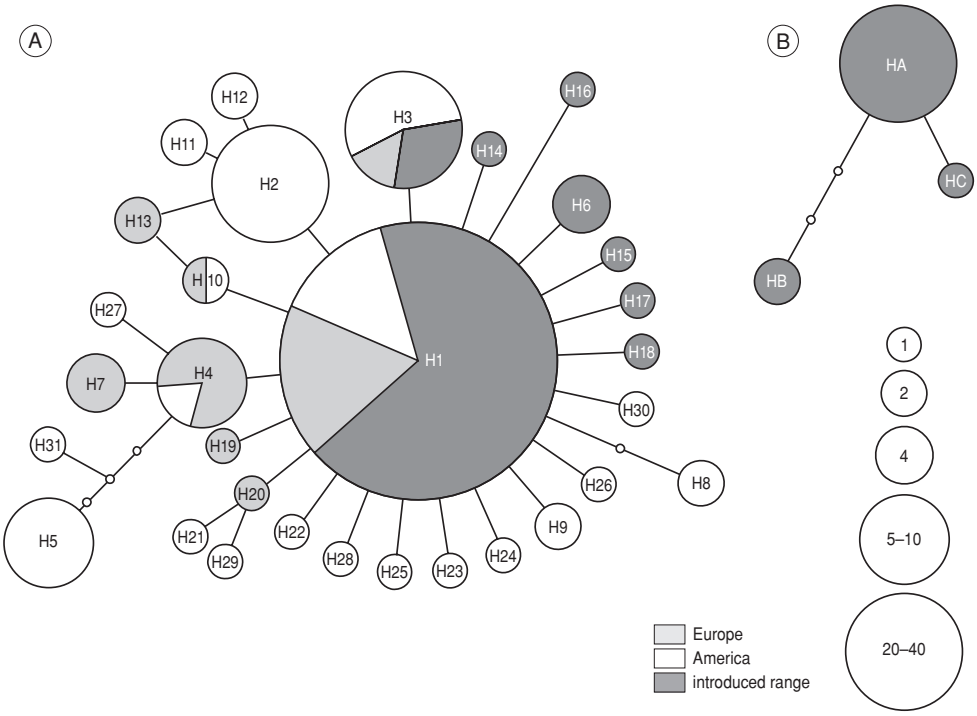

Figure 5.5 Haplotype networks for *M. manhattensis* (A) and *M. socialis* (B). Haplotype networks were created using TCS v. 1.13 (Clement et al. 2000). Numbers represent haplotype identities (see Table 4.1). Haplotype bubbles are proportional to haplotype frequency, see legend. European haplotypes (or proportions of haplotype occurrence) are indicated in dark gray, Northeast American haplotypes are white and haplotypes in the introduced range are light gray.

monophyletic groups (Hadfield *et al.* 1995, Huber *et al.* 2000). The closest relative of *M. manhattensis* is *M. provisionalis*, followed by *M. socialis*. On the Northwest Atlantic coast, *M. manhattensis* is replaced by *M. provisionalis* north of Cape Cod and has only been recognized as a separate species (based on morphological criteria) since 1945 (Van Name 1945). Its monophyly based on 18S data is here confirmed, and the single M. provisionalis individual shared the ancestral haplotype H1. On the Northeast Atlantic coast *M. manhattensis* and its close relative *M. socialis* occur in the same area and habitat, sometimes even in the same location. *M. socialis* has frequently been misidentified as *M. manhattensis* (Arenas *et al.* 2006), due to the fact that specific characters of the anatomy of these species can only be seen after a detailed dissection (Monniot 1969). The sequence data allowed us to rule out any morphologically-based misidentifications in our samples.

Figure 5.1 shows that the distribution patterns of species are not correlated with sister clades. However, based on the distribution of *M. provisionalis*, a Northwest Atlantic origin of *M. manhattensis* can be inferred. The timing and mode of dispersal

across the Atlantic cannot be inferred from these data, and the question remains whether *M. manhattensis* was dispersed by humans across the Atlantic, or has a natural disjunct amphi-Atlantic distribution.

Historical records

In 1762, Baster described a *Molgula* species from lock-doors in the Southwest Netherlands (Baster 1762), which could very well be the earliest record of *M. manhattensis* in European waters. More generally, *M. manhattensis* was reported in both Europe and Northeast America from the 19th century onward (in Europe as *M. ampulloides* (Van Beneden, 1846) and *M. macrosiphonica* Kupffer, 1872), as was confirmed by morphological comparison of 19th century specimens from both sides of the Atlantic (Monniot 1969). We here confirm 19th century presence of *M. manhattensis* in Europe based on the COI sequence of the museum specimen collected in 1878 from The Netherlands.

Phylogeography and haplotype diversity in the North Atlantic

A comparison of haplotype richness across the Atlantic (Fig. 5.3) and other diversity statistics in Table 5.1, shows a consistently higher mean corrected diversity of nearly three-fold on the Northeast American side as compared with the European side. Only the central ancestral haplotype (H1) and closely related H3 are found on both sides.

Northwest Atlantic populations show a latitudinal gradient of diversity from the south to the north (Figs. 5.2 and 5.3), which is consistent with post-LGM expansion from a southern refugium, possibly in the Chesapeake Bay region. At the same time, the dominant northern haplotype decreases in frequency southwards. This suggests a northern refugium, possibly in some ice-free areas of Nova Scotia and Newfoundland, and a subsequent contact zone to the south in the Long Island Sound region. As a hard substrate species, survival of *M. manhattensis* in southern refugia would have been difficult given the predominantly sandy coastlines (Wares & Cunningham 2001; Wares 2002; Maggs *et al.* 2008). However, it may have taken advantage of hitch-hiking on the shells of the American oyster, *Crassostrea virginica*, which has been present in Northwest Atlantic waters since before the Pleistocene glaciations (Vermeij 2005). In any case, the high diversity of the western Atlantic combined with nearly three times the number of haplotypes and, most significantly, the greater phylogeographic depth of American haplotypes, is consistent with North American native residency long before and after the LGM.

Northeast Atlantic populations of *M. manhattensis* do not show a latitudinal diversity gradient (Figs. 5.2 and 5.3). The central haplotype H1 accounts for 86% of the total number of sequences. This pattern is atypical for European rocky shores, where a number of high-diversity refugia have been identified, especially in Northwest Iberia, Brittany and Southwest Ireland (reviewed in Maggs *et al.* 2008). There are no confirmed records of current populations of *M. manhattensis* in the British Isles, Brittany or the Iberian Peninsula, and we were unable to find *M. manhattensis* at Oléron Island (S Atlantic France), where all sampled individuals were *M. socialis*.

Patterns of haplotype diversity in introduced populations

Low genetic diversity due to a population bottleneck was long thought to be a characteristic of introduced populations (Geller *et al.* 1994; Holland 2000). However, recent studies have shown that diversity of introduced populations can be similar to the level of diversity in native populations, and may even exceed native diversity levels due to admixture or high propagule pressure (Lockwood *et al.* 2005; Darling *et al.* 2008; Dlugosch & Parker 2008). *M. manhattensis*, in its introduced range, shows both patterns: low haplotype diversity in Japan and the Black Sea, and high haplotype diversity in San Francisco Bay (Table 5.1, Figs 5.2 and 5.3).

The high level of haplotype diversity in San Francisco Bay is comparable to that found in Chesapeake Bay and Long Island Sound (Figs. 5.2 and 5.3) and given that four of the seven haplotypes are unique to these two locations, it is quite certain that the San Francisco Bay introductions came from these sources. Not only have thousands of ships - with rich fouling communities - sailed from the East to the West coast of the USA (Carlton 1979) but between 1869 and 1940, large quantities of live oysters were transported by the transcontinental railway from New York and Long Island Sound to San Francisco, where they were placed in local waters for storage or growth and maturation (Ingersoll 1881; Carlton 1979; Miller 2000). The ships and oyster translocations were also responsible for introductions of associated fauna (Carlton 1979; Miller 2000).

In the case of *M. manhattensis*, sustained, high propagule pressure from oyster transplants to San Francisco Bay surely explains the high observed diversity. This pattern is in sharp contrast with the other introduced populations in Japan and the Black Sea, which showed low diversity and dominance of the ancestral haplotype H1. The origin of *M. manhattensis* in Japan was originally suggested to have been Atlantic Europe (Tokioka & Kado 1972). This hypothesis is not supported by our data. The haplotypes present in Japan include H1, but also H4, which occurs in Chesapeake Bay and San Francisco Bay, and not in Europe. It is, therefore, more likely that *M. manhattensis* was introduced to Japan from the Pacific coast of the USA and indirectly from the US Atlantic coast. The origin of *M. manhattensis* in the Black Sea is Atlantic Europe as five of the six individuals were H1 and one individual was H19, a derived, local haplotype. The vector of introduction in these populations is most likely hull fouling, which is reflected by the low haplotype diversities. Populations of sessile species that were introduced as hull fouling are more likely to show evidence of a founder effect, as the propagule pressure is much smaller as compared with large-scale oyster translocations.

Private haplotypes

Geographically restricted or private haplotypes are an indicator of longer-term residency far exceeding the timeframe of human introductions (Wares, 2002). Private haplotypes were found in both North America and Atlantic Europe, which is consistent with long-term residence. However, we also found four putatively private alleles in introduced populations.

As shown in Fig. 5.4, the sampling effort did not completely capture the diversity estimated to be present - mainly in Northeast America. This means that low-frequency Northwest Atlantic haplotypes have been missed. Further intensive sampling could reveal that the putatively private haplotypes found in the introduced populations also occur in the source populations of the Northwest Atlantic and thus did not evolve *in situ*. Likewise, if the European populations are the result of an introduction, then their putative private haplotypes could also be artifacts. However, the main argument against a European introduction is the fact that we would also have expected to see the medium and high frequency Atlantic American haplotypes in Europe (given the number of locations sampled in Europe), and we do not. Below we weigh the empirical and genetic evidence together.

Introduced or glacial relict?

Distinguishing between an anthropogenic introduction and post-glacial recolonization requires multiple lines of evidence. Empirical evidence based on life-history traits, preferred habitat and patchy distribution argue for an introduction of *M. manhattensis* from America to Europe. *M. manhattensis* has a low natural dispersal potential, and it is not clear how it would have spread naturally to the other side of the Atlantic. If it was introduced to Europe, the vector of introduction would have been hull fouling, as the first records of *M. manhattensis* in Europe precede the first American oyster transfers to Europe, which took place in the 1870s (Carlton & Mann 1996; Wolff & Reise 2002).

Moreover, though common in oyster culture areas (e.g. in the "Spuikom" in Oostende, Belgium and the Oosterschelde Estuary, The Netherlands), where it grows on docks, pylons and other structures typical of harbors and marinas, *M. manhattensis* is not found on the shells of the oysters *Ostrea edulis* (Korringa 1951) and *Crassostrea gigas* (D. Haydar, unpublished data) that are present in European waters, as would be expected given that it commonly occurs on American oysters in the Northwest Atlantic. Finally, for most invertebrates, the European coast is more diverse in the number of species as compared with the North American coast (Briggs 1974; 1995). Post-glacial recolonization of the North American coast occurred from Northwest Atlantic refugia, as well as from refugia in Europe via Iceland (Wares & Cunningham 2001; Vermeij 2005; Maggs *et al.* 2008). Dispersal and recolonization in the other direction has not been documented, nor are there examples of species that have a naturally disjunct amphi-Atlantic distribution. Taken together, these arguments support an anthropogenic origin of *M. manhattensis* in Europe.

The genetic data, however, present some challenges to the empirical data. Low haplotype diversity could be natural if the range and refugia on the European side were small. For example, comparison of the haplotypes networks of *M. manhattensis* and *M. socialis* indicate a similar evolutionary history and low diversity (Table 5.1, Fig. 5.5). However, the number of *M. socialis* populations sampled was low, making this comparison somewhat weak. More importantly, the absence of the medium-to-high frequency, Northeast American haplotypes in Europe is puzzling (Fig. 5.5a). These should have been present, even within the sampling error for Europe (Fig. 5.4) and, in

fact, were found in introduced populations where only single population sampling took place. As discussed above, the presence of private haplotypes could be an artifact; but not necessarily. Taken together these data suggest native residence in Europe.

Resolution of the question rests on two inter-related factors: 1) the degree to which the total haplotypic diversity was sampled; and 2) the relative role of vectors and propagule pressure at a given location. First, more intensive sampling of the Northwest Atlantic would certainly reveal more low frequency haplotypes, which could include the putative private haplotypes found in Europe. More intensive sampling in Europe might reveal additional private haplotypes and possibly the missing medium-to-high frequency American haplotypes, though the latter is much less likely. This leaves us with propagule pressure and its effects. Given hull transport and relatively low propagule pressure over many decades in Europe, the haplotype diversity of European populations of *M. manhattensis* could remain low. In the most extreme case the introduction may have been successful only a single time involving the most common haplotypes, H1 and H3. In contrast, the extremely-high propagule pressure (involving tons of tunicate-carrying oysters) that occurred in San Francisco Bay over many decades, certainly accounts for the high diversity. While more intensive sampling of the Northwest Atlantic might help to resolve some of the issues around low-frequency private alleles, it would not explain the absence of the higher frequency alleles in Europe. Thus, in conclusion, we cannot say with certainty that *M. manhattensis* was introduced to Europe, and it remains a cryptogenic species in Atlantic Europe.

Synthesis

Deniz Haydar

6

6

In this thesis the extent to which natural patterns of diversity and distributions in coastal marine ecosystems have been affected by biological invasions is examined. In Part I, the focus is on established non-indigenous and cryptogenic species, the magnitude of invasions and the predominant vectors in the North Sea.

Introduced species in the North Sea

The number of introduced and cryptogenic species known from the North Sea region (**Chapter 2**) has doubled since the previous checklist of 1999 (Reise *et al.* 1999), amounting to 167 introduced and cryptogenic species. This increase in the number of invasions in the North Sea is not entirely due to newly arriving invaders, but is also the result of the inclusion of previously overlooked invasions and cryptogenic species. Not all of these introduced species are known to have established in the North Sea; only 124 non-indigenous and cryptogenic species have actually established self-sustaining populations. Paleoenvironmental history (post-glacial recolonization of the North Atlantic in general and of the North Sea in particular) and disturbance due to strongly transformed modern coastal environments have contributed to relatively low species richness in the North Sea. This may be one of the explanations of the high number of introduced species. Many of the species that were introduced became established, increased local diversity and considerably modified ecosystem functioning in the nearshore zone (Reise *et al.* 2006). Matching the world-wide pattern, the rate of invasions has increased in the North Sea (Reise *et al.* 1999), and it will probably continue to increase as a consequence of climate change and ongoing globalization.

The majority of non-indigenous and cryptogenic species have localized distributions; only ten of these are known from all seven countries bordering the North Sea. However, continuing shipping and shellfish translocations may result in rapid secondary spread of these introduced species to other North Sea coasts and beyond. The checklist of non-indigenous and cryptogenic species presented in Chapter 2 may serve as a basis for future studies; it will have to be periodically updated with newly arriving invaders and spreading established invasions. New introductions will inevitably occur and established introductions will spread by natural or anthropogenic means in the coming years. In Chapter 2 the number of cryptogenic species is severely underestimated because only those species were included for which there is a strong indication that they were introduced in some part of their range, but for which the origin remains unknown. The actual list of cryptogenic species is undoubtedly much longer (see Chapter 4).

The effect of invasions in the North Sea was not the subject of this chapter, but three case-histories of non-indigenous species with significant ecological and economic impact on coastal systems in the North Sea were presented in addition to the checklist of introduced species. The slipper limpet *Crepidula fornicata*, the Chinese mitten crab *Eriocheir sinensis* and the shipworm *Teredo navalis* are examples of well-known and extensively studied non-indigenous species. For the majority of non-indigenous

species the impact on North Sea ecosystems remains unknown due to the lack of scientific studies on the effect of invasions in this region.

The predominant vectors for introduction of non-indigenous species in the North Sea are hull fouling and aquaculture (in particular oyster translocations), which are each responsible for 25% of the total number of introduced species.

Oysters as a vector

Oyster transports are one of the strongest vectors in the North Sea (Chapter 2), and in Dutch coastal waters. In **Chapter 3**, a detailed analysis of this vector was presented in order to explain its high share of introductions into The Netherlands. The frequency and scale of oyster translocations and the characteristics of this vector - the rugged shells of oysters offer ample opportunities for epiflora and –fauna to settle and survive transport to other regions - may explain the relatively high number of oyster-associated introductions, and were investigated here.

A literature review of oyster-associated species introductions resulted in a list of 35 non-indigenous oyster-associated species that have become established in The Netherlands. Of all associated introductions 45% originate from the Northwest Pacific and most these are assumed to have been introduced with Pacific oyster (*Crassostrea gigas*) imports. Some may have been introduced with hull fouling to Europe and have subsequently spread in European waters with oyster transports. The Northwest Atlantic is the origin of 20% of oyster-associated introductions; these species are supposed to have been introduced with shipments of the American oyster *C. virginica*.

The increased rate of oyster-associated introductions in the past 30 years is consistent with world-wide patterns of increased introduction rates, but does not coincide with increased commercial oyster imports. Instead the oyster import data, obtained from Statistics Netherlands (CBS), show a decrease in the amount of oysters imported since the 1970s, in particular of seed oysters or smaller oysters, which are imported to restock local culture. However, not all oyster imports are reported to the authorities. *C. gigas* is known to have been introduced to The Netherlands in the 1960s directly from Japan and via British Columbia (Shatkin *et al.* 1997; Drinkwaard 1999; Wolff & Reise 2002), but we did not find any official records of these imports. Unreported translocations of oysters are suspected to occur in other countries as well (Verlaque 2001), and the patterns of secondary spread of associated non-indigenous species provide evidence of ongoing transports. Even though adult oyster imports that are currently occurring may not be intended for restocking local oyster culture, the oysters may still be relaid on culture plots or in basins on the shore from where the introduction and secondary spread of associated species is still possible.

In Dutch coastal waters, the Pacific oyster has formed extensive intertidal and subtidal reefs that are continuing to increase in size. *C. gigas* is being cultured on plots in the Oosterschelde estuary. In order to analyze oysters as a vector, we collected and identified the epiflora from live Pacific oysters from these culture plots that were

treated as if they were transported to other European culture sites for relaying. Red seaweeds were the most common group of macroalgae retrieved from the oyster shells, and they also constituted the largest fraction of species introduced with this vector. The oyster shells provided a substrate for 41 macroalgal taxa, of which 36 were identified to species level. These macroalgae were native, cryptogenic and non-indigenous species; of the most common species, 50% were introduced. A single oyster may harbor up to 14 macroalgal taxa, the maximum species richness on oysters from the Oosterschelde estuary was estimated to be 44 species of macroalgae. Relatively small numbers of oysters (~500) may carry large numbers of associated species and individuals, resulting in a high potential of introducing non-indigenous species. The introduction of these species with their substrate further facilitates their establishment, which may partly explain the increase in oyster-associated introductions in the past decades.

Furthermore, the rapidly growing Pacific oyster reefs form a new habitat for fouling species in The Netherlands, and are likely to facilitate the establishment of associated non-indigenous species, possibly accelerated by other factors such as climate change and increased disturbance in the recipient region.

Pacific oysters in the Oosterschelde estuary are a substrate for native and introduced species, and for a large number of cryptogenic species. Historical introductions with oyster transports may have resulted in cosmopolitan or disjunct distributions of species we now call native, as pre-19th century movements of non-indigenous and native species with oysters within Europe are likely to have occurred. Oyster-associated introductions are not only a phenomenon of the past: the ongoing shellfish movements within Europe contribute to introduction and rapid secondary spread of non-indigenous species, and to exchange between populations of native and cryptogenic species. Oyster translocations are thus blurring natural distribution patterns and homogenizing diversity of algae and invertebrates in coastal waters.

The scale of cryptogenesis

Part II of this thesis is devoted to the scale of cryptogenesis in the North Atlantic Ocean. In general, the share of invasions is hypothesized to be highly underestimated (see Chapter 1), because species are assumed to be native, unless they are proven to be introduced. Regional checklists of introduced species often include cryptogenic species (see Chapter 2), but the cryptogens in these checklists are very likely to have been introduced, although their origin remains unknown. In Chapter 3, many of the macroalgae occurring on oyster shells were assigned to the cryptogenic species category, based on their association with anthropogenic vectors (oysters and hull fouling), and their disjunct or cosmopolitan distribution patterns. Although large-scale shipping across the Atlantic had been taking place long before the first comprehensive biological surveys were conducted, in contemporary marine ecology historical invasions are generally ignored, assuming that the 19th century coastal biota was natural or indigenous (Carlton 1989; 2003b).

Cryptogenic species are by definition all species that are neither demonstrably native nor introduced (Carlton 1996), and include species that are falsely viewed as native. In order to get a more robust estimate of the number of cryptogenic species, in **Chapter 4** lists of all reported species from three taxonomic groups of shallow-water invertebrates in the North Atlantic Ocean were compiled. For each species, not only world-wide distribution data were included, but natural dispersal potential, habitat characteristics and association with anthropogenic vectors were also noted. The three taxonomic groups that were analyzed (Ascidiacea, Hydrozoa and Bivalvia) differ in their natural dispersal potential. Ascidiacea are poor dispersers with a short pelagic larval phase. Hydrozoa have a high dispersal potential: they have pelagic larvae, sometimes a free-swimming medusa and the polyps may be able to raft. Bivalvia have a long-lived larval phase; they can be divided in three groups: infaunal bivalves, that are able to disperse only via pelagic larvae, and epifaunal- and boring bivalves, both of which may additionally be able to raft. All groups except most infaunal bivalves are potentially dispersed as ship hull fouling. The infaunal bivalves were used as a control group, because it was hypothesized that all introductions of infaunal bivalves are known since they are likely to have mostly occurred with ballast water, which has only been in use since 1870. Thus, unknown historical introductions are not expected to occur in infaunal bivalves.

One of the characteristics of cryptogenic species is a disjunct distribution pattern, which we used as a proxy for cryptogenesis. A disjunct amphi-Atlantic distribution pattern, defined as a distribution pattern that spans European and American North Atlantic coasts, but is interrupted in Arctic or sub-Arctic waters, can be explained by four scenarios: natural trans-oceanic dispersal, post-glacial recolonization, cryptic speciation and human-mediated introductions. Literature on the four proposed scenarios was reviewed and relative numbers of cryptogenic species were compared among and within groups with disjunct amphi-Atlantic distributions.

Natural dispersal by either planktotrophic larvae or rafting on various substrates does not explain disjunct amphi-Atlantic distribution patterns of individual species of Hydrozoa, Bivalvia and Ascidiacea. Dispersal by these mechanisms is highly unlikely and has not been demonstrated for any organism in the North Atlantic Ocean. Additionally, based on the surface-current regime the direction of transport would have to be from west to east, which is in conflict with patterns of migration and invasions in the North Atlantic Ocean.

Infaunal bivalves have the lowest proportion of disjunct amphi-Atlantic distributions (10 out of 299 species). There are no cryptogenic infaunal disjunct amphi-Atlantic bivalves, nor are there infaunal bivalves that have a disjunct amphi-Atlantic distribution that can be explained by pelagic larval dispersal. The disjunct distributions of infaunal bivalves are either explained by known anthropogenic introductions, or by other factors, such as occurrence in deep or warm waters. In Hydrozoa, a free-swimming medusa does not guarantee long-distance dispersal and a wide, possibly disjunct, distribution. Rafting may be a possible mechanism for regional dispersal of Hydrozoa, but those species that are able to raft are also likely to be able to attach to

ships. Long-distance dispersal is more effective on ships than on rafts; ships travel fast, are independent of surface currents, provide more space and travel farther.

Cryptic species are common in all taxonomic groups and could explain some of the disjunct amphi-Atlantic distributions, as has previously been shown for several invertebrate species.

The role of Pleistocene glaciations in the creation of disjunct amphi-Atlantic distributions is assumed to be the same for all groups. However, there are no infaunal bivalves with a natural disjunct distribution pattern, nor are there cryptogenic infaunal bivalves. Additionally, for none of the groups do studies exist that give conclusive evidence for a disjunct amphi-Atlantic distribution pattern that is the result of post-glacial dispersal across the North Atlantic. It is difficult to distinguish between genetic diversity patterns caused by the LGM and by an anthropogenic invasion, as both processes can result in either high or low levels of genetic diversity (see Chapter 5). There is only one example of a species with a natural disjunct amphi-Atlantic distribution in the North Atlantic Ocean (*Mytilus edulis*), but this species may actually have an amphi-Atlantic distribution pattern. There are no other examples of naturally disjunct amphi-Atlantic distributions that have been demonstrated using a combination of phylogeographic, palaeobiological and historical data.

Disjunct amphi-Atlantic distributions are not common: 10% (108 species) of all shallow-water species of Ascidiacea, Bivalvia and Hydrozoa (n = 1054) have a disjunct distribution pattern, and about half of these are categorized as cryptogenic (38 species) and introduced species (22 species). The other half include species that also occur in deep or warm waters, species complexes, species with disjunct distributions that are based on single or doubtful records on either coast, or species of which the taxonomy is uncertain, and may therefore not be truly disjunct amphi-Atlantic species. For 8 species the disjunct distribution appeared to be natural.

The proportion of disjunct distributions differs among groups with differing life-history characteristics and relative natural dispersal potential, and ranges from 3% to 48% (Table 6.1). The relative number of cryptogenic and known introduced species within the disjunct amphi-Atlantic category ranges from 1.3% to 28% of the total number of species per group. This wide range of relative numbers is caused by differing dispersal potential of the studied groups, which were selected for the reason that they span a large part of the "natural dispersal scale". For the epifaunal groups (i.e. all groups except the infaunal bivalves), the relative number of cryptogenic species ranges from 1.3% to 24% of the total number of species per group. This could amount to at least 38 overlooked invasions of ascidian, bivalve and hydrozoan species. Extrapolating this to all invertebrates and macroalgae in the North Atlantic Ocean, this suggests that between 1.3% and 24% of the shallow-water epifaunal flora and fauna is cryptogenic and may historically have been introduced; hence, the number of overlooked invasions could be of the order of magnitude of hundreds of species. This is still a conservative estimate: we have only considered species with a disjunct amphi-Atlantic distribution, although species occurring on either Atlantic coast or with a continuous amphi-Atlantic distribution may also be cryptogenic or introduced.

The traditional assumption of limited influence of historical shipping on dispersal of coastal organisms has great implications for understanding modern-day distributions of species in North Atlantic waters. The estimated 1.3 to 28% of species that may have been introduced is not limited to inconspicuous species, rare species or species playing a minor role in coastal communities. Key species of coastal communities may have been introduced by ships centuries before the onset of biological surveys, but are now falsely viewed as native (Carlton 2003b). Known anthropogenic introductions of key structuring taxa are resulting in similar faunas on both sides of the Atlantic, blurring some of the key differences in assemblage composition and community organization (Jenkins *et al.* 2008). The perception of the natural state of the system has shifted: historical introductions are regarded as natural components of the studied community. Assigning species the cryptogenic status creates possibility for further studying the scale of historical invasions. A multidisciplinary approach, combining palaeoecological, archaeological and historical resources, as well as molecular techniques is essential in order to gain insight into the scale and consequences of marine invasions.

Table 6.1 Summarized numbers and relative numbers (between brackets) of species with disjunct-amphi Atlantic distribution patterns and numbers and relative numbers of introduced or cryptogenic species for all taxonomic groups.

	Ascidiacea	Hydrozoa	Boring Bivalvia	Epifaunal Bivalvia	Infaunal Bivalvia
Total # of species	184	397	25	149	299
Disjunct amphi-Atlantic	16 (8.7%)	68 (17.1%)	12 (48.0%)	11 (7.4%)	10 (3.3%)
Introduced	8 (4.3%)	7 (1.8%)	1 (4%)	2 (1.3%)	4 (1.3%)
Cryptogenic	3 (1.6%)	27 (6.8%)	6 (24%)	2 (1.3%)	0 (0%)
Introduced + Cryptogenic	11 (5.9%)	34 (8.6%)	7 (28%)	4 (2.6%)	4 (1.3%)

The cryptogenic ascidian *Molgula manhattensis*

In **Chapter 5** the history of a cryptogenic species was investigated using molecular tools. The ascidian *Molgula manhattensis* is one of the species with a disjunct amphi-Atantic distribution (Chapter 4); its disjunct distribution pattern cannot be explained by larval dispersal or rafting. Furthermore, *M. manhattensis* has a recent history of introductions (in Australia, Japan, Russia, the Mediterranean and Black Seas, and the Pacific coast of the USA). The possible vectors responsible for these introductions were oyster transports and hull fouling, *M. manhattensis* is a common fouling species on American oysters (*C. virginica*) and ship hulls. Like other sea-squirts, *M. manhattensis* is a poor natural disperser, larvae are very short-lived and the adults are sessile and are not known to raft. The disjunct distribution pattern in the North Atlantic Ocean can therefore not be explained by natural long-distance dispersal mechanisms. In order to

investigate whether the European and North American populations of *M. manhattensis* are both relicts of the Last Glacial Maximum (LGM), we analyzed variation in the mtDNA COI subregion and reconstructed *M. manhattensis*'s phylogeographic history by comparing haplotypes in samples from both coasts of the North Atlantic, as well as samples collected from known introductions in other parts of the world.

The comparison of haplotype diversity across the Atlantic shows a nearly three-fold higher diversity on the Northeast American side as compared with the European side. The Northeast American populations showed a latitudinal diversity gradient, which is consistent with recolonization after the LGM from a southern refugium, and possibly also a northern refugium. *M. manhattensis* probably survived the LGM in the Northwest Atlantic with its natural substrate, the American oyster. Oddly, although *M. manhattensis* is very common on American oysters, it has not been recorded from shells of the European oyster *Ostrea edulis* or the Pacific oyster *C. gigas*. European populations did not show a latitudinal diversity gradient, but all samples were consistently low in haplotype diversity.

In the introduced range, two patterns were found. In Japan and the Black Sea diversity was low, which is in accordance with the classical view of introduced populations. However, San Francisco Bay haplotype diversity levels were comparable to those on the Northeast American coast. This is probably due to high propagule pressure. The vector of introduction into San Francisco Bay was the transfer of millions of oysters, and single oysters may introduce a number of individuals of *Molgula*. In contrast, the populations in the Black Sea and Japan were most likely introduced as hull fouling, which typically involves fewer individuals in an introduction event, and may result in low-diversity introduced populations.

Private haplotypes, which are an indication for long-term residence (i.e. preceding the LGM) of a species, occurred in all regions, except Japan. The presence of private haplotypes in introduced populations was unexpected, but may be explained by undersampling of northeast American populations (where these introduced populations originated), and high propagule pressure.

M. manhattensis is clearly native on the northwest Atlantic coast. However, distinguishing between an anthropogenic introduction and post-glacial recolonization in European waters requires multiple lines of evidence. Empirical evidence based on life-history traits, preferred habitat and a patchy European distribution (*M. manhattensis* being mostly restricted to harbors and marinas) argue for an introduction of *M. manhattensis* from North America to Europe. If it was introduced to Europe, the vector of introduction would have been hull fouling, as the first records of *M. manhattensis* in Europe precede the first American oyster transfers to Europe, which took place in the 1870s (Carlton & Mann 1996; Wolff & Reise 2002). For most invertebrates, the European coast is more diverse in the number of species than the North American coast. Post-glacial recolonization of the North American coast occurred from Northwest Atlantic refugia, and from refugia in Europe via Iceland (Wares & Cunningham 2001; Vermeij 2005; Maggs *et al.* 2008). Dispersal and recolonization in the other direction has not been documented, nor are there examples of species that are demonstrated to have a

naturally disjunct amphi-Atlantic distribution (Chapter 4). Taken together, these arguments support an anthropogenic origin of *M. manhattensis* in Europe.

The genetic data, however, present some challenges to the life-history and distributional data. Low haplotype diversity could also be the result of the LGM and does not necessarily mean that the European populations were introduced. The haplotype network showed the presence of nested, high frequency putative American haplotypes in introduced populations, but these were absent from Europe, which is unexpected given that there would have been a high chance of their presence had European populations been introduced, suggesting native residence in Europe.

Resolving the question rests on two inter-related factors: the degree to which the total haplotype diversity was sampled; and the relative role of vectors and propagule pressure at a given location. More intensive sampling of the Northwest Atlantic would certainly reveal more low frequency haplotypes, which could include the putative private haplotypes found in Europe. More intensive sampling in Europe might reveal additional private haplotypes and possibly the missing medium-to-high frequency American haplotypes, though the latter is much less likely. If European populations had been introduced, the vector would have been hull fouling, which is a vector with low propagule pressure when compared to oyster transports (Chapter 3). This could explain the low diversity of European populations. While more intensive sampling of the Northwest Atlantic might help to resolve some of the issues around low-frequency private haplotypes, it would not explain the absence of the higher frequency haplotypes in Europe. Thus, in conclusion, we cannot say with certainty that *M. manhattensis* was introduced to Europe, and it remains a cryptogenic species in Atlantic Europe.

Conlusions

The aim of this thesis was to determine to which extent natural patterns of diversity and natural distributions have been affected by anthropogenic species introductions. This question has been approached from different points of view, with different techniques and on different scales and levels of diversity.

The checklist of introduced species in the North Sea and the analysis of oyster associated introductions showed that the number of non-indigenous species is increasing. In fact, the North Sea is one of the most invaded seas in the world, with a high percentage of potentially harmful non-indigenous species (64%, Molnar *et al.* 2008). The most important vectors for species introductions in this region are hull fouling and shellfish translocations. Although the invasion process is highly unpredictable, and there are many examples of invasions with negative ecological and economic impact, oyster translocations are still taking place within European waters. Moreover, oysters are not the only commercial bivalves that are imported and relaid as is shown for the Oosterschelde estuary in The Netherlands. *Mytilus edulis* seed is imported from other European countries for restocking Dutch mussel culture, and despite a risk analysis

(Wijsman & De Mesel 2009), at least two non-indigenous species, the Atlantic oyster drill *Urosalpinx cinerea* and the Manila clam *Ruditapes philippinarum*, have recently been introduced by mussel imports from the United Kingdom and Ireland (Faasse & Ligthart 2008; 2009). Since established introduced species in marine environments usually are almost impossible to eradicate, management efforts of introductions in the sea should aim at preventing invasions by management of vectors (e.g. by banning shellfish translocations), rather than at reducing the impact of established invasions.

Determining propagule pressure for a single vector may aid in these management efforts. Detailed knowledge of vector characteristics, in particular propagule pressure, has been shown to be essential in explaining patterns of diversity. High propagule pressure explained the high diversity in introduced *M. manhattensis* populations, and the analysis of oyster-associated introductions and the epiflora of oyster shells revealed that even small numbers of imported oysters can result in large numbers of associated introductions. This process was further facilitated by the establishment of the vector itself, the non-indigenous oyster *C. gigas*, in receiving waters, which serves as a substrate for an increasing number of non-indigenous species.

Although most invasions have not been studied, and many of those that have been studied have shown that invasions fundamentally alter community structure and functioning, the intentional introduction of non-indigenous species as a biodiversity conservation method is again being considered (e.g. Briggs 2008). Assisted colonization is highly debated, as we do not know enough about the potential impact of non-indigenous species introductions to do a thorough risk assessment and predict the possible outcome of such intentional introductions (Ricciardi & Simberloff 2009). Assisted colonization and invasive species are two of the fifteen most important issues that were identified to potentially affect the conservation of biological diversity in coming years (Sutherland *et al.* 2010).

By estimating the scale of cryptogenesis, it has been demonstrated that the number of invasions is indeed highly underestimated. After analyzing distributions, natural and anthropogenic dispersal potential of individual species in detail, many of the species that we consider native should in fact be labeled as cryptogenic species, until their status is cleared up by thorough multidisciplinary studies that include molecular data. However, the phylogeography of *M. manhattensis* shows that it is not always easy to get conclusive results using molecular techniques. Although we did show that undersampling may have been an explanation for some of the discrepancies found in this study, other studies, based on fewer individuals and populations sampled, draw conclusions that are much more far-reaching, and the possibility of anthropogenic dispersal is rarely considered. This has great consequences for our understanding of the rate of evolution in the sea, as undoubtedly introduced species are included in some of these studies which are assumed to be native. Genetic diversity patterns within species caused by anthropogenic dispersal and by natural causes (such as the effects of the LGM) can be strikingly similar.

One question remains to be answered: **What is natural?** When studying marine communities, we should not by default assume that those species that are present in

our coastal waters are native and we should accept the possibility that many of these have been introduced in the past. Only those species that are proven to be native have natural distributions. Marine bioinvasions have been altering biological communities for centuries, and will continue to do so in the future. Completely natural ecosystems do not exist along North Atlantic coasts.

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Dutch summary – Nederlandse samenvatting

Deniz Haydar

Mariene benthische ecosystemen zijn, zoals alle ecosystemen, voortdurend onderhevig aan verandering: er komen soorten bij en er verdwijnen soorten, de grootte en genetische diversiteit van populaties fluctueren en veranderende omstandigheden verkleinen of vergroten het potentiële areaal van soorten. De geografische distributie van de mariene benthische flora en -fauna wordt bepaald door tolerantie voor abiotische factoren, voorkeur voor habitat, en het vermogen van een soort om zich te verspreiden. In de geologische geschiedenis zijn geografische distributies sterk veranderd doordat landmassa's en zeeën ontstonden en weer verdwenen en het zeeniveau en de temperatuur fluctueerden. De biogeografie onderzoekt deze distributiepatronen en de manier waarop zij in de geologische geschiedenis zijn ontstaan. Op basis van huidige distributiepatronen, overeenkomsten en verschillen in soortensamenstelling van gebieden, de mate van endemisme en de aanwezigheid van geografische barrières zijn biogeografische provincies of regio's gedefinieerd. Deze biogeografische provincies liggen niet geheel vast; de huidige distributies van mariene organismen zijn nog altijd onderhevig aan verandering. Deze verandering kan natuurlijk zijn, maar kan tevens door de mens teweeg worden gebracht.
De mens heeft op verschillende manieren invloed op diversiteits- en distributiepatronen van mariene organismen. Eén van de manieren waarop natuurlijke patronen door de mens worden veranderd is de introductie van exoten. De mate waarin de natuurlijke diversiteit en distributies in de zee zijn beïnvloed door antropogene introducties van exotische soorten is het onderwerp van dit proefschrift.

Invasiebiologie

Enkele van de eerste biologen en natuurliefhebbers waren zich reeds bewust van de mogelijkheid van transport van soorten buiten hun natuurlijk verspreidingsgebied door menselijk handelen (zie bijvoorbeeld Darwin's Origin of Species). Invasiebiologie als tak van de biologie is echter relatief jong; de invloed van biologische invasies wordt pas sinds de jaren '50 algemeen erkend en er is pas sinds de jaren '80 ruim aandacht voor de introductie van exoten in zee. Sindsdien is er steeds meer publieke en wetenschappelijke belangstelling voor mariene bioinvasies. Biologische invasies worden nu gezien als een belangrijk gevolg van mondialisering en klimaatverandering en een bedreiging van de natuurlijke biodiversiteit.
De termen exoot, introductie, immigrant, bioinvasie, invasief en uitheems worden alle gebruikt om soorten aan te duiden die door menselijk handelen buiten hun natuurlijk areaal zijn verplaatst en zich elders hebben gevestigd. Invasieve soorten of bioinvasies worden vaak gedefinieerd als uitheemse soorten die zich niet alleen buiten hun natuurlijk verspreidingsgebied hebben gevestigd, maar zich vervolgens ook hebben ontwikkeld tot een plaag met negatieve ecologische of economische gevolgen. De definitie van "negatieve gevolgen" is echter niet eenduidig; daarnaast zijn de gevolgen van de meeste introducties niet bekend. Invasieve soort en bioinvasie worden in dit proefschrift als neutrale termen gebruikt; alle bovengenoemde termen beschrijven soorten

die zich door menselijk handelen buiten hun natuurlijk verspreidingsgebied hebben gevestigd.
Voor een soort zich elders succesvol kan vestigen, moet een aantal barrières worden overwonnen. Allereerst moet een organisme opgenomen worden door zich beschikbaar te maken voor een vector (transportmiddel). Deze opname is afhankelijk van kenmerken van het organisme zelf en van de vector, zo zullen bijvoorbeeld vissen niet in staat zijn zich te vestigen op de buitenkant van een schip. Na de daadwerkelijke opname door een vector moet het organisme het transport zien te overleven, en ook hier bepalen kenmerken van de vector (bijvoorbeeld de duur van het transport, de competitie met andere soorten, de abiotische factoren) en de kenmerken van het organisme zelf de overlevingskans tijdens het transport. Na aankomst in een nieuw gebied zal het organisme moeten overleven onder omstandigheden die heel anders kunnen zijn dan in het gebied van herkomst en voor een succesvolle introductie zal vervolgens het organisme of een deel daarvan zich moeten vrijmaken van de vector. Dit kan door zelf weg te zwemmen, kruipen of drijven, maar ook door te reproduceren. De omstandigheden in het gebied waar het organisme terecht is gekomen kunnen heel anders zijn dan in het gebied van herkomst en er moet een geschikte habitat voor het organisme aanwezig zijn. In je eentje een nieuwe populatie beginnen is meestal onmogelijk en de aanwezigheid van soortgenoten is dus vaak een vereiste voor een succesvolle eerste introductie. Tijdens het uitgroeien tot een grotere populatie die zich voor langere termijn vestigt speelt competitie met andere soorten om ruimte en voedsel een grote rol. Sommige soorten zijn zo succesvol in deze competitie dat zij uit kunnen groeien tot een plaag. Vaak is er sprake van een incubatietijd en duurt het even voordat een soort zich vestigt na een eerste introductie. Dit verklaart dat veel soorten pas worden waargenomen nadat zij al enige tijd geleden zijn geïntroduceerd. Slechts een deel van de soorten die verplaatst worden vestigen zich daadwerkelijk in een nieuw leefgebied; van deze succesvolle introducties ontwikkelt zich slechts een deel tot een plaag.

Wat bepaalt het succes van een introductie?

Het succes van een introductie is afhankelijk van kenmerken van het organisme zelf, van de vector en van het ontvangende gebied. Introducties blijken meer succes te hebben in verstoorde gebieden. De aanwezigheid van reeds gevestigde exoten vergroot de kans op vestiging van nieuwe exoten en de negatieve invloed van nieuwe en reeds geïntroduceerde exoten en kan er voor zorgen dat het proces zichzelf versnelt, wat in de literatuur wordt aangeduid als een “invasional meltdown”. Vele studies zijn gewijd aan het identificeren van kenmerken van soorten die hun succes als exoot verklaren. Helaas heeft dit niet geleid tot een algemene set van kenmerken die van toepassing zijn op alle exoten en gebruikt kunnen worden als voorspellers van invasiesucces. Fysiologische tolerantie, aanpassingsvermogen en vruchtbaarheid zijn belangrijk, maar zijn op zichzelf geen goede voorspellers. Alleen de mate waarin de habitat in donor- en ontvangstregio’s overeen komt, en een recente geschiedenis van succesvolle

invasies van de soort in andere gebieden zijn goede voorspellers van een succesvolle introductie. Een eigenschap van een vector die een goede voorspellende waarde heeft voor succesvolle introducties is het aantal individuen dat wordt geïntroduceerd: een groot aantal individuen is positief gecorreleerd met de vestigingskans. De genetische diversiteit van geïntroduceerde soorten vergroot op deze wijze ook het kolonisatiesucces, waarschijnlijk doordat een groter aantal geïntroduceerde genotypen aanpassing aan nieuwe omstandigheden faciliteert.
Samengevat is het succes van invasies moeilijk te voorspellen omdat het afhankelijk is van eigenschappen van organismen, vectoren en donor- en ontvangstgebieden, die allemaal veranderen in de tijd.

Vectoren

De kenmerken van de vector waardoor een soort wordt getransporteerd kunnen zoals gezegd bepalend zijn voor het succes van introducties. Daarnaast kunnen veranderingen in diversiteit en distributiepatronen van organismen verklaard worden door vector-eigenschappen. De belangrijkste activiteiten waardoor organismen worden verplaatst zijn scheepvaart en aquacultuur.
Schepen zijn al sinds eeuwen een belangrijke vector voor exoten. In de aangroei op de scheepshuid (hull-fouling) leven sessiele, borende en ook mobiele organismen. Hull-fouling is een probleem voor schepen omdat het de weerstand van het schip vergroot en zo de snelheid verlaagt, en vroeger werden schepen daarom regelmatig gekield en schoongeschraapt. Tegenwoordig worden schepen gemaakt van staal, wat de kans voor borende organismen aanzienlijk vermindert. Door de opkomst van gemotoriseerde vaartuigen is de snelheid van schepen toegenomen, wat ertoe leidt dat minder organismen het schip kunnen koloniseren en bij aankomst geïntroduceerd kunnen worden in een nieuw gebied. De kans voor organismen om van het schip afgespoeld te worden tijdens een zeereis neemt ook toe met hogere snelheden. Daarnaast zijn er tegenwoordig effectieve anti-fouling verven en andere behandelingen, die het aantal introducties als aangroei op de scheepshuid de laatste decennia hebben verminderd. Kleinere pleziervaartuigen, die zich voornamelijk op regionale schaal verplaatsen maar ook een relatief lage snelheid hebben, vormen nog altijd een belangrijke vector voor de primaire introductie en secundaire verspreiding van exoten. Na het verbieden van TBT-houdende verven (vanwege negatieve effecten op mariene organismen) en de verbeterde waterkwaliteit in havens zou deze vector in de toekomst wel weer op grotere schaal van invloed kunnen zijn.
Schepen die geen lading aan boord hebben gebruiken ballast om stabiel te blijven. Voor 1900 werd droge ballast gebruikt, die bestond uit stenen en zand die werden ingeladen in de haven van vertrek. In de haven van aankomst werd de ballast gedumpt, met alle organismen die er op of in zaten, wat heeft geleid tot de introductie van exoten. Sinds 1870 wordt in plaats van droge ballast ballastwater gebruikt. Ballastwater wordt ook opgenomen in de haven van vertrek en geloosd in de haven van aankomst, maar

vergeleken met droge ballast biedt het mogelijkheden voor een veel grotere variatie aan organismen om verplaatst te worden buiten hun areaal. Verschillende levensstadia van organismen uit alle phyla zijn aangetroffen in ballastwater en de condities in ballasttanks zorgen er voor dat de overlevingskans vrij hoog is, net als de overlevingskans na aankomst in een gebied waarvan de abiotische variabelen erg kunnen lijken op die in het donor-gebied. Ballastwater is op het moment één van de belangrijkste en meest risicovolle vectoren, aangezien er grote hoeveelheden water worden verplaatst en er dus enorme aantallen organismen meekomen. Er is dan ook veel aandacht voor het behandelen van ballastwater om introducties te voorkomen. De meest gangbare methode om het risico te verminderen is het verversen van ballastwater op open zee waar de organismen die aan de kust voorkomen een kleine overlevingskans hebben. De organismen die vervolgens worden opgenomen in open zee hebben waarschijnlijk op hun beurt een kleine kans om in de haven van aankomst te overleven en zich te vestigen.
Het transport van schelpdieren is een andere activiteit die een groot aantal introducties tot gevolg heeft gehad. Door overbevissing en de grote vraag naar schelpdieren voor de consumptie ontstond de behoefte om schelpdieren te importeren die vervolgens in het wild worden uitgezet. Op deze manier hebben zich niet alleen de uitheemse schelpdiersoorten gevestigd, maar ook de geassocieerde flora en fauna. Er worden volwassen schelpdieren geïmporteerd die een diversite epiflora en -fauna meebrengen. De meest frequent geïntroduceerde schelpdieren zijn oesters, vooral Japanse oesters, *Crassostrea gigas*, afkomstig uit de noordwestelijke Stille Oceaan. De Japanse oester komt nu wereldwijd voor en is de meest gekweekte consumptie-oester. Verplaatsingen van schelpdieren hebben op grote schaal plaatsgevonden in het verleden en zijn ook nu nog algemeen. Deze vector is verantwoordelijk voor introducties van exoten en de versnelde verspreiding van reeds gevestigde exoten in een gebied.
Er zijn nog vele andere vectoren, die op minder grote schaal opereren. Dit betekent echter niet dat zij geen grote invasies kunnen veroorzaken. Ter illustratie: de alg *Caulerpa taxifolia* "ontsnapte" uit een aquarium in Monaco in de Middellandse zee, waar een grote invasie het gevolg was. Het aantal vectoren dat organismen verplaatst neemt toe met de mondialisering en daarmee neemt ook het aantal introducties van exoten toe.

Toename van invasies

Wereldwijd zien we een toename van het aantal geïntroduceerde soorten, met name in de laatste 30 jaar. Deze toename wordt toegeschreven aan toegenomen handel en het daarmee samenhangende transport en de toename van het aantal vectoren. In slechts 17% van de mariene bioregio's zijn geen exoten gerapporteerd, wat echter zou kunnen liggen aan het feit dat deze regio's weinig onderzocht zijn. Sommige gebieden herbergen meer exoten dan andere en uit bepaalde gebieden zijn meer exoten afkomstig. Dit eenrichtingsverkeer van exoten kan verklaard worden door verschillende

factoren. Invasies vinden vooral plaats vanuit diverse naar minder diverse gebieden, zoals dat ook het geval was bij natuurlijke invasies in de geologische geschiedenis. De verklaring hiervoor kan gevonden worden in enerzijds een langere evolutionaire geschiedenis met als resultaat diverse, stabiele systemen en anderzijds systemen met een lage diversiteit en veel lege niches die kunnen worden ingenomen door exoten, waardoor het aandeel van exoten erg hoog kan zijn.

Gevolgen van exoten

De gevolgen van introducties kunnen op verschillende niveaus worden bestudeerd. Ecologische gevolgens van exoten kunnen de verandering van fundamentele processen zijn, zoals de kringloop van anorganische stoffen, primaire en secundaire productie, het verstoren van ecologische interacties, habitat verandering en competitie met inheemse soorten, met als resultaat een locale functionele extinctie van inheemse soorten. Het succes van exoten wordt toegeschreven aan de afwezigheid van natuurlijke vijanden (predatoren en parasieten) in het nieuwe gebied, en antropogene verstoring van ecosystemen, waardoor ze gevoeliger zijn voor invasies.
Exoten kunnen zich ontwikkelen tot een plaag en dan grote negatieve gevolgen hebben, bijvoorbeeld door te interfereren met visserij of inheemse soorten te overwoekeren. In de zee is het bijna onmogelijk om exoten te bestrijden als zij zich eenmaal hebben gevestigd. Dit ligt aan het feit dat mariene ecosystemen verschillen van terrestrische systemen in de levensstrategieën van organismen, de mogelijkheden voor verspreiding en aan het drie-dimensionale karakter van het milieu. Er zijn geen gevallen bekend van de extinctie van een soort in het mariene milieu door de introductie van een exoot en daarom is er discussie over de negatieve gevolgen van mariene bioinvasies. De meeste introducties lijken onschuldig en worden daarom vaak gezien als een toevoeging aan de lokale biodiversiteit. Er zijn echter veel voorbeelden van invasies met negatieve gevolgen en daarnaast zijn de meeste invasies niet bestudeerd en weten we dus niet wat de gevolgen zijn. We kunnen er daarom niet vanuit gaan dat mariene invasies positieve gevolgen hebben en biologische invasies worden algemeen gezien als een bedreiging van de biodiversiteit in de zee.

Onderschatting van het aantal exoten

Van de meeste invasies is niet alleen het gevolg onbekend, maar we onderschatten ook het aantal gevestigde introducties. Dat komt doordat nieuwe soorten niet altijd opgemerkt of gemeld worden en de geregistreerde exoten voornamelijk behoren tot goed bestudeerde taxonomische groepen. Kleine en minder bestudeerde groepen van organismen, zoals protisten, meiofauna en microalgen kennen weinig exoten. Ook wordt verondersteld dat alle kleine organismen zich ook zonder hulp van de mens over de hele wereld kunnen verspreiden en dus toch al overal voorkomen. Dit blijkt echter niet

zo te zijn: in het mariene milieu zijn diversiteitsgradiënten aangetoond in alle phyla. Daarnaast komen er in zee relatief veel cryptische soorten voor. Dergelijke organismen kunnen op basis van morfologische kenmerken niet van elkaar te onderscheiden zijn, maar met behulp van moleculair-genetische technieken kan worden aangetoond dat zij toch tot verschillende soorten behoren.
Een meer fundamentele kwestie heeft ook gezorgd voor de onderschatting van het aantal exoten. Over het algemeen wordt aangenomen dat soorten inheems zijn, tenzij er bewijs is dat ze zijn geïntroduceerd. Vanwege deze aanname worden de historische introducties, die plaats hebben gevonden voordat de eerste biologische studies werden uitgevoerd, over het hoofd gezien. Trans-Atlantische scheepvaart vindt al plaats sinds het jaar 1000, toen de Vikingen voor het eerst voet aan land zetten in Noord-Amerika. Na de (her)ontdekking van Amerika door Columbus kwam grootschalige scheepvaart op gang. De schepen uit die tijd hadden rijke fouling-gemeenschappen op en in de scheepshuid. Het is bekend dat er in die tijd soorten zijn geïntroduceerd, zoals de strandgaper *Mya arenaria* en de Portugese oester *Crassostrea angulata*. Dit zijn slechts twee voorbeelden, maar er moeten veel meer introducties hebben plaatsgevonden waarvan wij geen weet hebben. Dit zijn de cryptogene soorten van vandaag: soorten waarvoor geen bewijs is voor hun geïntroduceerde, noch voor hun inheemse status. De categorie van cryptogene soorten wordt veelal conservatief gebruikt, soorten worden als zodanig aangeduid als er een sterk vermoeden is van een introductie, maar niet als een introductie mogelijk zou kunnen zijn geweest. Als kenmerken van distributiepatronen en natuurlijke- en antropogene verspreidingsmechanismen zouden worden afgewogen, zouden lijsten van cryptogene soorten veel langer zijn. Op deze lijsten zouden ook de historisch geïntroduceerde soorten staan die over het hoofd zijn gezien. De onderschatting van het werkelijke aantal invasies heeft grote gevolgen voor onze kennis en begrip van niet alleen biologische invasies en hun consequenties, maar ook van evolutionaire mechanismen en biogeografie.

De centrale vraag die gesteld wordt in dit proefschrift is wat de werkelijke omvang en consequenties van mariene bioinvasies in gematigde kustwateren zijn.

Exoten in de Noordzee

De lengte van de gepresenteerde lijst met bekende geïntroduceerde en cryptogene soorten in de Noordzee uit Hoofdstuk 2 is verdubbeld ten opzichte van de eerdere publicatie van een vergelijkbare lijst (Reise *et al.* 1999). Deze toename van het aantal exoten is niet alleen het gevolg van een toename van het aantal introducties in de afgelopen tien jaar, maar ook van de toevoeging van geïntroduceerde en cryptogene soorten die niet eerder als zodanig geregistreerd waren. De paleologische geschiedenis van de Noordzee en de verstoring door de mens hebben ertoe geleid dat de Noordzee relatief soortenarm is, wat een verklaring zou kunnen zijn voor het grote aantal introducties. Een groot aantal exoten heeft zich permanent gevestigd, op die manier de

biodiversiteit vergroot en het functioneren van ecosystemen gewijzigd. De frequentie van introducties is toegenomen in de Noordzee, zoals deze ook wereldwijd is toegenomen en zal blijven toenemen als gevolg van klimaatverandering en mondialisering.
De meerderheid van de exoten heeft een lokale distributie en komt dus niet in de gehele Noordzee voor. Dit betekent echter niet dat dat zo zal blijven: scheepvaart en schelpdiertransporten vinden nog altijd op grote schaal plaats binnen dit gebied en kunnen resulteren in een snelle secundaire verspreiding van nu nog lokaal voorkomende exoten. De lijst met exoten die hier is gepresenteerd is een basis voor toekomstige studies en zal regelmatig moeten worden bijgewerkt met nieuwe soorten die arriveren en de verdere verspreiding van reeds gevestigde exoten.
Het aantal cryptogene soorten dat in de lijst is opgenomen is een onderschatting van het werkelijke aantal cryptogene soorten; alleen die soorten zijn opgenomen waarvoor er sterke aanwijzingen zijn dat zij in een deel van hun verspreidingsgebied geïntroduceerd zijn. Ter illustratie zijn de invasiegeschiedenis en de gevolgen van drie beruchte exoten met een grote invloed op ecosystemen in de Noordzee besproken. Voor de meerderheid van de exoten is de invloed op ecosystemen in de Noordzee onbekend.
De belangrijkste vectoren in de Noordzee zijn hull-fouling en schelpdiertransporten (voornamelijk oestertransporten), die elk verantwoordelijk zijn voor 25% van de gevestigde exoten.

Oesters als een vector

Oestertransporten worden in detail geanalyseerd in Hoofdstuk 3. Levende, volwassen oesters, met een rijke epiflora en –fauna, zijn in het verleden op grote schaal geïntroduceerd om lokale oesterbestanden aan te vullen of nieuwe oestercultuur op te starten na instorten van de kweek van inheemse oesters. In Nederland zijn verschillende uitheemse oestersoorten geïntroduceerd, waarvan alleen de Japanse oester, die voor het eerst werd geïntroduceerd in de jaren ’60, zich heeft gevestigd en nu algemeen voorkomt in onder andere de Oosterschelde en de Waddenzee. Met de verschillende soorten oesters, afkomstig uit verschillende gebieden, zijn vele exoten meegelift. Uit de literatuur is een lijst met oester-geassocieerde introducties in Nederlandse kustwateren samengesteld. Er zijn 35 bekende introducties die worden toegeschreven aan deze vector. Van deze 35 soorten is 45% afkomstig uit de noordwestelijke Stille Oceaan, waarvan de meeste hoogstwaarschijnlijk zijn geïntroduceerd met Japanse oesters, rechtstreeks vanuit Japan of via andere centra van schelpdiercultuur. 20% van de soorten is afkomstig uit de noordwest Atlantische Oceaan en is waarschijnlijk geïntroduceerd met de Amerikaanse oester, *Crassostrea virginica*. De afgelopen 30 jaar zien we een toename van het aantal introducties met oesters.
Deze toename in het aantal oester-geassocieerde exoten in de afgelopen 30 jaar komt overeen met de wereldwijde toename van introducties van exoten. Er is echter in de afgelopen dertig jaar geen toename geweest van oesterimporten. Sterker nog, sinds de jaren ’70 zien we een afname in de oesterimporten, vooral van oesterzaad (kleine

oesters die uitgezet worden op de percelen om daar te groeien). Niet alle oestertransporten blijken echter gerapporteerd te worden bij de autoriteiten, zo is er geen melding gemaakt van oesterimporten uit Japan of Canada in de jaren '60, hoewel we zeker weten dat deze hebben plaatsgevonden en verantwoordelijk zijn voor de invasie van de Japanse oester in Nederlandse kustwateren. Hoewel de geïmporteerde oesters dankzij de succesvolle introductie van de Japanse oester niet langer bedoeld zijn om te zaaien op de percelen, worden zij soms toch voor de verkoop verwaterd in de Oosterschelde of in bassins op de kant, wat de introductie van epiflora en fauna alsnog mogelijk maakt.

Succesvolle invasies zijn niet alleen afhankelijk van de hoeveelheden oesters die geïmporteerd worden, maar ook van het aantal individuen en het aantal soorten dat op een oester aanwezig is. Deze twee vectoreigenschappen worden samengevat in de term "propagule pressure". "Propagule pressure" wordt gedefinieerd als het aantal individuen dat tijdens één introductie wordt vrijgelaten, gecombineerd met het aantal introducties dat plaatsvindt. Het geeft een indicatie van de kracht van een vector en de daaropvolgende kans op succesvolle introductie van exoten. Hoe groter het aantal individuen per introductie en hoe meer introducties in de tijd, hoe groter de kans dat een exoot zich vestigt. De importgegevens en het overzicht van bekende oester-geassocieerde exoten laten zien dat grote hoeveelheden oesters niet per se resulteren in een groot aantal introducties. Daarom is de epiflora van schelpen van Japanse oesters van de kweekpercelen in de Oosterschelde nader bestudeerd. De Japanse oester is nu de belangrijkste kweekoester in de Oosterschelde en levende oesters worden geëxporteerd naar andere landen. De oesters die werden gemonsterd werden behandeld alsof zij getransporteerd zouden worden, waarna de epiflora van de schelpen werd verzameld. Op de oesterschelpen werden 41 verschillende macroalgen aangetroffen, waarvan er 36 op naam zijn gebracht. Onder deze macroalgen waren geïntroduceerde, cryptogene en ook inheemse soorten, maar van de meest voorkomende soorten was 50% geïntroduceerd. Een enkele oester kon tot 14 soorten algen op zijn schelp dragen, het aantal verschillende soorten algen op oesters in de Oosterschelde is geschat op 44. Op relatief kleine aantallen oesters (~500) kunnen relatief veel soorten en nog veel meer individuen voorkomen, wat zodoende zorgt voor een grote "propagule pressure" en verklaart waarom oestertransporten zoveel succesvolle introducties veroorzaken. De vestiging van exoten wordt vergemakkelijkt doordat ze geïntroduceerd worden met hun substraat en de groeiende Japanse-oesterbanken zorgen voor een verdere toename van de kans van vestiging.

Oesterimporten hebben in het verleden een grote rol gespeeld bij de introductie van exoten, zelfs voor wij daar weet van hadden. Oestertransporten binnen Europa vonden al plaats voor de 19e eeuw en met deze transporten kunnen exoten zijn geïntroduceerd die nu als inheems worden beschouwd. Ook nu vindt transport van oesters nog plaats, wat niet alleen zorgt voor een snelle verspreiding van exoten, maar ook uitwisseling tussen populaties van inheemse en cryptogene soorten mogelijk maakt. Dit resulteert in een verdere homogenisering van de diversiteit in de zee.

Cryptogene soorten in de Noord-Atlantische Oceaan

De in Hoofdstuk 2 gepresenteerde lijst van geïntroduceerde en cryptogene soorten in de Noordzee is een onderschatting van het werkelijke aantal cryptogene soorten. In Hoofdstuk 4 van dit proefschrift wordt daarom een meer realistische schatting gemaakt van het aantal cryptogene soorten in de Noord-Atlantische Oceaan. In de Noord-Atlantische Oceaan heeft de laatste ijstijd, die duurde van 116.000 tot 11.500 jaar geleden, een belangrijke invloed gehad op patronen van diversiteit en distributie van organismen. Tijdens de koudste periode van de laatste ijstijd, zo'n 21.000 jaar geleden, was een groot deel van Noord-Amerika, Europa en de Noord-Atlantische Oceaan door een ijskap bedekt. De Noordzee lag droog en het grootste deel van de Noord-Atlantische Oceaan was te koud voor gematigde soorten om te kunnen overleven. Deze soorten trokken zich terug in refugia, ijsvrije gebieden, waar zij in relatief kleine populaties overleefden en vanwaar zij hun huidige areaal koloniseerden (zie Fig. 1.2). Aan de Amerikaanse kust waren met name voor hard-substraatsoorten minder mogelijkheden om te overleven tijdens de laatste ijstijd. Ten zuiden van Cape Hatteras komen weinig rotskusten voor en er waren daar daarom weinig refugia vergeleken met de Europese kust. Daarnaast was en is de latitudinale temperatuurgradiënt aan de Amerikaanse oostkust veel scherper dan in Europa, wat betekent dat er een minder groot potentieel verspreidingsgebied is voor gematigde soorten. Aan de Europese kust is de diversiteit daardoor groter, en het wordt aangenomen dat de kolonisatie van de Amerikaanse kust niet alleen vanuit lokale refugia plaatsvond, maar ook vanuit refugia aan de overkant van de Atlantische Oceaan, wat zou kunnen resulteren in een disjunct (onderbroken) verspreidingspatroon.

Eén van de kenmerken van cryptogene soorten zou een disjuncte distributie kunnen zijn; daarom wordt dit hier werd gebruikt als een indicator voor een cryptogene status. Een disjuncte amfi-Atlantische distributie wordt gekenmerkt door de aanwezigheid van een organisme aan Europese- en Amerikaanse Atlantische kusten, maar afwezigheid in het arctische of sub-arctische gebied. Een disjunct amfi-Atlantisch distributiepatroon kan worden verklaard door vier scenario's: natuurlijke trans-oceanische verspreiding, kolonisatie na de laatste ijstijd, cryptische speciatie en introductie door de mens. De waarschijnlijkhied van de vier scenario's werd onderzocht door lijsten met alle bekende soorten uit ondiepe kustwateren van de Atlantische Oceaan van drie goed bestudeerde taxonomische groepen van evertebraten samen te stellen op basis van de literatuur. Voor elke soort werd niet alleen de wereldwijde distributie genoteerd, maar ook de natuurlijke verspreidingsmechanismen, voorkeur voor habitat en associatie met antropogene vectoren. De drie groepen waar het om gaat zijn de Ascidiacea (zakpijpen), Hydrozoa (hydroïdpoliepen) en Bivalvia (tweekleppige schelpdieren). Deze groepen werden gekozen op basis van verschillen in verspreidingsmechanismen. Ascidiacea kunnen zich slechts over een kleine afstand verplaatsen: het larvale stadium van soorten in deze groep is extreem kort. Hydrozoa hebben een groot vermogen tot natuurlijke verspreiding door hun ingewikkelde levenscyclus: ze hebben pelagische larven, sommige soorten hebben een vrij-zwemmend stadium in de vorm

van een medusa (kwal) en ze kunnen in staat zijn om in het poliepenstadium drijvende substraten te koloniseren en daarop mee te liften. Bivalvia hebben een langdurig larvaal stadium. Deze groep kan verder worden opgedeeld in drie groepen die verschillen in hun habitat en verspreidingsmechanismen. De ingegraven bivalven kunnen zich alleen verplaatsen in de larvale fase. De epifaunale- en borende bivalven kunnen zich daarnaast ook vestigen op natuurlijke vlotten. Met uitzondering van de ingegraven bivalven hebben alle groepen de potentie om zich te verspreiden als aangroei op schepen. De ingegraven bivalven worden daarom als controlegroep gebruikt: zij verplaatsen zich alleen in het larvale stadium. De enige antropogene vector waarmee zij geïntroduceerd zouden kunnen worden is ballastwater. Aangezien ballastwater pas sinds 1870 in gebruik is, wordt aangenomen dat alle introducties van ingegraven bivalven recent en bekend zijn. Het aandeel van cryptogene en geïntroduceerde soorten is vergeleken tussen de groepen en op basis van de kenmerken van de cryptogene soorten en literatuuronderzoek is de waarschijnlijkheid van de vier scenario's in de verklaring van de disjunct amfi-Atlantische distributiepatronen bepaald.

De ingegraven bivalven hebben het kleinste aandeel in de soorten met disjunct amfi-Atlantische distributies en er zijn geen cryptogene ingegraven bivalven. De disjuncte distributies van ingegraven bivalven kunnen alle worden verklaard door introductie door de mens, of door andere factoren zoals een distributie die ook diepe of warmere wateren omvat. Voor de Hydrozoa blijkt het hebben van een vrijzwemmende medusa in de levenscyclus geen garantie te geven voor een wijde of disjuncte verspreiding. Regionale verspreiding van Hydrozoa op natuurlijke vlotten is mogelijk, maar is geen verklaring voor de disjunct amfi-Atlantische verspreidingspatronen. Daarnaast zijn de Hydrozoa die zich kunnen vestigen op natuurlijke drijvende substraten ook in staat schepen te koloniseren. Verspreiding over grotere afstanden op schepen is effectiever dan op natuurlijke susbtraten: schepen reizen relatief snel, zijn onafhankelijk van zeestromingen en overbruggen grotere afstanden. Natuurlijke verspreiding door larven of op natuurlijke vlotten leidt niet tot disjunct amfi-Atlantische distributies.

In alle groepen kan cryptische soortvorming sommige disjunct amfi-Atlantische verspreidingspatronen verklaren. Nader onderzoek is noodzakelijk om te bepalen of het in deze gevallen inderdaad om een soortencomplex gaat.

De kolonisatie na de laatste ijstijd zou op alle groepen een even groot effect moeten hebben gehad dat in alle groepen resulteerde in disjunct amfi-Atlantische distributies. Er zijn echter geen cryptogene ingegraven bivalven met een disjunct amfi-Atlantische distributie, hoewel je dat wel zou verwachten als de invloed van de laatste ijstijd even groot zou zijn als voor de andere groepen. Daarnaast zijn er geen studies die voor een soort uit één van de onderzochte groepen aantonen dat het verspreidingspatroon het resultaat is van natuurlijke processen tijdens en na de laatste ijstijd. Er zijn geen duidelijke voorbeelden van natuurlijke disjuncte verspreidingen in de Noord-Atlantische Oceaan die kunnen worden onderbouwd met paleobiologische, moleculaire en historische gegevens.

Disjunct amfi-Atlantische distributies zijn niet algemeen: 1 op de 10 soorten (108 soorten van het totaal van 1054 soorten van alle groepen bij elkaar) heeft een disjuncte

distributie. Ongeveer de helft van deze soorten is cryptogeen of geïntroduceerd. De andere helft bestaat uit soorten die geen strikt amfi-Atlantische distributie hebben: ze komen ook voor in diepe of warmere wateren, zijn eigenlijk soortencomplexen, hebben disjuncte distributies die zijn gebaseerd op enkele waarnemingen of hebben een onzekere taxonomische status. Voor 8 soorten van het totaal is geen reden gevonden om aan te nemen dat zij binnen een van de hierboven genoemde categorieën vallen en lijkt de disjuncte distributie natuurlijk te zijn.
Het aandeel van disjunct amfi-Atlantische distributies verschilt tussen de groepen, en varieert van 3% tot 48%. Het relatieve aantal cryptogene en geïntroduceerde soorten varieert van 1.3% tot 28%. Deze grote spreiding tussen groepen is te wijten aan de verschillen in eigenschappen van de groepen, die juist om deze reden waren gekozen. Voor de epifaunale groepen varieert het aantal cryptogene soorten tussen 1.3 en 24%. Dit betekent dat er minstens 38 historische invasies over het hoofd zijn gezien. Als we dit extrapoleren naar alle benthische evertebraten en algen in de Noord-Atlantische Oceaan, dan zijn er honderden soorten die geïntroduceerd zouden kunnen zijn. Dit is nog altijd een conservatieve benadering van het werkelijke aantal introducties: we hebben alleen gekeken naar soorten met een disjunct amfi-Atlantische distributie, maar ook in de andere distributiecategorieën kunnen soorten zitten die cryptogeen of geïntroduceerd zijn.
Dit zijn niet allemaal onopvallende, onbelangrijke of zeldzame organismen. Ook soorten die een belangrijke rol spelen in ecosystemen kunnen abusievelijk als inheemse biota worden gezien. Onze perceptie van de natuurlijke staat van de zee is verschoven: historische introducties worden gezien als natuurlijke componenten van ecosystemen. Verder onderzoek aan cryptogene soorten waarbij paleobiologie, archeologie en moleculair genetische technieken worden gecombineerd is essentieel voor het bepalen van de werkelijke omvang en gevolgen van mariene bioinvasies.

Introductie of relict van de ijstijd?

De zakpijp *Molgula manhattensis* is één van de soorten uit Hoofdstuk 3 waarvan disjunct amfi-Atlantische distributie niet verklaard kan worden door natuurlijke verspreiding van de larven of adulten. Daarnaast heeft *M. manhattensis* een recent verleden van wereldwijde introducties in onder andere San Francisco Bay, Japan en de Zwarte Zee. De mogelijke vectoren voor deze introducties zijn aangroei op schepen en oestertransporten; *M. manhattensis* komt algemeen voor in fouling gemeenschappen op schepen en op Amerikaanse oesters. Om uit te zoeken of *M. manhattensis* in de Noord-Atlantische Oceaan als resultaat van de laatste ijstijd een onderbroken distributie heeft, of dat er menselijke factoren in het spel zijn, is een deel van het mitochondrieel DNA van individuele zakpijpen gesequenced. Deze DNA fragmenten worden op basis van de verschillen tussen de individuele sequenties in haplotypen gegroepeerd. Haplotypen zijn DNA-sequenties die in minstens één basenpaar van elkaar verschillen. De haplotypen-diversiteit van populaties afkomstig van beide zijden van de Atlantische

Oceaan en uit gebieden waar *M. manhattensis* is geïntroduceerd werd vergeleken.
De diversiteit is aan de noordoost Amerikaanse kust drie keer zo groot als aan de Europese kust. Daarnaast is er in Amerika een latitudinale gradiënt in diversiteit aanwezig: de diversiteit neemt toe van noord naar zuid. Dit is conform de verwachting indien sprake is van herkolonisatie sinds de laatste ijstijd uit een refugium in het zuiden; waarschijnlijk heeft *M. manhattensis* de laatste ijstijd in Noord Amerika overleefd met zijn substraat, de Amerikaanse oester. In Europese wateren komt *M. manhattensis* niet voor op oesters. De diversiteit in haplotypen is in Europa uniform laag.
In de geïntroduceerde populaties waren twee patronen te zien. In Japan en de Zwarte Zee was de diversiteit laag. Dit klopt met het klassieke idee dat geïntroduceerde populaties het resultaat zijn van vestiging van een klein aantal individuen, waardoor populaties ontstaan met lage genetische diversiteit. In San Francisco Bay werden echter zeer hoge aantallen haplotypen aangetroffen: de diversiteit was vergelijkbaar met die in de meest diverse noordoost-Amerikaanse populaties. Dit kan verklaard worden door de vectoren waarmee *M. manhattensis* in de verschillende gebieden is geïntroduceerd. De introductie in San Francisco Bay is het resultaat van grootschalige oestertransporten van de Amerikaanse oostkust. Miljoenen levende oesters, die een rijke epifauna droegen, werden verplaatst. Zodoende zijn er grote aantallen *M. manhattensis* individuen geïntroduceerd met hun substraat, wat heeft geresulteerd in een hoge genetische diversiteit in de huidige populatie.
Het voorkomen van private haplotypen (haplotypen die maar in één populatie zijn gevonden) is een indicatie voor een lange geschiedenis in een bepaald gebied, in dit geval al sinds voor de laatste ijstijd. De verwachting was daarom dat private haplotypen niet in geïntroduceerde populaties voor zouden komen, maar dit was niet het geval: in alle populaties (behalve Japan) werden private haplotypen aangetroffen. Dit kan verklaard worden door een te klein aantal individuen dat gesequenced is van de noordoost-Amerikaanse kust: de private haplotypen van de geïntroduceerde populaties zouden daar wel aanwezig zijn, maar zijn in de monsters niet aangetroffen omdat de diversiteit in dat gebied zo groot is.
Het is evident dat *M. manhattensis* van nature aan de noordoost-Amerikaanse kust voorkomt. Het is helaas niet zo gemakkelijk om uit de data af te leiden of de Europese populaties natuurlijk of geïntroduceerd zijn. De habitatvoorkeur en distributie van *M. manhattensis* suggereren een antropogene introductie in Europa: *M. manhattensis* komt in Europa niet op natuurlijke substraten maar vooral op artificiële substraten voor en de Europese distributie is onregelmatig. De vector waarmee *M. manhattensis* geïntroduceerd zou kunnen zijn is scheepvaart aangezien de eerste waarnemingen van *M. manhattensis* dateren van voor de tijd dat Amerikaanse oesters in Europa werden geïmporteerd.
Voor de meeste evertebraten is de genetische diversiteit in Europa groter dan aan de Amerikaanse oostkust. De richting van trans-oceanische herkolonisatie na de laatste ijstijd was van Europa naar Amerika, en niet de andere kant op, zoals hier het geval zou moeten zijn, wat een introductie in Europa ondersteunt.
De genetische data laten echter wat discrepanties zien. Lage genetische diversiteit kan ook een resultaat zijn van expansie na de laatste ijstijd en hoeft niet altijd het gevolg te

zijn van een introductie. Daarnaast waren veel voorkomende Amerikaanse haplotypen niet aanwezig in Europese populaties, hoewel dat wel volgens verwachting zou zijn bij een introductie. De kans is groot is dat veelvoorkomende haplotypen geïntroduceerd worden. Dit kan niet verklaard worden door het nemen van te weinig monsters in Amerika en is dus een argument voor de inheemse status van *M. manhattensis* in Europa. Op basis van deze gegevens is er geen eenduidig antwoord te geven op de vraag of *M. manhattensis* in Europa is geïntroduceerd of er van nature voorkomt. *M. manhattensis* blijft voorlopig een cryptogene soort in de Noordoost-Atlantische Oceaan.

Conclusies

Het doel van dit proefschrift was te bepalen wat de werkelijke omvang en consequenties van mariene bioinvasies in gematigde kustwateren zijn. Deze vraag is vanuit verschillende invalshoeken benaderd, met verschillende technieken en op verschillende niveaus van diversiteit.

Het aantal geïntroduceerde soorten in de Noordzee is de laatste decennia toegenomen. Wereldwijd is de Noordzee één van de koplopers op het gebied van het aantal exoten dat voorkomt in een regio en een groot deel van de exoten in de Noordzee is mogelijk schadelijk. De belangrijkste vectoren zijn oestertransporten en scheepvaart. Hoewel invasies onvoorspelbaar zijn en er veel voorbeelden zijn van introducties met nadelige ecologische en economische gevolgen, vinden transporten van levende oesters nog steeds plaats binnen Europa. Daarnaast wordt in Nederland mosselzaad geïmporteerd vanuit andere Europese landen om verder te kweken op percelen in de Waddenzee en de Oosterschelde. Ondanks een voorafgaande risico-analyse zijn door die transporten onlangs twee roofslakken in Nederlandse wateren geïntroduceerd. Het voorkomen van dit soort invasies is slechts mogelijk door vroeg in te grijpen in het invasieproces, bijvoorbeeld door het verbieden van schelpdiertransporten.

Het bepalen van "propagule pressure" van een vector helpt bij het maken van plannen voor beheer en is essentieel voor het verklaren van diversiteitspatronen. De verschillen in diversiteit van de geïntroduceerde *M. manhattensis* populaties waren te verklaren door verschillen in "propagule pressure" van de vectoren die de invasies veroorzaakt hebben. De analyse van de oester-epiflora liet zien dat kleine aantallen geïmporteerde oesters een groot aantal introducties kunnen veroorzaken. Deze grote aantallen introducties met oesters in de Oosterschelde werden vergemakkelijkt door de vestiging van de Japanse oester in Nederlandse wateren. De Japanse oesterbanken vormen een substraat voor een toenemend aantal geïntroduceerde soorten.

De analyse van cryptogene soorten in de Noord-Atlantische Oceaan toont aan dat het aantal invasies inderdaad ernstig is onderschat. Vele soorten die wij als inheems beschouwen zouden eigenlijk cryptogeen genoemd moeten worden, totdat hun status

opgehelderd is door middel van multidisciplinaire studies. Dit blijkt niet altijd eenvoudig, zelfs met moleculair-genetische technieken hebben wij de status van *M. manhattensis* in de Noord-Atlantische Oceaan niet geheel op kunnen helderen.

Wat is er dan wel natuurlijk? We moeten niet langer per definitie aannemen dat soorten inheems zijn, maar de mogelijkheid in het achterhoofd houden dat ze in het verleden geïntroduceerd zouden kunnen zijn. Alleen die soorten waarvoor er bewijs is dat ze van nature aan een bepaalde kust voorkomen hebben natuurlijke distributies. Door de mens veroorzaakte mariene bioinvasies veranderen ecosystemen al eeuwenlang en zullen dat in de toekomst blijven doen. Natuurlijke ecosystemen bestaan niet in de Noord-Atlantische Oceaan.

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