Population genetics and morphometric variation of blue mussels in the western Baltic Sea

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Master Thesis
in the subject 'Biological Oceanography'

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October 2012

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1 Abstract

The investigation of hybridizing taxa can provide intriguing insights into the process of speciation and the adaptive potential of populations to environmental change. Due to its gradual nature in terms of ecology and genetics, the European blue mussel hybrid zone between North Sea *Mytilus edulis* and Baltic *M. trossulus* is an ideal study system to analyse the significance of inter-specific hybridization in evolution. Although much research has already focussed on the genetic and phenotypic structures in the Baltic Proper, less is known about the situation in the western transition area.

By means of a multi-locus genotypic assessment and multivariate morphometric analyses I have examined the genetic and morphological constitutions of several Baltic *Mytilus* populations. Paying special attention to the poorly resolved outer parts, I find that most blue mussels in the western Baltic Sea and Skagerrak are higher generation backcross hybrids (99 % in the Kiel Fjord; 81 % in Tjärnö). While my results suggest that gene variants of *M. edulis* still prevail over that of *M. trossulus* in these mytilids, they also demonstrate that a minority of individuals in the Kiel Fjord are introgressed by *M. galloprovincialis* alleles. Although the origin of these alien polymorphisms cannot be told from my data, I postulate that their movement into the Baltic gene pool is most likely human-mediated (e.g. ship traffic). Considering the increasing records of anthropogenic introductions of *Mytilus* species to non-native habitats and the limited power of the applied molecular markers to discriminate *M. galloprovincialis*, it is to expect that more cryptic invasions are detected in future, technically advanced investigations.

My analysis of *M. edulis* allele frequency changes from the North Sea to the Baltic Proper extends and supports an earlier work by Stuckas et al. (2009), providing evidence for discordant patterns of gene flow across the hybrid zone. These discrepancies probably arise from the concerted action of direct selection, genetic hitchhiking, stochastic evolutionary forces and shifts in the geographical position of the secondary contact area. Different to this previous study and in line with the observations by Kijewski et al. (2006), I find that introgression into the central Baltic is restricted at least for some *M. edulis* maternal haplotypes (D-loop), indicative of cytonuclear incompatibilities between inner and outer Baltic mussels.

While differential environmental pressures might contribute to the maintenance of semi-permeable genetic barriers between *M. edulis*-like and *M. trossulus*-like hybrid populations, they can also account for the observed dissimilarities in shell morphology and phenotypic diversity. In contrast to individuals of the Baltic Proper, which express a

population-specific, *M. trossulus*-like morphotype, mussels of the transition zone build a morphological continuum between parental forms. Following the reasoning by Gardner (1996) I propose that the strong environmental variability in the western Baltic selects for multiple phenotypes, while the constant and extreme conditions (e.g. low salinities, absence of predators) in the eastern Baltic favour only a single shell morphotype. It remains to be shown whether these opposing patterns are consequences of environmentally-induced discrepancies in genetic variability at fixed causal loci (level of heterozygosity, selection for multiple alleles) and/or differences in phenotypic plasticity.

2 Zusammenfassung

Die Untersuchung von hybridisierenden Taxa kann nicht nur wichtige Einblicke in Artbildungsprozesse, sondern auch die Adaptationsfähigkeit von Populationen geben. Aufgrund ihrer graduellen genetischen und ökologischen Natur bietet die nordeuropäische Miesmuschelhybridzone zwischen *Mytilus edulis* und Baltischer *M. trossulus* ein ideales Studiensystem, um die evolutionäre Rolle von inter-spezifischer Hybridisierung zu analysieren. Während bereits viel über die genetische und phänotypische Struktur in der inneren Ostsee bekannt ist, ist die westliche Transitionszone weniger gut erforscht.

Mithilfe einer Multilocus-Genotypisierung und multivariaten morphometrischen Analysen habe ich die genetische und morphologische Konstitution verschiedener baltischer *Mytilus*-Populationen untersucht. Indem ich verstärkt auf den Status in den äußeren Regionen eingehe, kann ich zeigen, dass Miesmuscheln in der westlichen Ostsee und dem Skagerrak mehrheitlich Rückkreuzungshybride höherer Generation sind (99 % in der Kieler Förde; 81 % in Tjärnö). Meine Resultate deuten darauf hin, dass Genvarianten von *M. edulis* gegenüber denen von *M. trossulus* vorherrschen, wobei einige Individuen aus der Kieler Förde in geringem Ausmaß Introgression durch *M. galloprovincialis*-Allele aufweisen. Obwohl meine Daten keinen Rückschluss auf den Ursprung dieser nicht-nativen Polymorphismen zulassen, ist es wahrscheinlich, dass menschliche Aktivitäten, wie starker Schiffsverkehr, den Alleleintrag begünstigt haben. Wenn man die zunehmenden, durch Menschen verursachten Translokationen von Miesmuscheln in fremde Habitate bedenkt und berücksichtigt, dass die hier verwendeten molekularen Marker nur begrenzt *M. galloprovincialis*-Allele diagnostizieren konnten, kann davon ausgegangen werden, dass in zukünftigen, technisch fortgeschrittenen Studien weitere kryptische Invasionen detektiert werden.

Meine Untersuchungen über Änderungen der *M. edulis*-Allelfrequenzen zwischen Nordsee und innerer Ostsee bestätigen und erweitern die bereits durch Stuckas et al. (2009) aufgezeigten diskordanten Introgressionsmuster. Diese sind vermutlich das Ergebnis der simultanen Wirkung verschiedener evolutionärer Kräfte, wie direkte und indirekte Selektion, Gendrift sowie geographische Verschiebungen der Hybridzone. Im Gegensatz zu dieser früheren Arbeit und in Übereinstimmung mit den Beobachtungen durch Kijewski et al. (2006) zeigen meine Analysen, dass der Genfluss zumindest einiger mütterlich vererbter *M. edulis* Haplotypen (D-loop) in die zentrale Ostsee limitiert ist, was auf cyto-nukleare Inkompatibilitäten zwischen Mytiliden der westlichen und östlichen Regionen schließen lässt.

Während differenzielle Umweltbedingungen zur Aufrechterhaltung der semi-permeablen Barriere zwischen *M. trossulus*- und *M. edulis*-ähnlichen Hybridpopulationen

beitragen dürften, können sie ebenso die beobachteten Unterschiede in Schalenmorphologie und phänotypischer Diversität erklären. Anders als Individuen der inneren Ostsee, welche durch einen distinkten, *M. trossulus* vergleichbaren Morphotyp definiert sind, bilden Muscheln der Transitionszone ein morphologisches Kontinuum zwischen parentalen Arten. In Einklang mit der Hypothese Gardners (1996) schlussfolgere ich, dass die starke Umweltvariabilität in der westlichen Ostsee für mehrere Phänotypen selektiert, wohingegen die konstanten und extremen Bedingungen (z.B. niedrige Salinität, geringer Prädationsdruck) in den östlichen Regionen nur einen bestimmten Schalenmorphotyp zulassen. Für weiterführende Forschungen wird es herauszufinden bleiben, ob diese Gegensätze die Folgen von Umwelt-induzierten Differenzen in genetischer Variabilität an fixierten, kausalen Loci (Heterozygotie-Grad, Selektion von multiplen Allelen) sind und/oder durch unterschiedliche phänotypische Plastizität hervorgerufen werden.

3 Introduction

Due to their ambivalent role in biodiversity dynamics hybrid zones are of considerable interest for ecological and evolutionary research. While interspecific hybridization has the potential to increase diversification through hybrid speciation and reinforcement of reproductive isolation, it can also lead to species collapse by amalgamation of formerly distinct genetic material (Barton & Hewitt 1985; Arnold 1992; Rhymer & Simberloff 1996; Allendorf et al. 2001; Seehausen 2004; Mallet 2005; Seehausen et al. 2008). Not infrequently such reverse speciation events are consequences of human-mediated habitat homogenizations or species invasions (Rhymer & Simberloff 1996; Allendorf et al. 2001; Mallet 2005; Seehausen et al. 2008; Vonlanthen et al. 2012). Although hybrid zones should therefore be transitory stages, they can be stabilized, if hybrids experience a fitness advantage in a specific habitat (bounded hybrid superiority) or when extrinsic or intrinsic hybrid incompatibilities are balanced by parental dispersal (dynamic equilibrium in a tension zone) (Barton & Hewitt 1985; Arnold 1992). Seemingly contradicting each other, these cases are not mutually exclusive, as the type of selection can vary in time and space (Bert & Arnold 1995; Shields et al. 2008).

Apart from disintegrating genetic barriers, intergradation between two taxa often manifests itself in the erosion of morphological, eco-physiological and behavioural features as well as environmentally induced reaction norms that typify the parental lineages – with varying impacts on hybrid performance (e.g. Gardner 1996; Collins & Goldsmith 1998; Long et al. 1998; Repka et al. 1999; Silim et al. 2001; Seehausen 2004; Braby & Somero 2006a; Seiler & Keeley 2007; Beaumont et al. 2004, 2008; Penney et al. 2006, 2007, 2008; Gardner & Thompson 2009; Morris et al. 2011; Page et al. 2011). Analysing the direct consequences of interbreeding and the mechanisms that maintain species identities despite inter-specific gene flow is thus crucial for understanding diversification, but might likewise illuminate the molecular basis of adaptability to environmental perturbations (Seehausen 2004; Mallet 2005) – insights that will be essential given the increasing anthropogenic pressures (e.g. climate warming, ocean acidification, habitat destruction) on marine ecosystems (IPCC Fourth Assessment Report 2007; Fabry et al. 2008; Doney et al. 2009).

Characterized by a unique genetic structure and strong horizontal gradients in salinity conditions (Voipio 1981; Bonsdorff & Pearson 1999; Leppäranta & Myrberg 2009), the Baltic hybrid zone between the blue mussel species *Mytilus edulis* and *Mytilus trossulus* represents a promising model system for studying the functional and evolutionary implications of hybridization. Whereas populations in the marine Kattegat/Skagerrak are

believed to have a predominant *M. edulis* background with limited movement of *M. trossulus* alleles (mainly at locus EFbis) into the gene pool, pervasive, albeit asymmetric genetic exchange has prompted the formation of a *M. trossulus*-like hybrid swarm in the oligohaline eastern Baltic parts (Bierne et al. 2003b; Riginos & Cunningham 2005; Kijewski et al. 2006, 2011; Stuckas et al. 2009; Väinölä & Strelkov 2011; Figure 1). Even if it has been suggested that Baltic mussels should be considered as an own taxonomic unit (i.e. hybrid species) (Väinölä & Strelkov 2011), this pattern is in so far unusual, as it does not correspond to the conventional picture of contact zones, where the region of hybridization is delineated by pure populations of parental species (Barton & Hewitt 1985).

Introgression of *M. edulis* alleles into Baltic populations is particularly extensive for (some) neutral nuclear markers, but restricted for several allozymes that either confer (secondary) adaptation to the prevailing salinity regimes or are linked to the respective loci (Bulnheim & Gosling 1988; Johannesson et al. 1990; Väinölä & Hvilsom 1991; Borsa et al. 1999; Riginos & Cunningham 2005; Johannesson & André 2006; Kijewski et al. 2006, 2011; Stuckas et al. 2009). Likewise, genes involved in sexual reproduction, such as the acrosomal sperm protein M7 Lysin (Stuckas et al. 2009), are less able to cross the species barrier.

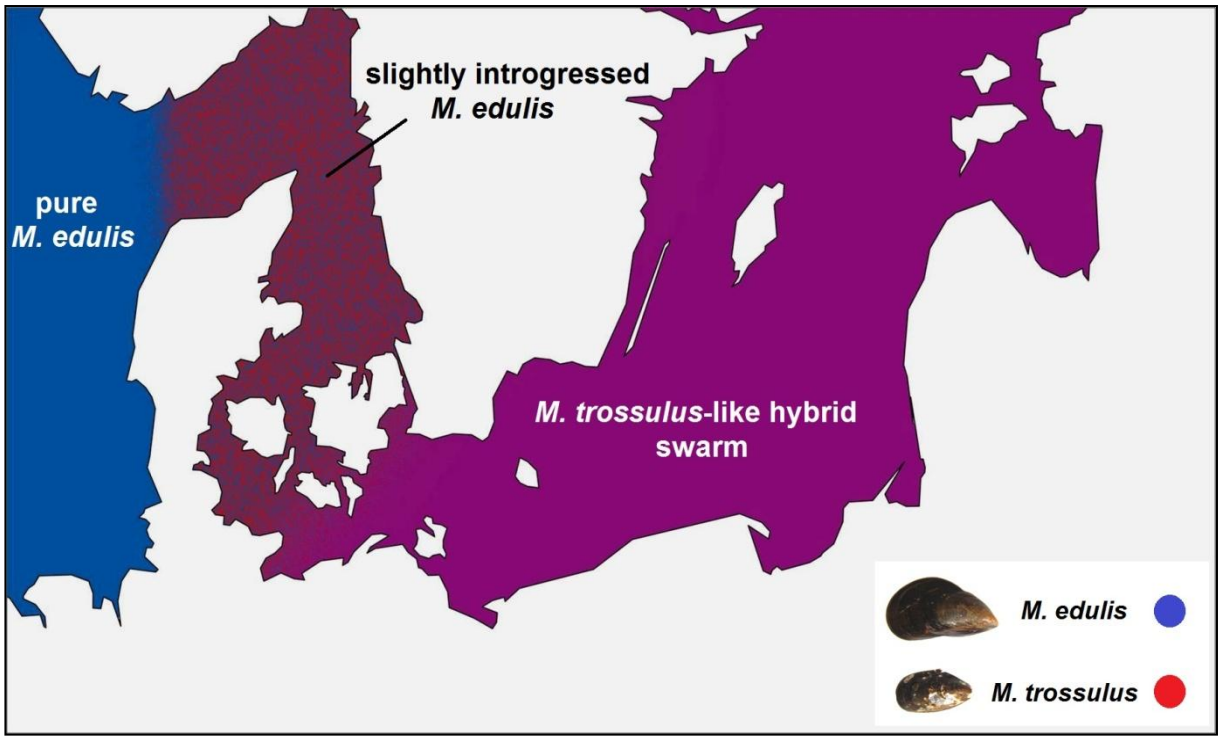

Figure 1. The genetic structure of the Baltic blue mussel hybrid zone. While North Sea populations consist of pure *M. edulis* (blue), mussels in the Kattegat/Skagerrak area are slightly introgressed by *M. trossulus* alleles (blue/red spotted). The degree of introgression increases with closer proximity to the inner Baltic, where mytilids form a *M. trossulus*-like hybrid swarm (violet). This gradient in genetic composition is tightly correlated with changes in seawater salinity from the marine North Sea to the fresh Baltic Proper. Image modified after H. Stuckas.

Although these findings evidence that not only ecological, but also weak reproductive isolation is operating in this area of secondary contact, it remains to be shown whether the two mechanisms should be seen as separately acting or mutually dependent processes.

Apart from this, Stuckas et al. (2009) have pointed out that differential clines among marker loci might be the result of hybrid zone movements related to salinity changes during the colonization process ~7500 years ago, when the last deglaciation built a connection between Baltic and North Sea (Donner 1995).

Discordances in introgression patterns have been observed for mitochondrial genomes as well, relying on whether they are maternally or paternally inherited.

Unlike most animal taxa, in which mitochondria are exclusively transmitted by females, mytilid mussels possess a system of doubly uniparental mtDNA inheritance (Skibinski et al. 1994; Zouros et al. 1994), where both parents contribute mitochondrial DNA to their progeny. Under the influence of maternal nuclear factors, all cells in daughters become dominated by mother-derived mitogenomes (F-mtDNA), whilst sons concentrate paternal mtDNA (M-mtDNA) in sperm and female mitochondria in somatic tissues (reviewed in Zouros 2012). In Baltic *M. trossulus* both original F and M genomes have been superseded by matrilinear *M. edulis* mtDNA (mitochondrial capture), which has undergone role reversals and masculinizations in male mussels, possibly through acquisition of the M-like mitochondrial control region via intermolecular recombinations (Quesada et al. 1999, 2003; Burzyński et al. 2003, 2006). In spite of this, gene exchange between inner and outer Baltic mussels seems to be reduced for paternal mitotypes (Stuckas et al. 2009), which might be due to the higher mutation rate of male compared to female mitogenomes (Riginos et al. 2004; Zouros 2012). Such an accelerated evolution is likely to disrupt coadapted gene complexes by negative epistatic interactions, thereby causing incompatibilities in hybrids (Stuckas et al. 2009). Interestingly, a recent study by Burzyński et al. (2006) found that large tandem duplications occur in the displacement (D) loops of both M- and F-like control regions in eastern Baltic mussels and first hints exist that introgression is blocked at least for this maternal locus (Kijewski et al. 2006).

Remarkably, this situation contrasts greatly with that in the Canadian Maritimes, where gene flow between sympatric *M. edulis* and *M. trossulus* is generally restricted and has resulted in a mosaic hybrid zone (reviewed in Riginos & Cunningham 2005).

The genetic structure of Baltic *Mytilus* populations could be further complicated by immigrations of allochthonous mytilids (*M. galloprovincialis*, North American *M. trossulus*) and their intergradation with native blue mussels, given the massive navigation between Baltic ports and harbours in various parts of the world. That these scenarios are realistic is

supported by recent reports about human-induced introductions of North American *M. trossulus* to several locations in Northern Europe (Väinölä & Strelkov 2011). Besides, Kijewski et al. (2006, 2011) provided first evidence for weak introgression of *M. galloprovincialis* alleles into Baltic *Mytilus* populations, though the exact invasion pathways have yet to be determined.

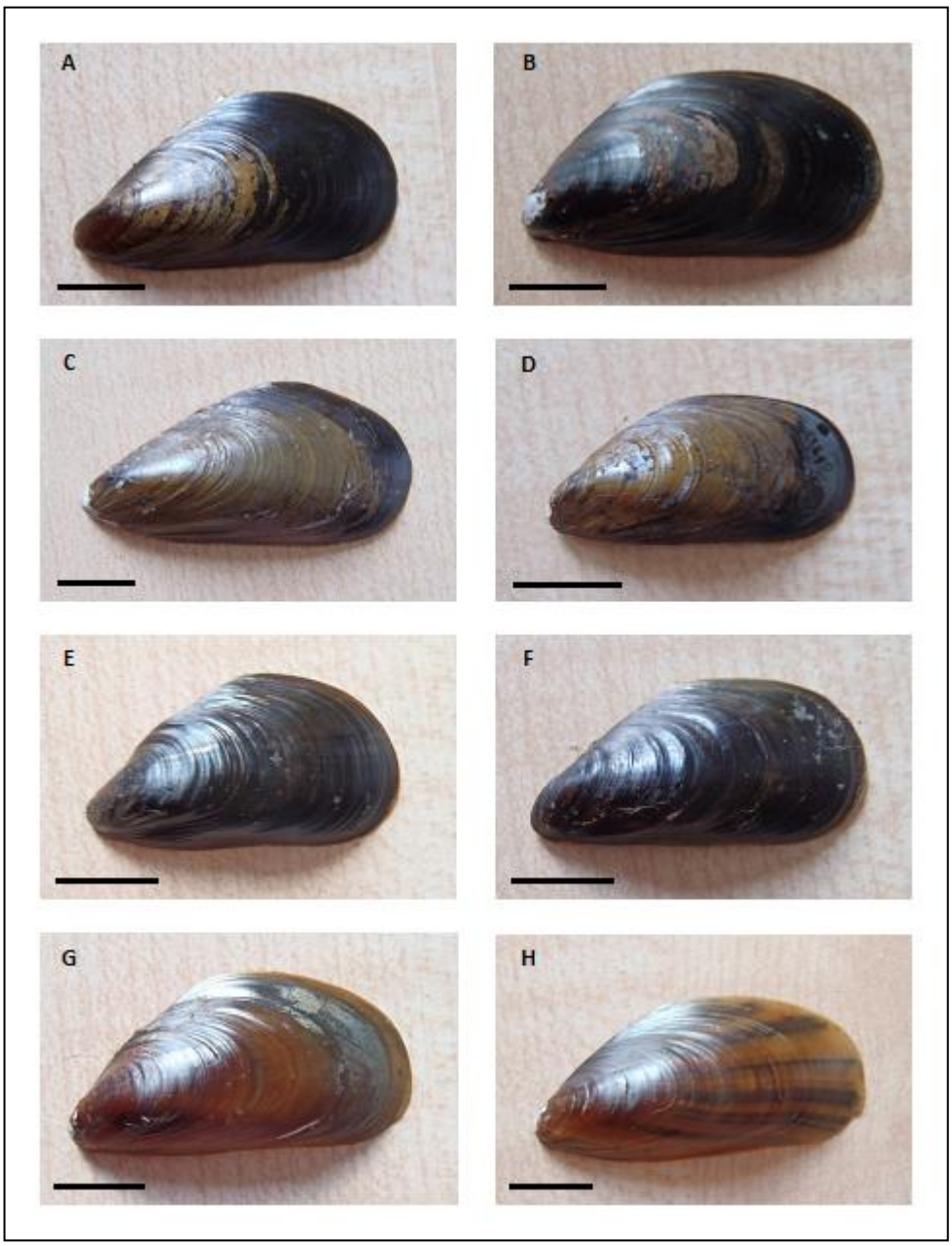

Figure 2. Examples of *Mytilus* specimens from the Kiel Fjord. Scale bar = 1 cm.

Conforming to allozymic characteristics, morphometric studies classify specimens from the northern Kattegat as *M. edulis* and those from the Baltic as *M. trossulus*, which seems to be typified by a more elongated and thinner shell than its congener (McDonald et al. 1991; Innes & Bates 1999; Beaumont et al. 2008; Penney et al. 2007, 2008). However, so far no study

could validate that the shell traits of Baltic mussels are related to the fixation of *M. trossulus* alleles at the causative loci.

In contrast to the situation at the outer edges of the hybrid zone, the geno- and phenotypic identities of mytilids from intermediate western Baltic regions like the Kiel Fjord remain largely obscure. Preliminary observations indicate that these mussels show a great morphological diversity (F. Melzner, pers. communication; Figure 2), but the links to genetic status and environmental conditions are not known. Whereas allozyme frequencies suggest that mytilids in this area are typical *M. edulis* (Theisen 1978; Bulnheim & Gosling 1988; Väinölä & Hvilsom 1991), Stuckas et al. (2009) noticed limited introgression at a few applied molecular markers. By contrast, Kossak (2006) has postulated that hybridization might theoretically be stronger due to the geographical proximity of the Kiel Fjord to the Danish Straits – the transiton zone between the hybrid swarm in the inner basin and the *M. edulis*-like populations in the Kattegat/Skagerrak area (Riginos & Cunningham 2005) – but failed to support her hypothesis with genetic data. Similarly, however, based upon his study on the effects of secondary contact between *M. edulis* and *M. galloprovincialis* in south-west England Gardner (1996) proposed that the environmental intermediacy of the North Sea/Baltic passage in terms of salinity should favour introgression and thus morphological variability in local mussel populations. The question therefore arises whether this might actually be the case in the Kiel Fjord.

Aside from its value for evolutionary investigations, knowledge about the extent and ramifications of hybridization in this region will be important for eco-physiological studies that are commonly conducted on local blue mussels (GEOMAR Helmholtz Centre for Ocean Research Kiel: http://www.geomar.de/en/). In addition, it would be profitable for these analyses if hybrids and pure species could be identified based on their shell morphology without the need of genotypic assessments.

4 Objectives of the Study

To determine the effects of hybridization on morphological variation and to set impulses for future scientific research on the *Mytilus edulis* species complex (*M. trossulus, M. edulis, M. galloprovincialis*), I will analyse the genetic and phenotypic structures of blue mussel populations in the Baltic Sea, thereby placing particular emphasis on the hitherto scarcely examined Kiel Fjord. In this context, a multilocus genotyping at eight diagnostic loci – including the maternal D-loop – will be conducted, utilizing allopatric populations of *M. edulis*, *M. trossulus* and *M. galloprovincialis* as references. Furthermore, I will perform a morphometric assessment of 14 shell traits that are commonly determined for the phenotypic differentiation between the three *Mytilus* sibling species. Precisely, the following issues will be addressed:

(1) How strong is the degree of introgression in the Kiel Fjord?
(2) Are there signs of invasive mytilids (*M. galloprovincialis*, North American *M. trossulus*)?
(3) To what extent are populations in Kiel differentiated from those of other Baltic and non-Baltic sites?
(4) Which genes show restricted *versus* relaxed flow across the Baltic hybrid zone?
(5) Do *Mytilus* populations exhibit distinct morphotypes or does a continuum exist?
(6) How similar are Baltic mussels to allopatric *M. edulis*, *M. trossulus* and *M. galloprovincialis* in terms of shell morphology?
(7) Does a particular genotype elicit a certain phenotype?
(8) How are shell characteristics influenced by salinity and hybridization?

5 Material & Methods

5.1 List of materials

Material	Supplier
PCR chemicals	
DFS-*Taq* DNA Polymerase	Bioron, Ludwigshafen, Germany
10X Complete Reaction Buffer	
Go*Taq* Flexi DNA Polymerase	Promega, Mannheim, Germany
5X Colourless Go*Taq* Flexi Buffer	
$MgCl_2$	Bioron/Promega
Primer	Biomers, Ulm, Germany
dNTP Set	Fermentas, St. Leon-Rot, Germany
Cycler	
Veriti 96 Well Thermal Cycler	Applied Biosystems, Darmstadt, Germany
vapo.protect Mastercycler proS	Eppendorf, Hamburg, Germany
Mastercycler epgradientS	
Centrifuges, Vortexer & Thermomixers	
Analog Vortex Mixer	VWR, Darmstadt, Germany
Multifuge 1 S-R Heraeus	Thermo Scientific, Schwerte, Germany
Heraeus Fresco 21 Centrifuge	
MC6 Centrifuge	Sarstedt, Nürnbrecht, Germany
Thermomixer comfort	Eppendorf, Hamburg, Germany
Mixing Block MB-102	Bioer, Hangzhou, China
Water destillation	
TKA Smart2Pure	TKA, Niederelbert, Germany
DNA isolation	
innuPREP DNA MiniKit	Analytik Jena, Jena, Germany
EtOH	Merck, Darmstadt, Germany
NanoDrop 1000	peqlab, Erlangen, Germany
NanoDrop Software V3.7.1	
Gelelectrophoresis	
peqGold Universal Agarose	peqlab, Erlangen, Germany
6X DNA Loading Dye	Fermentas, St. Leon-Rot, Germany
Lambda DNA/EcoRI+HindIII Marker, 3	
GeneRuler™ 100bp DNA Ladder	
Tris	Roth, Karlsruhe, Germany
EDTA	
NaCl	
NaAc	Merck, Darmstadt, Germany
GelRed Nucleic Acid Stain	Biotium/VWR, Darmstadt, Germany
Biorad PowerPac Basic 300V/400mA/75W	Biorad, Munich, Germany
Consort E835 300V/500mA	Consort, Turnhout, Belgium
Gene Snap	Syngene/VWR, Darmstadt, Germany
RFLP	
Perfect Blot	peqlab, Erlangen, Germany
*Hha*I	New England Biolabs, Frankfurt am Main, Germany
*Hinf*I	
*Spe*I	
*Rsa*I	
100X BSA	
10X NE Buffer 4	
Gel extraction	
NucleoSpin Gel & PCR CleanUp	Macherey Nagel, Düren, Germany

Material	Supplier
Sequencing & Fragment analysis	
Hi-Di™ Formamide	Applied Biosystems, Darmstadt, Germany
GeneScan™ 1200 LIZ® Size Standard	
BigDye® Terminator ready reaction mix	
BigDye® 5X Sequencing Terminator Buffer v1.1, v3.1	
3130*xl* Genetic Analyzer	
Sequence Scanner v1.0	http://www.appliedbiosystems.com/
Peak Scanner v1.0	
Sephadex™ G-50 Fine	GE Healthcare, Munich, Germany
ExoSAP-IT®	USB/Affymetrix, High Wycombe, UK
BioEdit Sequence Alignment Editor v7.1.3	Ibis Biosciences, Carlsbad, USA http://www.mbio.ncsu.edu/bioedit/bioedit.html
Statistics	
R versions i386 2.15.0 & 2.14.2	R Development Core Team 2012 http://www.R-project.org/
Arlequin v3.5	Excoffier et al. 2005 http://cmpg.unibe.ch/
STRUCTURE v2.3.3	Pritchard et al. 2000; Falush et al. 2003 http://pritch.bsd.uchicago.edu/structure.html
Isolation By Distance Web Service v3.23	Jensen et al. 2005; http://ibdws.sdsu.edu/
PRIMER v6	Clarke & Gorley 2006
Microsoft Office Excel 2007/2010	http:// www.microsoftstore.com/
Inkscape v0.48.2	http://inkscape.org/?lang=de
Shell morphometrics	
Point micrometre 9M05.1.76	Hogetex, Bad Wildbad, Germany
Stereomicroscope M205C	Leica, Wetzlar, Germany
Digital scales BP 220S	Sartorius, Göttingen, Germany
dialMAX analogous calliper	Wiha, Schonach, Germany
Milan angle metre 555	InterES, Nürnberg, Germany

5.2 Sample collection and preparation

Blue mussels were freshly collected at the end of January 2012 from five subtidal sites of the Kiel Fjord: Wendtorf Marina (54°25’25’’ N; 10°9’32’’ E), East Shore Harbour (54°19’49’’ N; 10°10’44’’ E), Hörn (54°18’39’’ N; 10°7’44’’ E), Museum of Ship Transport (54°19’25’’ N; 10°8’ 37’’ E) and GEOMAR West Shore Campus (54°19’42’’ N; 10°8’44’’ E). All animals were brought to the Helmholtz Centre of Ocean Research Kiel, where they were sorted and placed into 20 l flow-through aquaria that were supplied with filtered seawater from the Fjord. Subsequently, gills and inner mantles were excised with clean dissection sets, transferred into 1,8 ml cryo pure tubes and immediately put in liquid nitrogen, before being stored at -80 °C. The remaining soft tissue was removed and shells were dried at room temperature for a few days. At the beginning of March 2012, mussel shells and tissue samples were transported to the Senckenberg Institute Dresden for further investigation. Additional samples were obtained from the Skagerrak (Tjärnö) and the Baltic Proper (Askö), while mussels from putatively allopatric populations of *M. edulis* (Helgoland, North Sea), *M. galloprovincialis* (Vigo, Spanish West coast) and *M. trossulus* (Penn Cove, US North Pacific coast) were used as reference material.

Table 1. Sampling sites, sampling dates and sizes of mussel samples investigated in this study.

Species	Sampling site	Marine region	Sampling date	Sample size
Mytilus spp.	GEOMAR West Shore Campus (Germany)	Kiel Fjord (south-western Baltic)	2012	20
Mytilus spp.	Museum of Ship Transport (Germany)	Kiel Fjord (south-western Baltic)	2012	20
Mytilus spp.	Hörn (Germany)	Kiel Fjord (south-western Baltic)	2012	20
Mytilus spp.	East Shore Harbour (Germany)	Kiel Fjord (south-western Baltic)	2012	20
Mytilus spp.	Wendtorf Marina (Germany)	Kiel Fjord (south-western Baltic)	2012	20
Mytilus spp.	Tjärnö (Sweden)	Skagerrak	2005	20
Mytilus spp.	Askö (Sweden)	Baltic Proper	2005	20
M. galloprovincialis (reference)	Vigo (Spain)	East Atlantic	2005	20
M. trossulus (reference)	Penn Cove (USA)	North Pacific	2005	20
M. edulis (reference)	Helgoland (Germany)	North Sea	2005	20

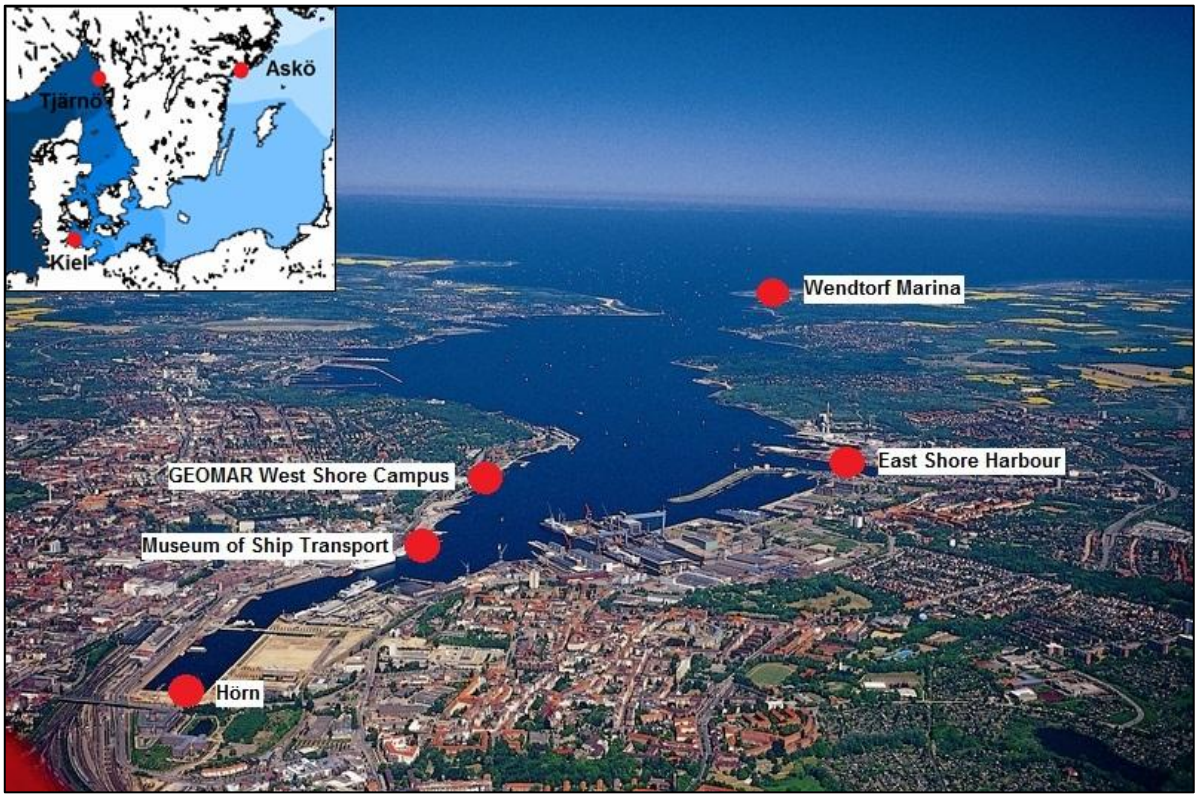

Figure 3. Mussel sampling locations in the Baltic Sea (modified after [4] and Kossak 2006). The small section in the upper left shows the sampling range spanning from Tjärnö in the Skagerrak over Kiel in the south-western Baltic to Askö in the Baltic Proper. Different shades of blue indicate the prominent salinity gradient, with decreasing salt content from West (Tjärnö, dark blue) to East (Askö, light blue). The great section shows the sampling sites in the Kiel Fjord.

All of these samples (frozen whole animals or selected tissues) were kindly provided by Dr. Heiko Stuckas (Senckenberg Natural History Collections Dresden). Details about the sampling dates, origins of the mussels and number of specimens used for the study can be found in Table 1, while Figure 3 gives an impression of the Baltic sampling stations.

5.3 Shell morphometrics

20 specimens with shell length > 2 cm were randomly selected from each site for morphometric and genetic analyses (200 individuals in total). The left valve of each specimen was measured for 12 shell characters that have been shown to be useful for the discrimination between *M. edulis*, *M. trossulus* and *M. galloprovincialis* (McDonald et al. 1991; Toro 1998; Innes & Bates 1999; Beaumont et al. 2008; Penney et al. 2007): length of anterior adductor muscle scar (aam), length of hinge plate (hp), distance between pallial line and ventral shell margin midway along the shell (pal), distance between ventral edge of posterior adductor muscle scar and ventral shell margin (pamv), distance between anterior edge of posterior adductor muscle scar and posterior shell margin (pamp), distance between umbo and posterior end of ligament (lig), length of posterior adductor muscle scar (pam), shell width (wid), point of maximum shell width (mwid), shell height (ht), shell length (l) and shell thickness (tck). In addition, the ligamentary angle (la), as suggested by McDonald et al. (1991) and references therein, and total shell weight (wg; Penney et al. 2008) were determined (Figure 4).

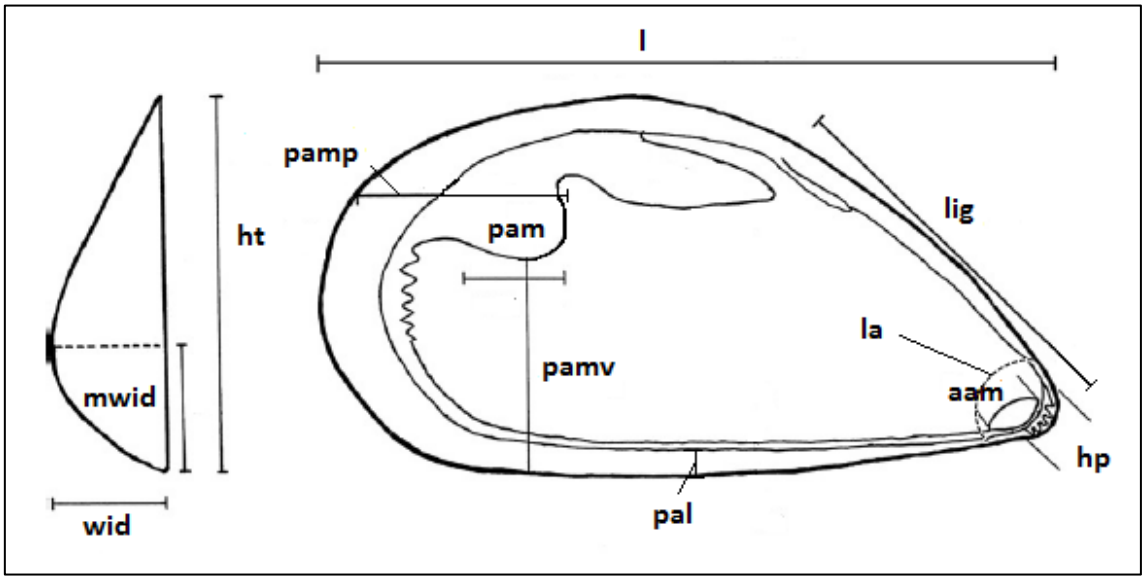

Figure 4. Shell morphometrics determined in this study (modified after Beaumont et al. 2008). l = shell length; ht = shell height; wid = shell width; mwid = point of maximum shell width; lig = distance between umbo and posterior end of ligament; hp = length of hinge plate; pal = distance between pallial line and ventral shell margin midway along the shell; la = ligamentary angle; aam = length of anterior adductor muscle scar; pam = length of posterior adductor muscle scar; pamv = distance between ventral edge of posterior adductor muscle scar and ventral shell margin; pamp = distance between anterior end of posterior adductor muscle scar and posterior shell margin.

Except for aam, hp, pal (ocular micrometre in Leica M205C stereomicroscope with ±0,01 cm precision, Wetzlar, Germany), la (Milan 555 angle metre with ±1° precision, InterES, Nürnberg, Germany), tck (Hogetex 9M05.1.76 point micrometre with ±0,001 mm precision, Bad Wildbad, Germany) and wg (Sartorius BP 220S digital scales with ±0,0001 g precision, Göttingen, Germany), all measurements were made with the help of a dialMax analogous calliper (precision ±0,01 cm, Wiha, Schonach, Germany). To account for age-dependent thickness differences along the shell, the mean of readings at the centre of the posterior adductor muscle scar, the point of maximum shell width and the midway point along the line of maximum shell height was taken for the analyses (Penney et al. 2007). Besides, as determination of the ligamentary angle seemed to be most liable to subjectivity, the average out of three replicated measurements was calculated to increase the accuracy of the results.

5.4 DNA isolation and genotypic assessment

Samples were briefly thawed and a piece of somatic tissue was directly incubated in lysis solution of the innuPrep DNA Mini Kit (Analytik Jena, Jena, Germany) for DNA extraction. To minimize contaminations, dissection sets were cleaned with 70% ethanol (Merck, Darmstadt, Germany) and flamed after each preparation. DNA from all specimens was isolated according to the manufacturer's instructions except that incubation in lysis solution with proteinase K was extended to overnight in a Thermomixer comfort (Eppendorf, Hamburg, Germany) or a Mixing Block MB-102 (Bioer, Hangzhou, China) and elution was repeated twice with 30 µl elution buffer. Nucleic acid concentrations were determined with the help of a NanoDrop 1000, using the NanoDrop software version 3.7.1 (peqlab, Erlangen, Germany), and samples were diluted to 20 ng DNA/µl. Subsequently, mussels were genotyped at seven nuclear loci (EFbis, Glu-5', ITS, mac-1, MAL-1, M7 Lysin, PLIIa) and one mitochondrial marker (maternal D-loop) that have been shown to be highly diagnostic between *M. edulis*, *M. galloprovincialis* and *M. trossulus* (Heath et al. 1995; Inoue et al. 1995; Rawson et al. 1996a, 1996b; Ohresser et al. 1997; Daguin & Borsa 1999; Daguin et al. 2001; Bierne et al. 2003b; Riginos & McDonald 2003; Wood et al. 2003; Riginos & Cunningham 2005; Kijewski et al. 2006; Stuckas et al. unpublished; Tables 2 & 3). Note, however, that only EFbis, Glu-5' and mac-1 can be used to differentiate between all three species of the *M. edulis* complex, while the other markers partly fail to distinguish *M. edulis* (all others) or *M. trossulus* (PLIIa) from *M. galloprovincialis* with the methods applied in this study. Genotypes were identified based on natural PCR product length variations (Glu-5', mac-1, D-loop), restriction fragment length polymorphisms (RFLP: EFbis, ITS, MAL-1, PLIIa) or presence/absence assays (M7 Lysin).

Table 2. Characteristics of the eight molecular markers used in this study.

Marker	Description	Type	Region	Copies
Mitochondrial				
D-loop	displacement loop in the control region of maternal mitochondrial DNA	-	non-coding	single
Nuclear				
EFbis	intron of elongation factor 1α	co-dominant	non-coding	single
Glu-5'	polyphenolic adhesive foot protein	co-dominant	coding	single
ITS	internal transcribed spacer	co-dominant	non-coding	multiple
mac-1	first intron of actin gene	co-dominant	non-coding	single
MAL-1	*Mytilus* anonymous locus 1	co-dominant	coding	single
M7 Lysin	acrosomal sperm protein	co-dominant	coding	single
PLIIa	protamine-like sperm packaging protein	dominant	coding	single

Table 3. Primer sequences for the eight molecular markers investigated in this study. All primers were ordered at Biomers (Ulm, Germany). In the case of D-loop, Glu-5' and mac-1 unlabelled forward primers were used for the model sequencing reactions.

Marker	Sequence	Author
D-loop		
Forward: VD1_Fb.for (6-FAM)	5'-GATTGTTAATGCCCAGGTCT-3'	Stuckas et al. (unpublished)
Reverse: RNAY.rev	5'-TTACAGCTCACCACCTATTC-3'	
EFbis		
Forward: EFbis_F	5'-ACAAGATGGACAATACCGAACCACC-3'	Bierne et al. (2003b)
Reverse: EFbis_R2	5'-CCTTCTGGATTTCCATGAATCGG-3'	
Glu-5'		
Forward: Me15_F (Atto 565)	5'-CCAGTATACAAACCTGTGAAGA-3'	Inoue et al. (1995)
Reverse: Me16_R	5'-TGTTGTCTTAATAGGTTTGTAAGA-3'	
ITS		
Forward: ITS_F	5'-GTTTCCGTAGGTGAACCTG-3	Heath et al. (1995)
Reverse: ITS_R	5'-CTCGTCTGATCTGAGGTCG-3'	
mac-1		
Forward: mac-1a_F (HEX)	5'-GCTGTATTTCCATCAATTGTTGG-3'	Bierne et al. (2003b) Daguin et al. (2001)
Reverse: mac-1b_R	5'-CGAAAATTGTAGTCTAGTTTTGTG-3'	
MAL-1		
Forward: MAL-1_JH2_F	5'-GCGCAGTGCTTATTGTAGACG-3'	Rawson et al. (1996a)
Reverse: MAL-1_PR9_R	5'-CTTCATGGCTTTGACTTTTGCTC-3'	
M7 Lysin		
Forward: M7_TR_exon5_1.for	5'-CCAGCTTTTACTAATCCTAAATGGA-3'	This study
Reverse: M7_TR_exon5_2.rev	5'-GTCTTGCAGACATAGTTGTATTGC-3'	
Forward: M7_ED_exon5_4.for	5'-CCAGCTTTTTCTAATCCTAAATGGT-3'	
Reverse: M7_ED_exon5_5.rev	5'-TCTTGCAGACATAGTTGTACTGA-3'	
PLIIa		
Forward: PLIIa_F	5'-GAGCCCAAGTAGGAAATCCCG-3'	Heath et al. (1995)
Reverse: PLIIa_R	5'-CCTTCGCATTGTTAGATTTATT-3'	

Even so, to verify the accuracy of the PCR reactions, amplified products of a few mussels from the three allopatric reference populations were Sanger sequenced in forward direction on an Applied Biosystems Genetic Analyzer model 3130*xl* with the ABI Prism BigDye® Terminator v1.1 Cycle Sequencing Kit (for further details of the general sequencing procedures see below) after purification with ExoSAP-IT® (USB/Affymetrix, High

Wycombe, UK). Obtained sequences were eventually compared with published sequences in the NCBI Nucleotide database using MEGABLAST.

5.5 PCR product length variations

In order to refine the investigation of natural length polymorphisms, variation at loci Glu-5', mac-1 and maternal D-loop was analysed by means of automated capillary electrophoresis. For this purpose, forward primers (Inoue et al. 1995: Glu-5'; Bierne et al. 2003b: mac-1; Stuckas et al. unpublished: D-loop) were labelled with three different fluorescent dyes (Glu-5': Atto 565 (red); mac-1: HEX (green); D-loop: 6-FAM (blue)) and DNA fragments were amplified in a multiplex polymerase chain reaction using 20-40 ng DNA, 2 µl 10X complete PCR-Buffer (Bioron, Ludwigshafen, Germany), 0,025 µmol $MgCl_2$ (Bioron, Ludwigshafen, Germany), 4 nmol dNTPs (Fermentas, St. Leon-Rot, Germany), 2,5 pmol of each primer for Glu-5' and D-loop, 7,5 pmol of each primer for mac-1, 1 unit of DFS-*Taq* Polymerase (Bioron, Ludwigshafen, Germany) and doubly distilled water in a total volume of 20 µl. To check for contaminations, negative controls without DNA were included in every run. After initial denaturation for 3 min at 94°C, DNA samples were put through 37 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Darmstadt, Germany), a vapo.protect Mastercycler proS or a Mastercycler epgradientS (Eppendorf, Hamburg, Germany). Fragments were finally elongated for 10 min at 72°C and afterwards cooled to 8°C.

For purity and efficiency controls a subset of PCR products was resolved on 2% agarose gels (peqGold Universal Agarose: peqlab, Erlangen, Germany; 1X TAE buffer (pH 7,8): 0,04 M Tris (Roth, Karlsruhe, Germany), 0,02 M NaAc (Merck, Darmstadt, Germany), 2 mM EDTA (Roth, Karlsruhe, Germany)) utilizing 5 µl GeneRuler™ 100bp DNA Ladder (Fermentas, St. Leon-Rot, Germany) and 5 µl PCR product mixed with 1 µl 6X DNA Loading Dye (Fermentas, St. Leon-Rot, Germany). Gelelectrophoresis was run at 75 V for approximately 45 min in 1X TAE buffer with the Biorad PowerPac Basic (Munich, Germany) or Consort E835 (Turnhout, Belgium) system. Eventually, gels were stained in a 3X GelRed bath (15 µl GelRed Nucleic Acid Stain (Biotium/VWR, Darmstadt, Germany) in 750 ml 0,1 M NaCl solution (Roth, Karlsruhe, Germany)) and images were captured with the Gene Snap software (Syngene/VWR, Darmstadt, Germany) after UV exposure. Based on the intensity of the gel bands, PCR products were diluted twentyfold with doubly distilled water and then 1 µl of each sample was mixed with 8,6 µl Hi-Di™ formamide (Applied Biosystems, Darmstadt, Germany) and 0,4 µl GeneScan™ 1200 LIZ® Size Standard (Applied Biosystems, Darmstadt, Germany). Following denaturation for 5 min at 95°C, samples were subjected to

capillary electrophoresis in a 3130*xl* Genetic Analyzer (Applied Biosystems, Darmstadt, Germany) with run settings given in Table 4. If required, electrophoresis was repeated with adjusted sample dilutions. For scoring species-specific alleles, fragments were examined with the help of the free ABI Peak Scanner software v1.0 (http://www.appliedbiosystems.com/) using the PP setting defaults, which consider primer peaks. Previous to analysis, size standards for each sample were checked for quality and corrected by hand, if necessary.

In the case of D-loop, alleles of around 694 bp were defined as the original *M. edulis* F genome (F_E; Burzyński et al. 2006), while longer fragments were pooled into one recombinant *M. edulis* haplotype (F_L). As primers were designed to amplify only *M. edulis*/*M. galloprovincialis* derived sequences, lack of PCR products was interpreted as native *M. trossulus* F-mtDNA (F_T).

Genotyping at the multi-allelic locus mac-1 is often done based on allele frequencies in putatively allopatric populations (e.g. Daguin & Borsa 1999; Daguin et al. 2001; Bierne et al. 2003a, 2003b, 2006). However, as allelic frequencies alone do not allow distinguishing between incomplete lineage sorting and introgression, I refrained from assigning alleles to individual species prior to population genetic analyses.

Characteristic fragment lengths for the Glu-5' marker were 177 bp (*M. edulis*), 165 bp (*M. trossulus*) and 124 bp (*M. galloprovincialis*). Interestingly, three individuals from Kiel and one individual from Helgoland appeared to be heterozygous for the *M. edulis* and *M. galloprovincialis* alleles. In order to confirm these observations, fragments of two specimens were exemplarily sequenced. For this, 50 ng DNA of each mussel were PCR re-amplified using 13,4 µl $_{dd}H_2O$, 2 µl 10X complete PCR-Buffer (Bioron, Ludwigshafen, Germany), 4 nmol dNTPs (Fermentas, St. Leon-Rot, Germany), 7,5 pmol of unlabelled primer and 1 unit of DFS-*Taq* Polymerase (Bioron, Ludwigshafen, Germany). Initial denaturation (94°C for 5 min) was followed by 38 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s as well as a final extension step for 10 min at 72°C. For separation and purification of the PCR products, fragments were extracted from a 2% agarose gel by means of the NucleoSpin Gel & PCR CleanUp Kit (Macherey Nagel, Düren, Germany). Subsequently, downstream sequencing reactions were performed after the BigDye® Terminator v1.1 Cycle Sequencing Manual (Applied Biosystems, Darmstadt, Germany) using 4 µl of pure product, 5 pmol of unlabelled forward primer, 1 µl BigDye® Terminator ready reaction mix and 2 µl BigDye® 5X Sequencing Buffer v1.1/v3.1 added to 2,5 µl $_{dd}H_2O$. Prior to capillary electrophoresis in the 3130*xl* Genetic Analyzer (Applied Biosystems, Darmstadt, Germany), products were purified with the Performa® DTR V3 96-Well Short Plate Kit (Edge Bio, Gaithersburg, USA). Sample processing was carried out as instructed by the manufacturer with one modification:

400 µl of 5% Sephadex™ G-50 Fine solution (GE Healthcare, Munich, Germany) were pipetted to the Short Plate before the first centrifugation step. Finally, samples were filled up with 0,5 M EDTA (Roth, Karlsruhe, Germany) to a total volume of 15-20 µl and then sequenced with run settings detailed in Table 4.

Table 4. Run settings for capillary electrophoresis.

Parameter	Fragment length polymorphism		Sequencing	
	Value	Range	Value	Range
Oven temperature	60	18-65°C	60	18-65°C
Poly fill volume	7300	7300-38000 steps	7300	7300-38000 steps
Current stability	5	0-2000 µA	5	0-2000 µA
Pre-run voltage	15	0-15 kV	15	0-15 kV
Pre-run time	180	1-1000 s	180	1-1000 s
Injection voltage	1,6	1-15 kV	1,6	1-15 kV
Injection time	20	1-600 s	60	1-600 s
Voltage number of steps	30	1-100 nk	30	1-100 nk
Voltage step interval	15	1-60 s	15	1-60 s
Data delay time	250	1-3600 s	250	1-3600 s
Run voltage	13	0-15 kV	13,4	0-15 kV
Run time	5500	300-14000 s	2800	300-14000 s

After checking sequencing data for quality with the Sequence Scanner software v1.0 from Applied Biosystems (http://www.appliedbiosystems.com/), they were aligned against published sequences of *M. galloprovincialis* using the BioEdit Sequence Alignment Editor v7.1.3 (Ibis Biosciences, Carlsbad, USA: http://www.mbio.ncsu.edu/bioedit/bioedit.html).

5.6 Restriction fragment length polymorphisms (RFLP)

RFLP procedures for EFbis (Kijewski et al. 2006), ITS, PLIIa (Heath et al. 1995) and MAL-1 (Rawson et al. 1996a) were carried out after previously published protocols. Generally, PCRs were performed according to conditions described in Table 5 and quality was checked on subsamples via agarose gelelectrophoresis (see above). In a few cases amplification of the ITS, MAL-1 and PLIIa loci was poor or failed completely, so that reactions were changed as follows: 20-100 ng DNA were incubated with 4 µl 5X Colourless Go*Taq* Flexi Buffer (Promega, Mannheim, Germany), 0,05 µmol $MgCl_2$ (Promega, Mannheim, Germany), 0-4 ng BSA (New England BioLabs, Frankfurt am Main, Germany), 4 nmol dNTPs (Fermentas, St. Leon-Rot, Germany), 7,5 pmol of each corresponding primer, 1 unit of Go*Taq* Flexi DNA Polymerase (Promega, Mannheim, Germany) and doubly distilled water in a final volume of 20 µl. Samples were initially denatured for 2 min at 95°C and then subjected to 38 cycles of 95°C for 30 s, 48-55°C for 30 s and 72°C for 1,5 min. The final extension step was performed

at 72°C for 5 min. For MAL-1 0,5 µl of products from these reactions were used in another PCR with the same conditions to increase fragment yield.

Table 5. PCR conditions for EFbis, ITS, PLIIa and MAL-1 with DFS-*Taq* polymerase (Bioron, Ludwigshafen, Germany). All reactions were run in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Darmstadt, Germany), a vapo.protect Mastercycler proS or a Mastercycler epgradientS (Eppendorf, Hamburg, Germany). To test for contaminations, negative controls were included in all runs by replacing sample DNA with doubly distilled water.

	EFbis		**ITS**		**PLIIa**		**MAL-1**	
Reaction mixture	1x in µl		1x in µl		1x in µl		1x in µl	
$_{dd}H_2O$	14,4-11,9		13,9		9,9-6,9		14,9-12,9	
10X complete PCR-Buffer	2		2		2		2	
$MgCl_2$ (25 mM)	0		0		1		0	
dNTPs (10 mM)	0,4		0,4		0,4		0,4	
Primer forward (10 pmol/µl)	0,75		0,75		0,75		0,75	
Primer reverse (10 pmol/µl)	0,75		0,75		0,75		0,75	
DFS-*Taq* polymerase	0,2		0,2		0,2		0,2	
DNA (20 ng/µl)	1,5-4		2		5-8		1-3	
Total	20		20		20		20	
Programme								
1) Initial denaturation	94°C	5 min	94°C	5 min	94°C	5 min	94°C	5 min
2) Denaturation	94°C	20 s	94°C	20 s	94°C	30 s	94°C	20-30 s
3) Annealing	54°C	20 s	55°C	20 s	48°C	30 s	52°C	20-30 s
4) Extension	72°C	45 s	72°C	45 s	72°C	45 s	72°C	45 s
5) Final extension	72°C	10 min	72°C	10 min	72°C	10 min	72°C	10 min
6) Cooling	8°C	∞	8°C	∞	8°C	∞	8°C	∞
Cycles (2-4)	38		35		38		35-38	

For assessing alleles specific to *M. edulis*, *M. trossulus* and *M. galloprovincialis*, PCR products of ITS, PLIIa and MAL-1 were cut with the restriction endonucleases *Hha*I, *Hinf*I and *Spe*I (New England Biolabs, Frankfurt am Main, Germany), respectively (Heath et al. 1995; Rawson et al. 1996a). By contrast, EFbis amplicons were digested with *Hha*I and *Rsa*I (New England Biolabs, Frankfurt am Main, Germany) in two separate assays (Kijewski et al. 2006), which are derived from the observation that *Hha*I cuts only alleles typical of *M. edulis*, whilst *Rsa*I cuts *M. trossulus* and *M. galloprovincialis* alleles in different ways. Briefly, 7,5 µl of each PCR product were mixed with 1,5 µl 10X NE Buffer 4 (New England Biolabs, Frankfurt am Main, Germany), 0-1,5 µl 10X BSA (New England Biolabs, Frankfurt am Main, Germany), 1 unit of the corresponding enzyme and water in a final volume of 15 µl (Table 6). After overnight incubation at 37°C, enzyme activities were stopped by adding 3 µl 6X Loading Dye (Fermentas, St. Leon-Rot, Germany) to each sample and restriction patterns were subsequently resolved on 3% agarose gels. Gelelectrophoretic procedures were performed as described above except that the total sample volume was applied to the gel wells to ensure fragment detection. A summary of the characteristic restriction fragment lengths for each species can be found in Table 7.

Table 6. Reaction mixtures for the RFLP assays.

	EFbis		**ITS**	**PLIIa**	**MAL-1**
	1x in µl	1x in µl	1x in µl	1x in µl	1x in µl
PCR product	7,5	7,5	7,5	7,5	7,5
10X NE Buffer 4	1,5	1,5	1,5	1,5	1,5
BSA (10X)	1,5	-	1,5	-	1,5
$_{dd}H_2O$	4,45	5,9	4,45	5,9	4,4
Enzyme	-	-	-	-	-
• *Hha*I (20 U/µl)	0,05	-	0,05	-	-
• *Rsa*I (10 U/µl)	-	0,1	-	-	-
• *Hinf*I (10 U/µl)	-	-	-	0,1	0,1
• *Spe*I (10 U/µl)	-	-	-	-	-
Total	15	15	15	15	15

Table 7. Characteristic RFLP patterns for *M. edulis*, *M. trossulus* and *M. galloprovincialis* found in this study. Smaller fragments in brackets were usually not observed.

	M. edulis **[bp]**	***M. trossulus*** **[bp]**	***M. galloprovincialis*** **[bp]**
EFbis			
uncut	380	300	350
cut	200 + 180	220 + 80	275 (+ 25)
ITS			
uncut	1200	1200	1200
cut	450 + 200 (+ <100)	250 + 200	450 + 200 (+ <100)
PLIIa			
uncut	475	475	475
cut	225 (+ 25)	475 + 225 (+ 25)	475 + 225 (+ 25) or 225 (+ 25)
MAL-1			
uncut	1050	1050	1050
cut	650 + 400	725 + 325	650 + 400

Importantly, restriction sites in some MAL-1 alleles were lost, which made genotyping partly ambiguous. Nonetheless, as uncut alleles were never observed in allopatric *M. trossulus*, but only in populations with strong *M. edulis* genetic background (Helgoland, Tjärnö, Kiel), I rated these alleles as originally *M. edulis*. Validation of this assumption will of course require cloning and sequencing of MAL-1, which was, unfortunately, out of the scope of this study due to time constraints.

5.7 M7 Lysin

For examining variability at locus M7 Lysin, species-specific primers targeting ~150 bp of the exon 5 region were developed based on GenBank sequences of allopatric *M. edulis* and *M. trossulus* from North America (Riginos & McDonald 2003). Since exon 5 nucleotide sequences of *M. galloprovincialis* are barely differentiated from those of its congener *M. edulis* (Stuckas et al. 2009), no attempt was made to design primers for the third species. Therefore, although almost no amplification occurred in individuals from Vigo, which could

be indicative of *M. galloprovincialis* alleles, I treated such results as missing data. PCRs were performed in two reactions containing 20 ng DNA, 2 µl 10X complete enzyme buffer (Bioron, Ludwigshafen, Germany), 4 nmol dNTPs (Fermentas, St. Leon-Rot, Germany), 15 pmol of the respective primer pair and 1 unit of DFS-*Taq* Polymerase (Bioron, Ludwigshafen, Germany) added with doubly distilled water to a final volume of 20 µl. Samples were initially denatured for 5 min at 94°C and then replicated in 37 cycles involving denaturation for 20 s at 94°C, annealing for 20 s at 55°C and extension for 45 s at 72°C. Final elongation was performed for 10 min at 72°C. Subsequently, amplicons were electrophoretically separated on a 3% agarose gel (see above) and mussels were genotyped as homo- or heterozygous for the *M. edulis* and *M. trossulus* allele depending on the presence of PCR products from only one or both reactions.

5.8 Statistical analyses

5.8.1 Population genetics

In order to estimate the degree of hybridization within the Baltic mussel populations, multi-locus hybrid indices were calculated for each individual by counting the number of *M. trossulus* alleles over the five co-dominant nuclear markers EFbis, ITS, Glu-5', MAL-1 and M7 Lysin. Due to the inability to accurately identify heterozygous individuals with dominant markers, PLIIa was excluded from this analysis. Hybrid scores ranging from 0 (pure *M. edulis*) to 10 (pure *M. trossulus*) were eventually plotted against their frequencies in every population and compared with genotype distributions in the allopatric reference populations of *M. edulis* (Helgoland) and *M. trossulus* (Penn Cove).

Complementary to this, changes in *M. edulis* allele frequencies from Helgoland to Askö were charted for cytoplasmic and nuclear markers to elucidate cline structures across the Baltic contact zone. Please keep in mind, however, that discrimination between *M. edulis/M. trossulus* and *M. galloprovincialis* was not feasible at some genetic loci. Therefore, results of this analysis must be seen as simplification, even if influence of *M. galloprovincialis* alleles can be anticipated to be low in the Baltic Sea. Following the approach of Riginos et al. (2002), Hardy-Weinberg equilibrium was presumed for assessing the total amount of heterozygotes at PLIIa. Since patterns for nuclear markers were similar among all five Kiel populations, the corresponding allele frequencies were pooled across samples. In contrast, *M. edulis* haplotype frequencies notably differed between the inner and outer Kiel Fjord, so that they were analysed separately for each sample. In the case of locus mac-1, frequencies of all alleles were examined across populations.

Statistical tests for genetic diversity and differentiation both within and between all populations were conducted in Arlequin v3.5 (Excoffier et al. 2005) based on variability at the co-dominant single-copy loci EFbis, Glu-5', M7 Lysin and mac-1 (standard format, unknown gametic phase). Besides, separate tests were done for the mitochondrial D-loop marker (standard haplotypic format). Nevertheless, as *M. edulis* could not be distinguished from *M. galloprovincialis* at this locus, mussels from Vigo were excluded from this second analysis to avoid false-positive detections of genetic admixture. Variation at the intra-population level was evaluated by calculating Hardy-Weinberg equilibria for each locus, using the default settings implemented in the software (Markov chain length: 1000000; dememorization steps: 100000) and adjusting p values for 10 samples. As a measure of population differentiation pairwise F_{ST}s were computed from weighted average F-statistics *sensu* Weir & Cockerham (1984), using non-parametric permutation procedures (10000 replications) for evaluating the significance of the results. In order to control type 1 error rates in multiple comparisons, obtained fixation indices were sequentially Bonferroni corrected (Rice 1989), choosing 0,05 as the α level. As results for nuclear data suggested a positive association between genetic differentiation and locality separation, $F_{ST}/(1-F_{ST})$ estimates (Rousset 1997) between Baltic and North Sea populations were subsequently regressed against geographic distance (computed from Stuckas et al. 2009). Linear relationships were assessed with the Isolation By Distance Web Service v3.23 (Jensen et al. 2005; http://ibdws.sdsu.edu/), where Mantel tests (10000 randomizations) and Reduced Major Axis regression were conducted to determine the statistical significance and parameters of the model, respectively. For determining the relative contribution of each molecular marker to the genetic variation both within and between populations standard and locus by locus AMOVAs were performed by calculating F_{ST}-statistics from squared Euclidean distance matrices on haplotypic frequencies and pairwise differences in allele numbers, respectively (Excoffier et al. 1992; Michalakis & Excoffier 1996). Haplo- or genotypes were permuted 10000 times among populations to test the significance of the extracted covariance components and F_{ST} values.

Although F_{ST}-statistics are still commonly used in population genetic approaches, they are increasingly criticized for having little test power and relying on unrealistic assumptions (Pearse & Crandall 2004). In-depth analysis of population structure was therefore carried out in the programme STRUCTURE v2.3.3 (Pritchard et al. 2000; Falush et al. 2003), which uses a sophisticated Bayesian inference algorithm to detect the number of populations K that best explains a multilocus genotype data set. Briefly, assuming Hardy-Weinberg and linkage equilibrium within populations (slight departures are allowed), a Markov Chain Monte Carlo (MCMC) iteration is implemented to find the highest posterior

probability of getting the data given variable values of K (Pritchard et al. 2000; Pearse & Crandall 2004). I chose the admixture model with correlated allele frequencies to infer the most likely number of genetic clusters based on the genetic information for loci EFbis, Glu-5', mac-1 and M7 Lysin. All parameters were set to default and a burnin length of 50000 was used, which was followed by 500000 repetitions of the MCMC simulation. Posterior probabilities were calculated for K ranging from 1 to 12 with five replications for each value. As noted by Evanno et al. (2005), Ks larger than the true number of populations cause the posterior probability to stagnate or even further increase, thereby impeding the detection of the real K. To find the accurate number of genetic clusters, I quantified the second order rate of change ΔK of the probability function, which was shown to reach its maximum at the optimal value of K (Evanno et al. 2005).

5.8.2 Morphology

Since most morphological characters are dependent on organism size, investigation of morphometric data can be severely biased, if study objects have different body sizes (Reist 1989; Berner 2011). For eliminating influences of variable size, mussel shells were therefore normalized to unit length prior to multivariate analyses. Precisely, all measured traits were converted to decadic logarithm and an ANCOVA using the log transformed length as the covariate was implemented for each logarithmized parameter to calculate population-specific linear growth relationships (Reist 1989; Innes & Bates 1999). To validate assumptions of normality and homogeneity of variances diagnostic normal q-q and residual-fit spread plots were carefully examined for each ANCOVA. Except for the ligamentary angle, which did not show any association with mussel length (ANCOVA: F = 1,4013; p = 0,2381; no curvilinear relation indicated in plots), all shell characters scaled linearly with size. Models were simplified as far as possible by standardizing slopes and intercepts that were not significantly different between mussel populations. Generally, slopes of the growth equations did not differ between groups with two exceptions in the case of the diameter of the anterior adductor muscle scar, where populations from Vigo (V) and the Museum of Ship Transport (SM) exhibited steeper regression lines than all others. However, as these discrepancies seemed to be biologically irrelevant (V: F = 2,355, p = 0,01962; SM: F = 2,309, p = 0,02205) and might be not fully reliable given the small sample sizes, slopes were pooled also for this shell parameter. Based on the obtained equations, size independent trait values were eventually calculated for each individual, using the logarithmized overall mean shell length as new predictor and adding the residual variation from the regression analyses to the outcomes. To facilitate detection of distinct morphotypic groups by increasing variability among mussels,

the antilogarithm of each value was used in all further statistical investigations. Moreover, all variables were standardized through division by the corresponding maximum (and multiplication with 100 in all analyses conducted in PRIMER), owing to unequal measurement scales in the data set.

For assessing the morphological variation within the Baltic Sea and determining the degree of phenotypic differentiation between sympatric and allopatric populations, hierarchical cluster dendrograms were constructed with the help of the software package *pvclust* within the statistical programme R v2.14.2 and 2.15.0 (R Development Core Team 2012: http://www.R-project.org/). This algorithm uses multiscale bootstrap resampling to calculate approximately unbiased p values (au), which have the advantage of being less skewed than conventional bootstrap probabilities (Suzuki & Shimodaira 2006). Multivariate morphometric data were entered as Euclidean distance matrix and agglomerated by means of the UPGMA method (unweighted pair-group method using arithmetic averages) with 10000 bootstrap resampling steps, as suggested by Suzuki & Shimodaira (2006). To indicate different mussel sampling stations, leaf labels were colour coded in Inkscape v0.48.2 (http://inkscape.org/?lang=de). Morphotype clusters were defined as reliable, when branches were supported with au values ≥ 80 and contained at least 15 (= 75 %) mussels from a station. However, usage of the whole data set resulted in a poorly supported breakdown of dendrogram branches, likely due to the predominance of data from the Kiel Fjord, which accounted for half of the morphometric measurements and thus introduced a high amount of variance to the analysis. With the aim of getting an unbiased resolution, I decided to repeat the procedure with random subsamples from Kiel. Besides, populations of the Kiel Fjord were investigated separately. These approaches elucidated the phenotypic integrity of mytilids from Helgoland, Vigo, Askö and Penn Cove, while the morphology of Tjärnö and Kiel mussels seemed to be more variable.

The significance of the differences between the morphological entities was tested by means of one-way ANOSIMs and subsequent multiple comparisons, using PRIMER v6 (Clarke & Gorley 2006). In order to prevent distortion of the analyses, sample sizes were equalized to 17 individuals per cluster beforehand. Distances between pairs of individuals were calculated by Euclidean metrics and test statistics were permuted 9999 times. To rectify increasing false discovery rates in multiple comparisons, p values were sequentially Bonferroni corrected (Rice 1989; $\alpha = 0{,}05$). Afterwards, one-way SIMPER analyses were applied for identifying the parameters that contributed most to the differences between morphotypes.

5.8.3 Relationships between genetic identity, shell morphology and salinity conditions

Correlative patterns between genotypes, phenotypic traits as well as habitat salinities were examined via conventional and partial Mantel tests based on Pearson's product moment correlation, utilizing the *vegan* package in R. For this purpose, data were assembled in four matrices containing information about:

(1) Genetic identity
   a. Individual by hybrid index matrix with the amount of *M. trossulus*, *M. edulis* and *M. galloprovincialis* alleles as cell entries based on loci EFbis and Glu-5' (mussels from Vigo included)
   b. Individual by hybrid index matrix with the amount of *M. trossulus* and *M. edulis* alleles as cell entries based on loci EFbis, Glu-5', ITS, MAL-1 and M7 Lysin (mussels from Vigo excluded)

(2) Morphology
   a. Individual by morphometric character matrix with antilogarithmic trait values standardized by maximum as cell entries

(3) Salinity
   a. Individual by salinity matrix having site-specific seawater salt content as cell entries. Typical salinity values for all localities were taken from Alvarez et al. (2005) (Vigo: 34), Kossak et al. (2006) (Askö: 6; Kiel: 17; Tjärnö: 29; Helgoland: 33) and Carvalho et al. (2010) (Penn Cove: 22).

The reason behind the construction of two genetic matrices was the fact that only loci EFbis and Glu-5' could be used for the creation of hybrid indices for all three species – an information content that is little representative of the true genetic status of an individual. Consequently, a second matrix without mussels from Vigo and with data on five molecular markers was built to accurately evaluate associations between genetic, morphological and environmental parameters. Since introgression of *M. galloprovincialis* alleles can be expected to be negligible in all populations but Vigo, I rated *M. edulis*/*M. galloprovincialis* restriction patterns at loci ITS and MAL-1 solely as *M. edulis* alleles for this analysis. To make a detailed interpretation possible, five data (sub)sets were investigated: (a) data from both Baltic/Skagerrak and reference populations (with or without Vigo), (b) only data from Baltic/Skagerrak populations (Kiel, Askö, Tjärnö), (c) only data from the reference populations (with or without Vigo), (d) only data from the North Sea/Baltic transition zone (Kiel and Tjärnö), (e) only data from the Kiel Fjord. Morphology and salinity matrices were

transformed into Euclidean distance matrices, while hybrid indices were converted to Bray-Curtis dissimilarities. To analyse genotype-phenotype relationships within the Kiel Fjord, a simple Mantel test was carried out, using 9999 permutations of the morphology distance matrix. All other data sets were examined with partial Mantel tests. Morphology distance matrices were permuted 9999 times, while removing the influences of either salinity or genetics.

6 Results

6.1 Population structure and hybridization

Calculations of hybrid indices using the nuclear loci EFbis, Glu-5', ITS, MAL-1 and M7 Lysin indicated that virtually all mussels in the Kiel Fjord are backcross hybrids (99 %), while parental taxa and F1 offspring seem to be almost non-existent (Figure 5). In fact, there was only a single individual at Hörn that had a hybrid score of 0, implying pure *M. edulis* genetic background, whereas no *M. trossulus* individuals were observed at all. Likewise, I did not find any mussel that was heterozygous for every locus analysed, although one individual from the Museum of Ship Transport carried equal proportions of *M. edulis* and *M. trossulus* alleles. The scarcity of molecular markers that could differentiate between *M. edulis* and *M. galloprovincialis* limited my ability to determine admixture of invasive mytilids or introgressive hybridization with the same. Nevertheless, fragment length polymorphisms of the Glu-5' marker were informative in that they identified three individuals in the Kiel Fjord as being *M. edulis/M. galloprovincialis* heterozygotes at this locus – a finding that was confirmed by sequence analysis and disclosed the fact that these mussels comprised alleles from all three species (Figure 6). If one assumes that nuclear genotypes based on five diagnostic markers are representative of the whole genome and ignores potential further movement of *M. galloprovincialis* alleles into the gene pool, mussels from Kiel, however, predominately possess genetic information from *M. edulis*, because hybrid scores of 2 were most numerous and those > 5 completely absent.

Analysed across the North Sea/Baltic transect, frequencies of *M. edulis*-like genotypes and alleles decreased from West to East. In the Skagerrak (Tjärnö) pure *M. edulis* parental types yet amounted to 19 % of the population and hybrid indices did not exceed values of 2. By contrast, mussels in the Baltic Proper (Askö) already carried 30-80 % *M. trossulus* alleles (hybrid scores 3 to 8), while genotypes in the middle region (Kiel) overlapped with both distributions (hybrid scores 0 to 5; Figure 5).

In spite of this general trend, the decline in *M. edulis* (E) allele and haplotype frequencies was discordant among molecular markers (Figure 7). The strongest decrease was usually observed between Kiel and Askö, although drops were more abrupt for MAL-1, M7 Lysin and D-loop than for PLIIa, ITS and Glu-5'. As opposed to this, locus EFbis showed an almost linear reduction in E allele/mitotype frequencies from reference North Sea (Helgoland) to inner Baltic populations. While no meaningful differences in E allele frequencies were observed between the five Kiel populations for any nuclear marker, an interesting pattern emerged for mitochondrial haplotypes. Populations in the inner Kiel Fjord seemed to be fixed

for the typical *M. edulis* variant, while the population in Wendtorf Marina at the entry to the inner Baltic carried both original and recombinant haplotypes.

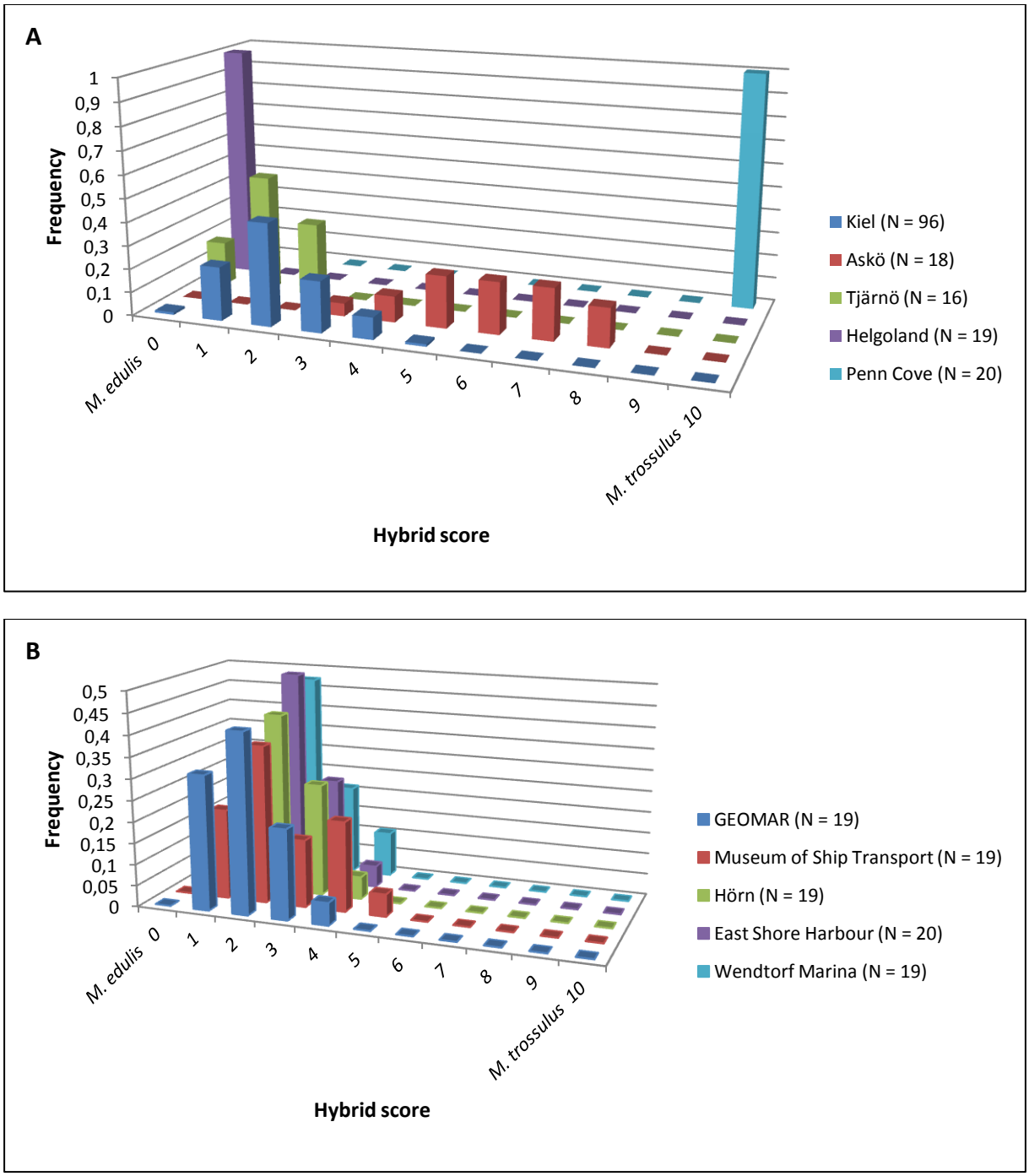

Figure 5. Genotype distributions in the Baltic Sea compared to those in the reference populations of *M. edulis* (Helgoland) and *M. trossulus* (Penn Cove). A) Hybrid indices pooled over all five Kiel populations. B) Hybrid indices plotted separately for the five Kiel populations.

Concerning locus mac-1, in total 30 different alleles ranging in size between 352 bp and 633 bp were identified. Although some polymorphisms were shared between species (i.e. 363 bp and 364 bp fragments both occurred in Vigo and Helgoland), the majority appeared to be fixed in allopatric *Mytilus* populations (Table 8). Alleles 354, 358, 360, 361, 367 as well as

393 seemed to be indicative of *M. edulis*, while alleles 359 and 397 to 424 were good predictors for *M. galloprovincialis*. Fragment lengths typical of *M. trossulus* were 368 bp, 371 bp and ≥ 588 bp.

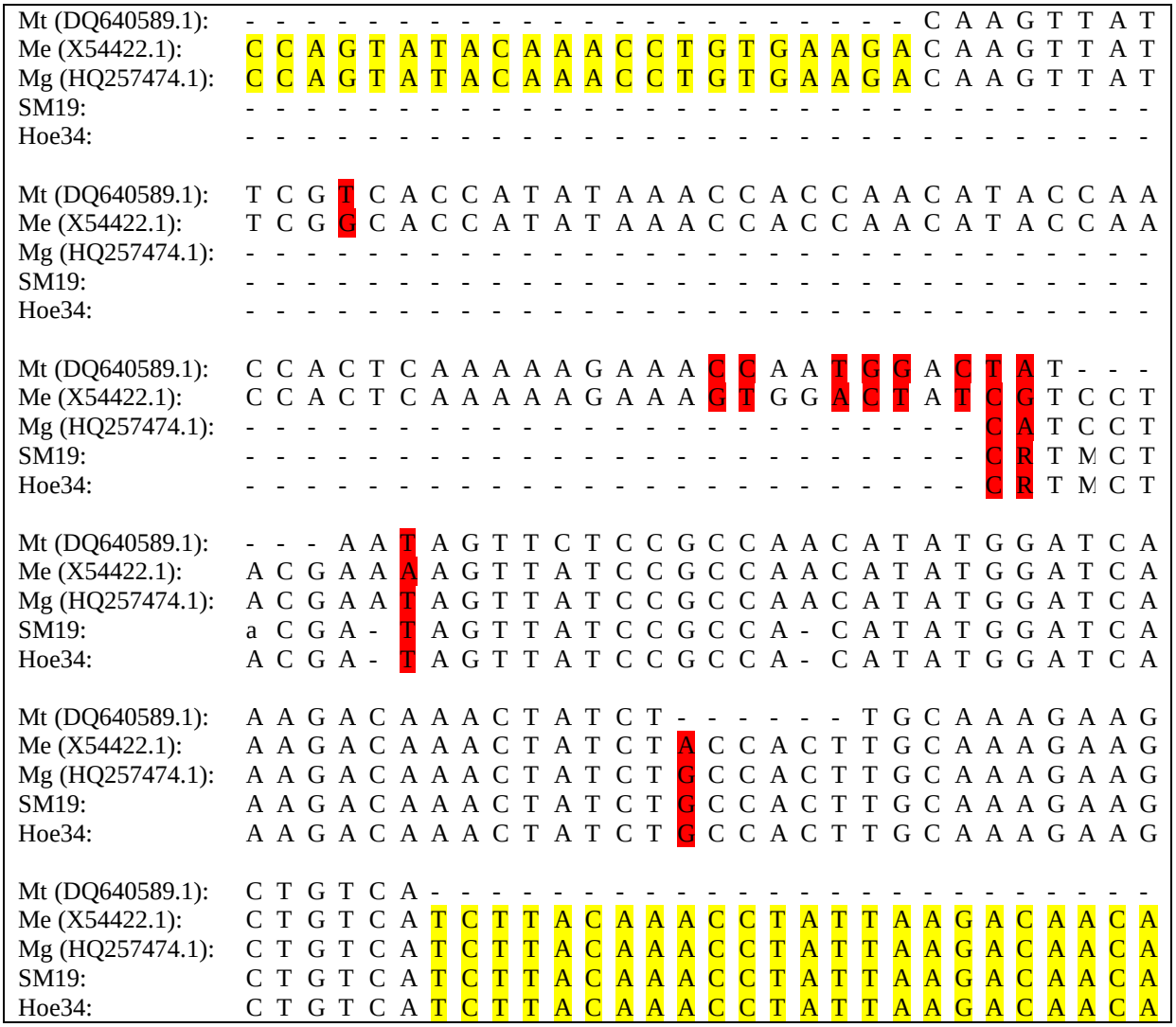

Figure 6. Alignment of Glu-5' nucleotide sequences in forward direction. Yellow labels indicate primer sequences, while red markings denote nucleotide substitutions. Hyphens were inserted to align the sequences. Me = *M. edulis*, Mg = *M. galloprovincialis*, Mt = *M. trossulus*, where codes in brackets represent accession numbers from NCBI. SM19 and Hoe34 are hybrid individuals from Kiel, which were found to carry each one *M. galloprovincialis* (shown) and one *M. edulis* (not shown) allele at Glu-5'.

With respect to the sympatric mussel populations, polymorphisms characteristic of Helgoland *M. edulis* were most abundant in Kiel and Tjärnö, whereas alleles found in North American *M. trossulus* were extremely sparse. Surprisingly, the rare allele 359, which was apparently specific for *M. galloprovincialis*, also occurred in low frequency within the Kiel Fjord. Moreover, Kiel populations exhibited two private polymorphisms (362 bp and 366 bp). As expected, mussels in Askö carried a higher proportion of putative *M. trossulus* alleles than other Baltic mytilids. Interestingly, however, the most common allelomorph (369 bp) was not

observed in allopatry, but seemed to be restricted to inner Baltic and Kiel populations, decreasing in frequency from East to West. In addition, allele 352 was exclusive to Askö.

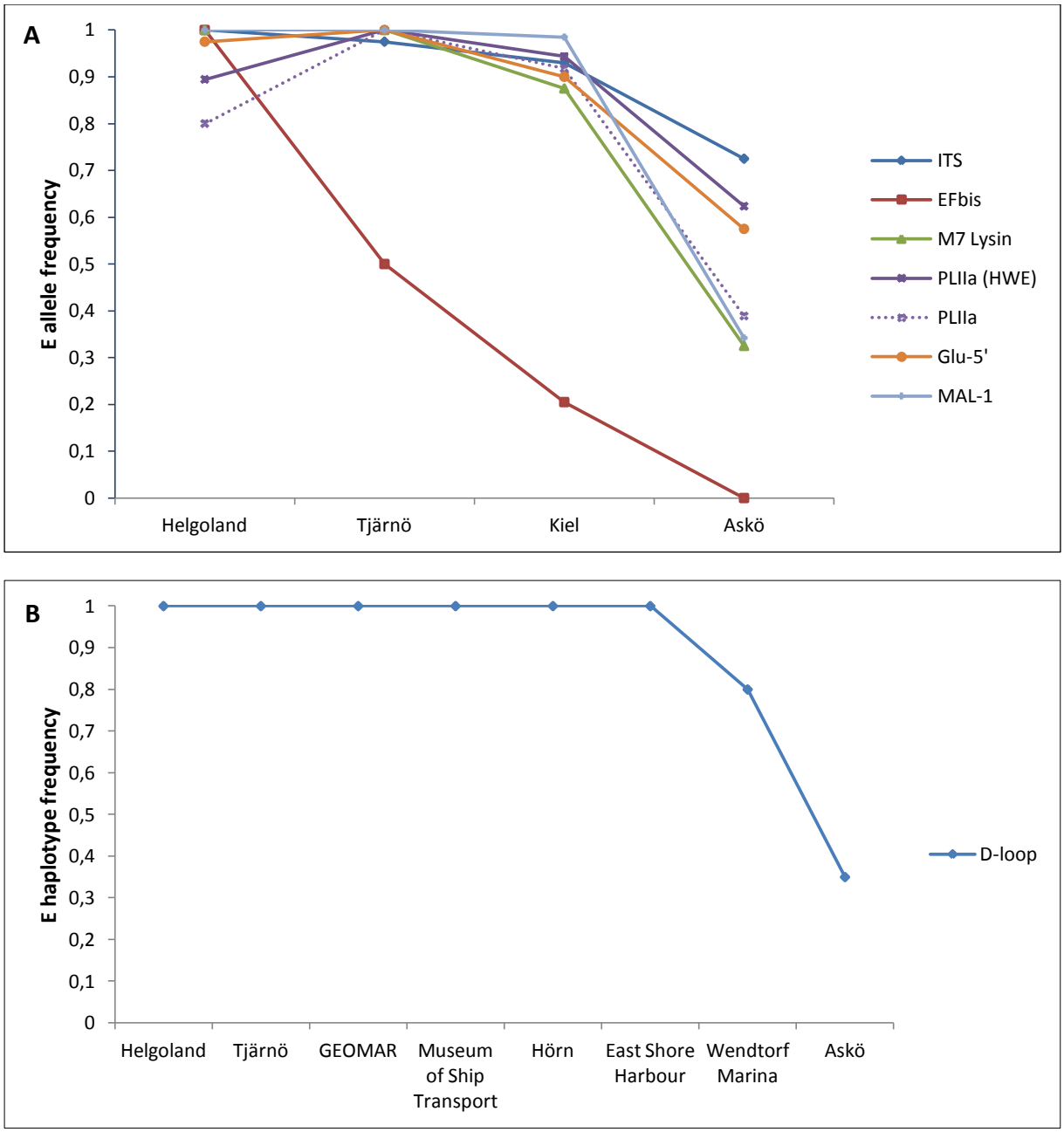

Figure 7. Frequencies of *M. edulis* alleles and haplotypes across the Baltic hybrid zone. A) Nuclear loci with pooled data for Kiel. In the case of PLIIa raw data as well as corrected values under the assumption of Hardy-Weinberg equilibrium (HWE) were plotted. B) Mitochondrial D-loop. Notable differences were observed between inner and outer Kiel Fjord populations.

The descriptive analysis of the population structure within the Baltic Sea was corroborated by Bayesian inference models, which evidenced (1) the existence of three genetic entities corresponding to *M. edulis*, *M. trossulus* and *M. galloprovincialis* in the total sample set, (2) the gradual introgression of *M. trossulus* alleles from western to eastern localities (Tjärnö <

Kiel < Askö) and (3) the slight movement of *M. galloprovincialis* alleles into the gene pool of the Kiel Fjord population (Figure 8).

Table 8. Frequencies of mac-1 alleles in the ten populations analysed. GEO = GEOMAR West Shore Campus, SM = Museum of Ship Transport, FH = East Shore Harbour, MAR = Wendtorf Marina, SC = Penn Cove, V = Vigo, H = Helgoland, AK = Askö, TJ = Tjärnö.

Allele [bp]	**Allele frequency**									
	GEO	SM	FH	Hörn	MAR	SC	V	H	AK	TJ
352	0	0	0	0	0	0	0	0	0,025	0
354	0	0	0	0	0	0	0	0,05	0	0
358	0	0,025	0	0,025	0,05	0	0	0,025	0	0
359	0,025	0	0	0	0	0	0,025	0	0	0
360	0	0	0	0,05	0,05	0	0	0,05	0	0
361	0,35	0,275	0,325	0,5	0,325	0	0	0,425	0,075	0,275
362	0,025	0,05	0	0,025	0	0	0	0	0	0
363	0,35	0,325	0,3	0,2	0,175	0	0,125	0,25	0,025	0,35
364	0,025	0,075	0,175	0,1	0,225	0	0,05	0,1	0	0,2
366	0,025	0,025	0,05	0,025	0	0	0	0	0	0
367	0,05	0,05	0	0	0,05	0	0	0,075	0	0,075
368	0	0	0,05	0	0	0,025	0	0	0,05	0,05
369	0,125	0,05	0,025	0	0,025	0	0	0	0,6	0
371	0	0	0	0	0	0,05	0	0	0	0
393	0,025	0,125	0,075	0,075	0,1	0	0	0,025	0	0,05
397	0	0	0	0	0	0	0,6	0	0	0
400	0	0	0	0	0	0	0,05	0	0	0
407	0	0	0	0	0	0	0,025	0	0	0
417	0	0	0	0	0	0	0,025	0	0	0
424	0	0	0	0	0	0	0,1	0	0	0
588	0	0	0	0	0	0,025	0	0	0	0
600	0	0	0	0	0	0,1	0	0	0	0
602	0	0	0	0	0	0,05	0	0	0,05	0
604	0	0	0	0	0	0,2	0	0	0,075	0
605	0	0	0	0	0	0,175	0	0	0	0
606	0	0	0	0	0	0,225	0	0	0,1	0
608	0	0	0	0	0	0,025	0	0	0	0
611	0	0	0	0	0	0,025	0	0	0	0
631	0	0	0	0	0	0,075	0	0	0	0
633	0	0	0	0	0	0,025	0	0	0	0

Table 9. Estimated allele frequencies for PLIIa (assuming Hardy-Weinberg equilibrium), ITS and MAL-1 in the reference populations of *M. trossulus* (Penn Cove), *M. edulis* (Helgoland) and *M. galloprovincialis* (Vigo). E/G = frequency of the *M. edulis*/*M. galloprovincialis* allele; G/T = frequency of the *M. galloprovincialis*/*M. trossulus* allele; T = frequency of the *M. trossulus* allele.

	PLIIa		**ITS**		**MAL-1**	
	E/G	G/T	E/G	T	E/G	T
Penn Cove	0,00	1,00	0,00	1,00	0,00	1,00
Vigo	0,39	0,61	1,00	0,00	1,00	0,00
Helgoland	0,89	0,11	1,00	0,00	1,00	0,00

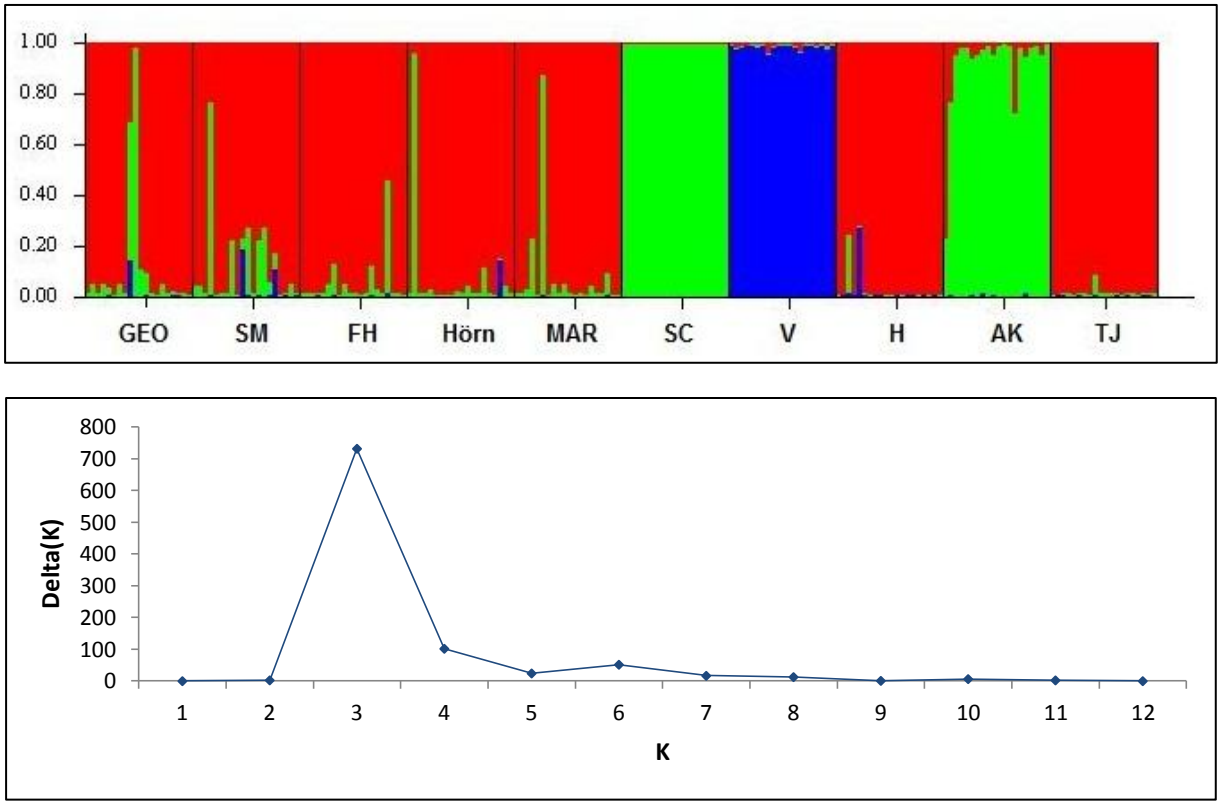

Figure 8. Results of the STRUCTURE analysis. Upper image: bar plot showing the level of admixture and introgression at the ten sampling sites. GEO = GEOMAR West Shore Campus, SM = Museum of Ship Transport, FH = East Shore Harbour, MAR = Wendtorf Marina, SC = Penn Cove, V = Vigo, H = Helgoland, AK = Askö, TJ = Tjärnö. One vertical line corresponds to one mussel sampled at the respective location, where the y axis indicates the proportion of alleles the individual carries from one or more genetic units, each of which is represented by a different colour. Lower image: determination of the true number of populations according to Evanno et al. (2005). The peak of ΔK implies the presence of three genetic clusters in the data set, which is consistent with allelic content stemming from the three species (*M. edulis, M. trossulus, M. galloprovincialis*) analysed in the study.

Moreover, this analysis clearly illustrated that the reference populations of *M. edulis* and *M. galloprovincialis* were not completely allopatric, but somewhat introgressed by non-native alleles. Given these observations, the extent of hybridization might be even higher, but not detectable owing to the weak discriminative power of some markers (Table 9).

Considering variation at both nuclear and cytoplasmic markers, the degree of population differentiation was further quantified by computations of pairwise F_{ST}s, which demonstrated that the five demes in the Kiel Fjord build a single, panmictic population that is distinct from populations of other Baltic and non-Baltic sites (Table 10). Regarding the mitochondrial D-loop, Helgoland, Tjärnö and Kiel showed no significant differences from each other, but had usually diverged from Askö, suggesting strong genetic shifts at the transition zone between inner and outer Baltic (Table 10). The only exception was Wendtorf Marina, where notable gene exchange with demes from the innermost basin resulted in insignificant deviations from the Askö population. F_{ST} values between Penn Cove and the other populations were constantly large, implying almost complete separation of North

American mussels, which is consistent with the capture of the *M. edulis* F genome by Baltic *M. trossulus*.

Table 10. Pairwise F_{ST}s according to Weir & Cockerham (1984). Lower diagonal: nuclear loci (EFbis, Glu-5', M7 Lysin, mac-1). Upper diagonal: mitochondrial D-loop. Significant values after Bonferroni correction are marked in red (see Appendix). GEO = GEOMAR West Shore Campus, SM = Museum of Ship Transport, FH = East Shore Harbour, MAR = Wendtorf Marina, SC = Penn Cove, V = Vigo, H = Helgoland, AK = Askö, TJ = Tjärnö.

	GEO	SM	FH	Hörn	MAR	Kiel	SC	H	AK	TJ	V
GEO		0,00	0,00	0,00	0,16	-	1,00	0,00	0,63	0,00	-
SM	0,00		0,00	0,00	0,16	-	1,00	0,00	0,63	0,00	-
FH	0,00	-0,01		0,00	0,16	-	1,00	0,00	0,63	0,00	-
Hörn	0,02	0,01	0,00		0,16	-	1,00	0,00	0,63	0,00	-
MAR	0,02	0,01	-0,01	0,00		-	0,83	0,16	0,31	0,16	-
Kiel	-	-	-	-	-		0,94	-0,01	0,72	-0,01	-
SC	0,45	0,46	0,48	0,54	0,51	0,46		1,00	0,76	1,00	-
H	0,34	0,41	0,38	0,39	0,37	0,34	0,72		0,63	0,00	-
AK	0,21	0,21	0,24	0,29	0,26	0,23	0,35	0,60		0,63	-
TJ	0,06	0,09	0,06	0,08	0,05	0,07	0,57	0,19	0,36		-
V	0,61	0,62	0,63	0,66	0,64	0,60	0,71	0,71	0,67	0,63	

Table 11. AMOVA results. SSD = sum of squared differences, Df = degrees of freedom, Va & Vb = variance components among and within populations, % Var = percentage of variation.

	Among populations				Within populations					
	SSD	Df	Va	% Var	SSD	Df	Vb	% Var	F_{ST}	p
Nuclear loci										
EFbis	53,82	9	0,15	55,00	46,75	390	0,12	45,00	0,55	0
M7 Lysin	38,11	9	0,12	58,48	28,77	348	0,08	41,52	0,58	0
Glu-5'	62,76	9	0,17	70,19	28,58	390	0,07	29,81	0,70	0
mac-1	26,29	9	0,06	14,45	146,93	390	0,38	85,55	0,14	0
Total	180,97		0,5	43,33	251,02		0,65	56,67	0,43	0
Mitochondrial loci										
D-loop	23,53	8	0,14	76,16	7,75	171	0,05	23,84	0,76	0

The genetic differentiation between populations was generally less pronounced for nuclear loci than for the cytoplasmic marker (43,33 % compared to 76,16 %; Table 11). Within the North Sea/Baltic transect divergence between populations increased linearly with geographic distance (Figure 9; Mantel test: p = 0,0407; r = 0,9383; Reduced Major Axis regression: y = 7,156e-04x – 0,4155; R^2 = 0,88). In agreement with the outcomes of the STRUCTURE analysis, stronger introgression of *M. trossulus* alleles into a population (Askö > Kiel > Tjärnö > Helgoland = Vigo) resulted in smaller differences from Penn Cove. Likewise coinciding, fixation indices between Vigo and other populations were consistently high, albeit smaller for hybrid populations (Kiel, Askö, Tjärnö) than for reference samples of *M. edulis* (Helgoland) and *M. trossulus* (Penn Cove). Analysed across all populations, the among-sample variation for nuclear loci was mainly caused by Glu-5', whereas mac-1 showed the highest variance within populations.

Table 12. Observed and expected heterozygosities according to Hardy-Weinberg. Significant p values after Bonferroni correction for 10 samples ($p < 0{,}005$) are highlighted in red, while missing data indicate monomorphy of a locus. H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity.

Locus	H_{obs}	H_{exp}	p	Locus	H_{obs}	H_{exp}	p
GEOMAR				**Penn Cove (*M. trossulus*)**			
EFbis	0,40	0,38	1,00	EFbis	-	-	-
M7 Lysin	0,05	0,15	0,08	M7 Lysin	-	-	-
Glu-5’	0,30	0,26	1,00	Glu-5’	-	-	-
mac-1	0,35	0,77	0,00	mac-1	0,45	0,88	0,00
Museum of Ship Transport				**Vigo (*M. galloprovincialis*)**			
EFbis	0,30	0,26	1,00	EFbis	0,25	0,22	1,00
M7 Lysin	0,26	0,37	0,24	M7 Lysin	-	-	-
Glu-5’	0,30	0,27	1,00	Glu-5’	-	-	-
mac-1	0,35	0,81	0,00	mac-1	0,45	0,62	0,03
East Shore Harbour				**Helgoland (*M. edulis*)**			
EFbis	0,40	0,33	0,55	EFbis	-	-	-
M7 Lysin	0,10	0,10	1,00	M7 Lysin	-	-	-
Glu-5’	0,20	0,18	1,00	Glu-5’	0,05	0,05	1,00
mac-1	0,60	0,78	0,02	mac-1	0,65	0,75	0,15
Hörn				**Askö**			
EFbis	0,30	0,33	1,00	EFbis	-	-	-
M7 Lysin	0,16	0,23	0,26	M7 Lysin	0,47	0,46	1,00
Glu-5’	0,10	0,10	1,00	Glu-5’	0,25	0,50	0,03
mac-1	0,40	0,71	0,00	mac-1	0,15	0,63	0,00
Wendtorf Marina				**Tjärnö**			
EFbis	0,35	0,36	1,00	EFbis	0,40	0,51	0,39
M7 Lysin	0,26	0,23	1,00	M7 Lysin	-	-	-
Glu-5’	0,10	0,10	1,00	Glu-5’	-	-	-
mac-1	0,55	0,82	0,01	mac-1	0,45	0,77	0,00

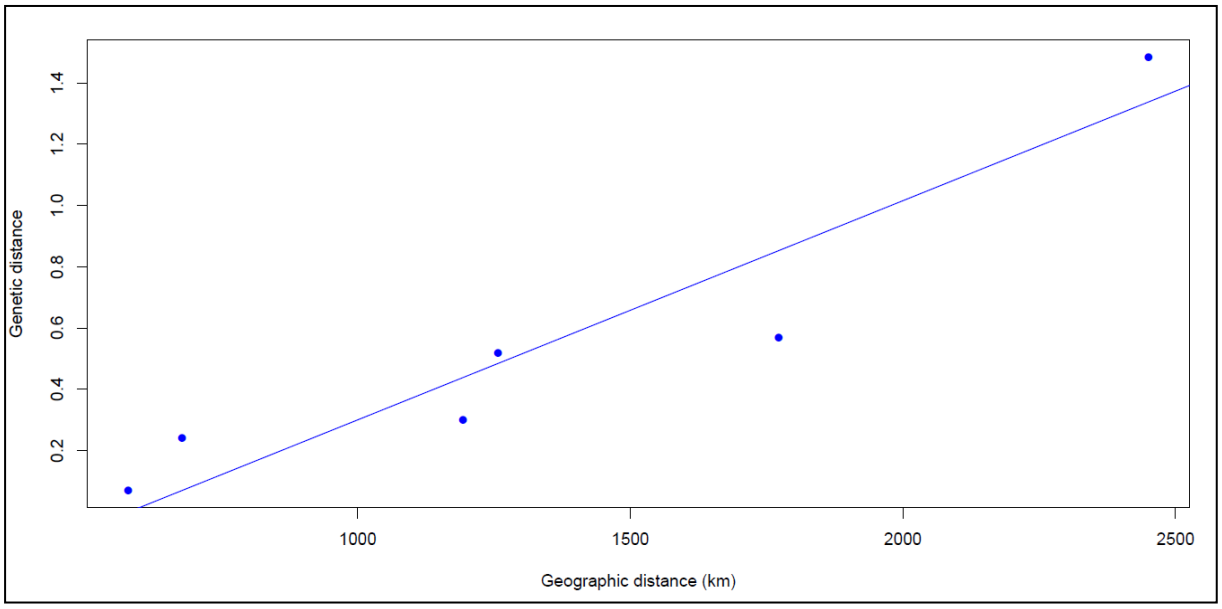

Figure 9. Change of genetic distance with increasing separation between localities for *Mytilus* populations along the North Sea/Baltic transect (nuclear data). Geographic distances were calculated from Stuckas et al. (2009): Tjärnö-Kiel (579 km), Helgoland-Tjärnö (678 km), Kiel-Askö (1193 km), Helgoland-Kiel (1257 km), Tjärnö-Askö (1772 km), Helgoland-Askö (2450 km).

By contrast, EFbis and M7 Lysin were similarly variable both between and within sites (Table 11). Local populations generally corresponded to random mating expectations, as no strong deviations from Hardy-Weinberg equilibrium were observed. However, a notable exception existed (Table 12). In almost all cases a significant heterozygote deficit was detected at locus mac-1, though this might be a statistical artefact attributable to an insufficient sample size compared with the multi-allelic status of this marker.

6.2 Morphological variation in the Baltic Sea & phenotypic similarity to reference species

UPGMA dendrograms showed that the reference populations of *M. edulis*, *M. trossulus* and *M. galloprovincialis* as well as mussels from the inner Baltic formed four distinguishable morphological groups, whereas Tjärnö and Kiel mytilids exhibited a higher phenotypic variability (Figure 10 to 12). This became evident by the following observations:

- Firstly, usage of randomly chosen subsamples provided no evidence for the morphotypic identity of western Baltic and Skagerrak populations (au ≤ 80).
- Secondly, assortment of Tjärnö individuals was not consistent, but depended on the choice of mussels from Kiel (Figure 11). In turn, this means that even smaller samples from the Fjord added enough variability to the statistics to strongly influence the outcome.
- Thirdly, phenotypes in Kiel were not related to sampling site, which is consistent with the panmictic nature of the five demes (Figure 10).

In spite of this, two lower nodes were not only supported (cluster 1: au = 97; cluster 2: au = 85), but also comprised a reasonable amount of individuals (> 15 % of all mussels from Kiel), indicating potential morphological subgroups. I subsequently tried to infer the relation of these mussels to the other populations, randomly choosing 17 individuals from each location to equalize sample sizes. While individuals of cluster 1 showed a higher similarity to Helgoland (Figure 12A), those of cluster 2 resembled the Penn Cove Type (Figure 12B). Although cluster 1 could indeed be described as a morphotype based on my definition (au ≥ 80; ≥ 15 individuals), these results clearly demonstrate that mussels of the western Baltic Sea and Skagerrak build a morphological continuum from *M. edulis*- to *M. trossulus*-like forms and that distinct population-specific identities do not exist for Tjärnö and Kiel.

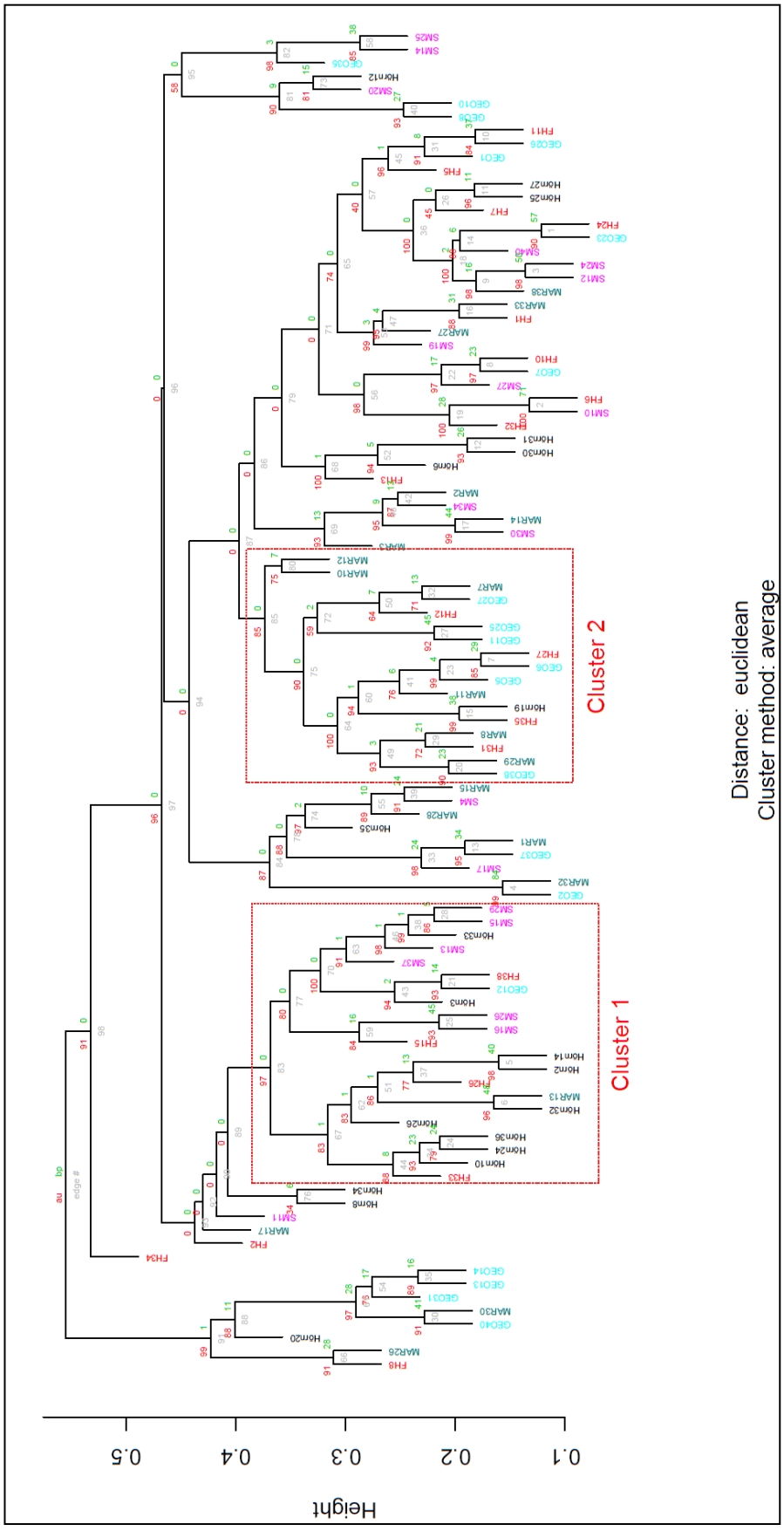

Figure 10. UPGMA dendrogram based on Euclidean distances between Kiel data. The five demes ● Wendtorf Marina, ● East Shore Harbour, ● Hörn, ● GEOMAR, ● Museum of Ship Transport were morphologically undifferentiated. Potential morphotypic clusters are framed in red.

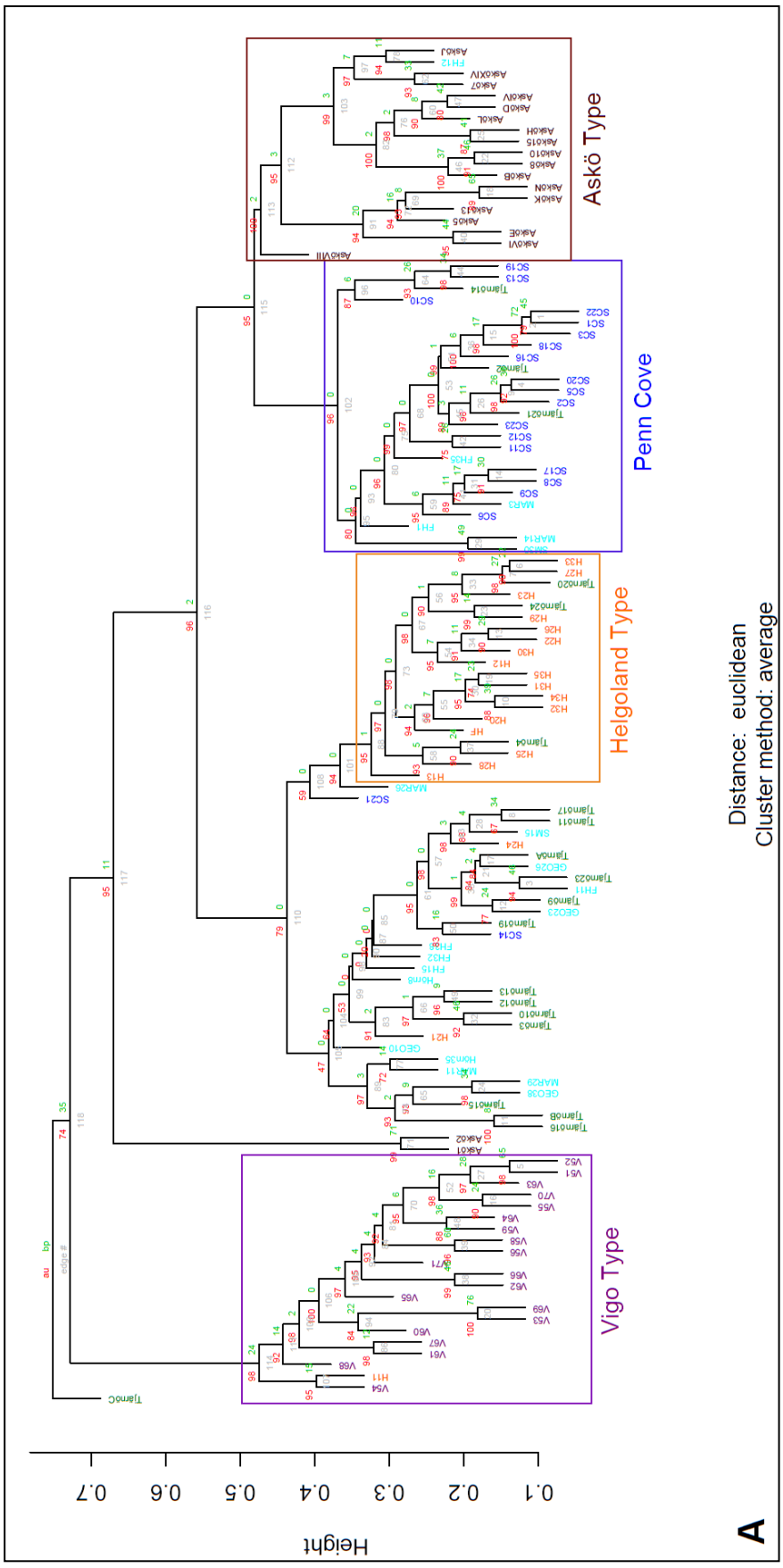

Figure 11. UPGMA dendrograms with random subsamples from Kiel. No distinct western Baltic morphotypes could be identified, as clusters were poorly supported and the selection of individuals from the Kiel Fjord influenced the affinities of Tjärnö mussels. ● Vigo, ● Helgoland, ● Penn Cove, ● Tjärnö, ● Kiel, ● Askö.

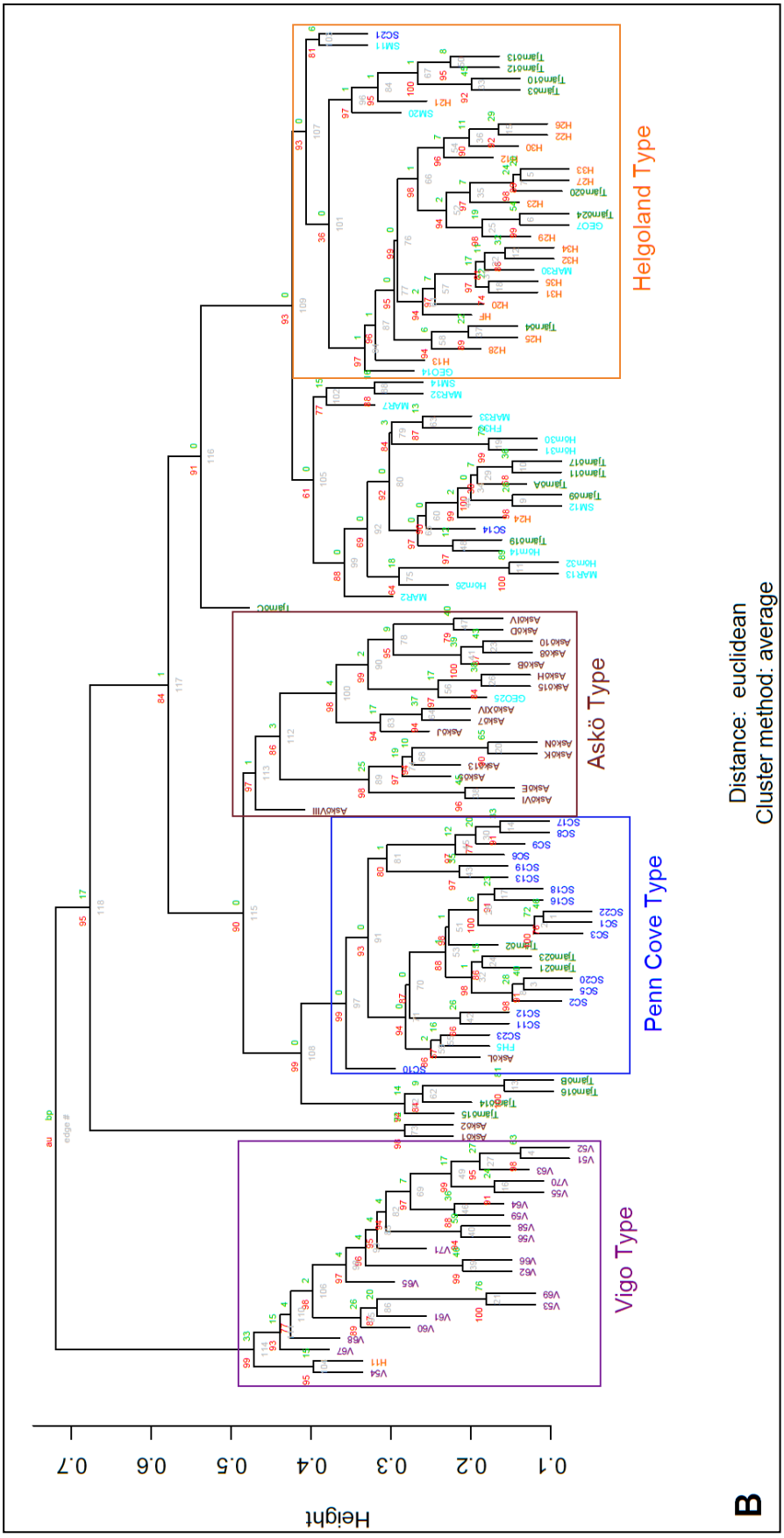

Figure 11 (continued). ● Vigo, ● Helgoland, ● Penn Cove, ● Tjärnö, ● Kiel, ● Askö.

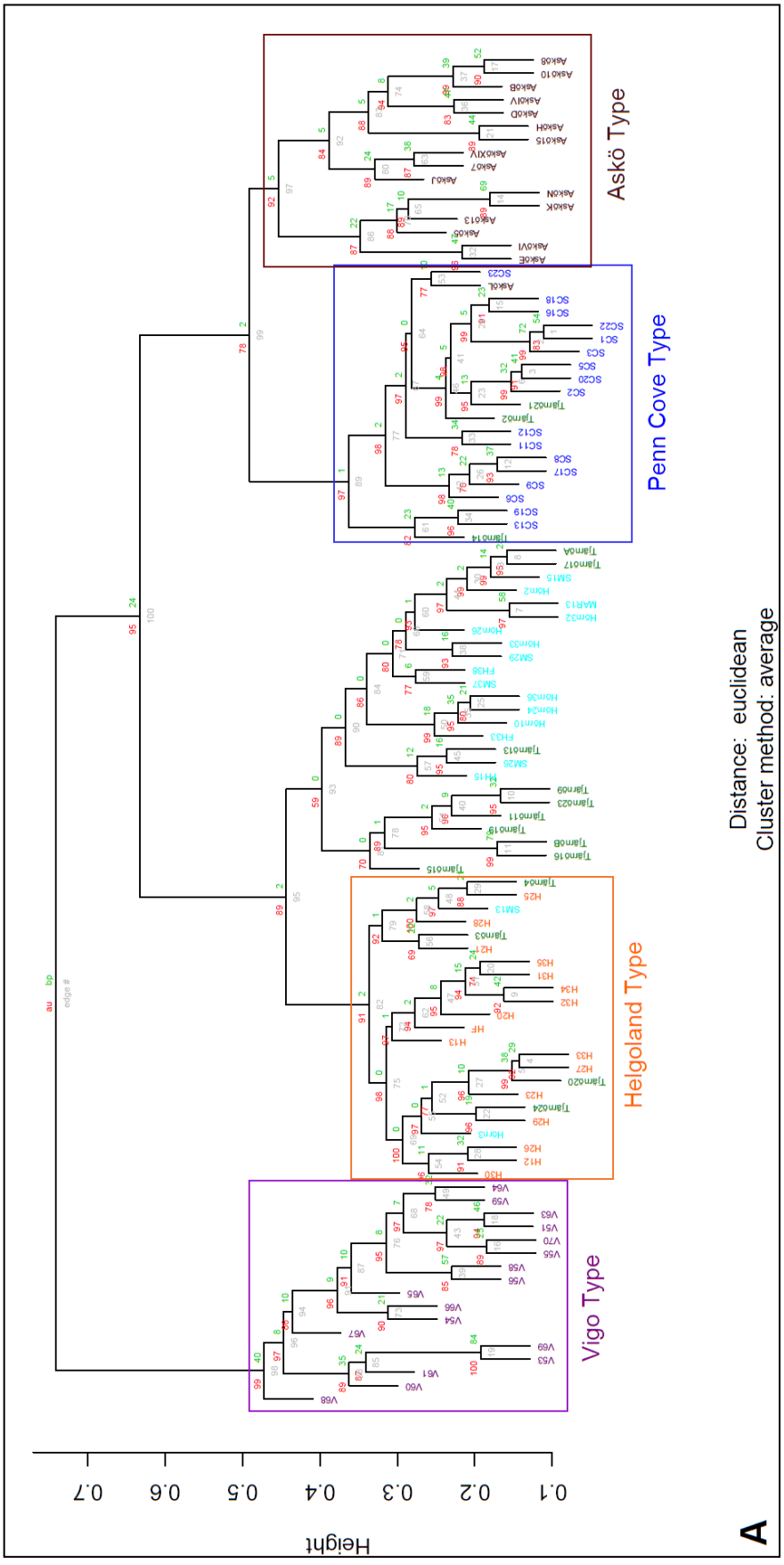

Figure 12. UPGMA dendrogram with potential morphotype subclusters from Kiel. While individuals of cluster 1 (A) were *M. edulis*-like, individuals of cluster 2 (B) showed a higher affinity to *M. trossulus*. ● Vigo, ● Helgoland, ● Penn Cove, ● Tjärnö, ● Kiel, ● Askö.

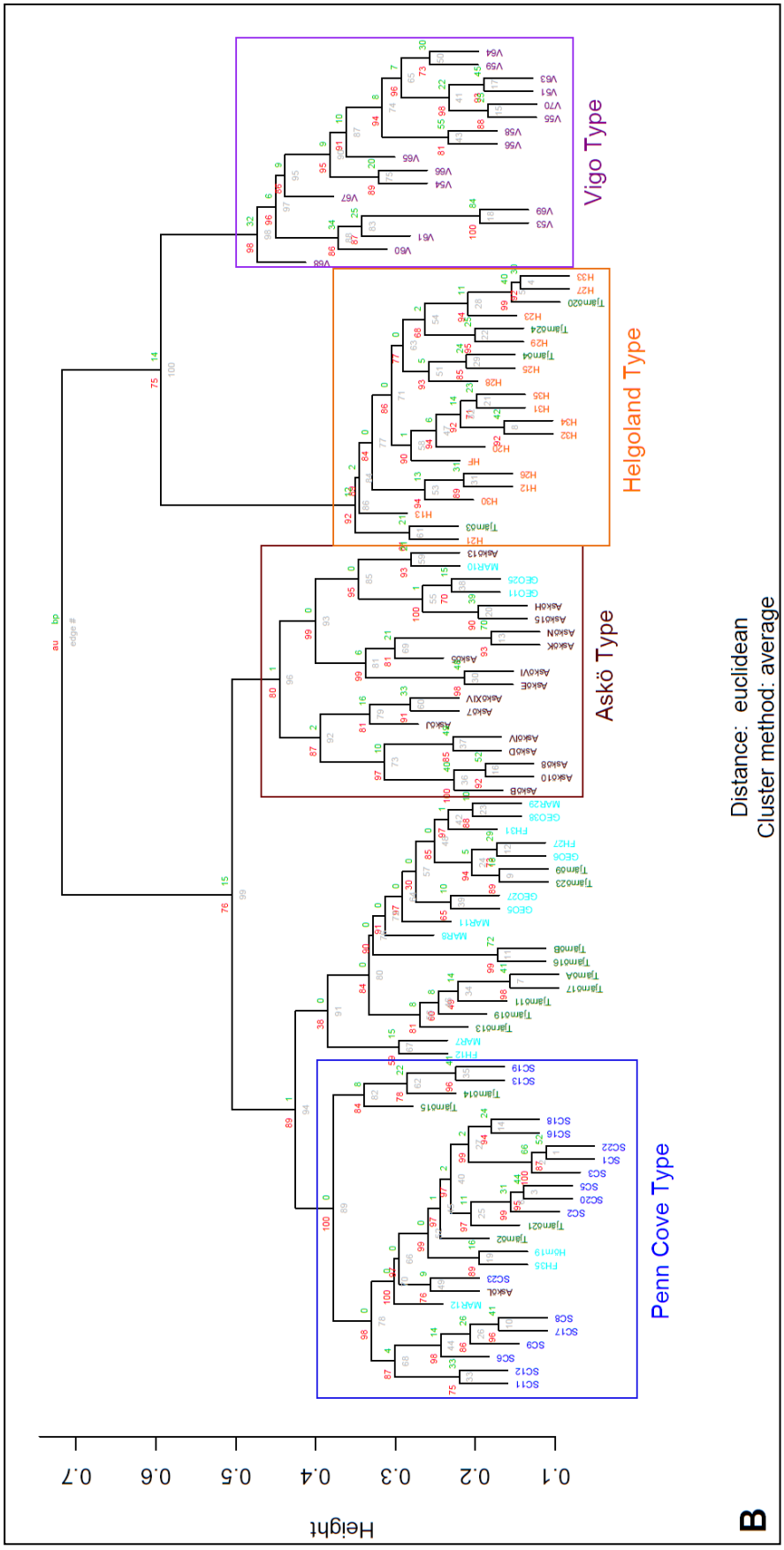

Figure 12 (continued). ● Vigo, ● Helgoland, ● Penn Cove, ● Tjärnö, ● Kiel, ● Askö.

Table 13. Global output of the ANOSIM.

Global test			
Global R	p	No. of permutations	No. of permutated statistics ≥ Global R
0,916	0,1%	9999	0

Table 14. Results of the pairwise comparisons following ANOSIM. To account for increasing type I error, multiple tests were Bonferroni corrected according to Rice (1983).

Pairwise comparisons					
Morphotype	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number ≥ Observed
Askö-Helgoland	0,997	0,01	Very large	9999	0
Askö-Penn Cove	0,699	0,01	Very large	9999	0
Askö-Vigo	0,993	0,01	Very large	9999	0
Helgoland-Penn Cove	0,997	0,01	Very large	9999	0
Helgoland-Vigo	0,901	0,01	Very large	9999	0
Penn Cove-Vigo	0,982	0,01	Very large	9999	0

In agreement with the genetic status Askö had the greatest resemblance to Penn Cove. Interestingly, despite the closer phylogenetic relationship between *M. edulis* and *M. galloprovincialis*, mussels from Helgoland usually bore more morphological similarity to Penn Cove and Askö than Vigo, which built an outgroup to all other clusters.

The morphological distinctiveness of Helgoland, Vigo, Penn Cove and Askö populations was supported by ANOSIM and SIMPER analyses (Table 13 to 15). In four out of six cases (Askö-Helgoland, Helgoland-Penn Cove, Askö-Vigo, Penn Cove-Vigo), the differences between morphotypes were mainly determined by shell thickness (tck) and weight (wg; Table 15). These morphometric characters did not only have a high single discriminative power (tck: ≥ 33,48 %; wg: ≥ 20,24 %), but usually explained > 50 % of the variances, when taken together. However, other parameters became more important, where shell stability was comparable between phenotypes. For instance, the dissimilarity between Askö and Penn Cove was predominately caused by the ligamentary angle (la) as well as the distance between the posterior adductor muscle scar and the ventral shell margin (pamv), which contributed each at least 10 % to the differences. Besides, the diameters of the anterior (aam) and posterior (pam) adductor muscle scars and the point of maximum shell width (mwid) were significant factors for the differentiation of Vigo from Helgoland morphotypes. In terms of these parameters, the four morphological groups can be defined as follows (Table 15):

- Vigo type: greatest shell weight and thickness, weakest anterior adductor muscle and comparatively small, high-positioned posterior adductor muscle, lowest point of maximum shell width, small ligamentary angle

- Helgoland type: thick, heavy shell, strongest adductor muscles, medium-sized ligamentary angle, distance between posterior adductor muscle and ventral shell margin rather short, highest point of maximum shell width
- Penn Cove type: thin and light shell, small anterior adductor muscle, posterior adductor muscle with intermediate strength and relatively low position, small ligamentary angle, intermediate point of maximum shell width
- Askö type: shell extremely fragile, large ligamentary angle, weak adductor muscles, longest distance between posterior adductor muscle and ventral shell margin, intermediate point of maximum shell width

Table 15. Results of the SIMPER analysis. Av.Value = average value of the standardized parameter for a morphotype; Av.Sq.Dist. = average squared distance; Sq.Dist/SD = squared distance divided by standard deviation; Contrib% = contribution of the parameter to the dissimilarity between morphotypes in per cent; Cum.% = cumulative contribution of parameters to the dissimilarity between morphotypes in per cent.

Groups Askö & Helgoland: Average squared distance = 7108,15						
Variable	Askö Av.Value	Helgoland Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
tck	27,9	76	2,38E+03	3,04	33,48	33,48
wg	27,9	64,9	1,44E+03	2,3	20,24	53,72
pal	69,1	90,9	591	1,25	8,32	62,03
aam	66	83,4	546	0,96	7,68	69,72
pamv	91,9	71,8	461	1,43	6,49	76,2
pam	69	87,1	433	1,07	6,1	82,3
lig	66	83,9	347	1,79	4,88	87,18
mwid	74,2	82,6	290	0,76	4,07	91,25
hp	74,3	81,2	185	0,7	2,61	93,86
la	83,2	75,2	171	0,82	2,41	96,27
ht	82,9	91,6	120	0,89	1,69	97,96
wid	81,4	75,3	74,5	0,92	1,05	99,01
pamp	82,5	80,1	70,5	0,6	0,99	100
Groups Askö & Penn Cove: Average squared distance = 2547,10						
Variable	Askö Av.Value	Penn Cove Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
pamv	91,9	67,8	639	1,76	25,1	25,1
la	83,2	69,1	320	0,89	12,56	37,67
mwid	74,2	72,3	219	0,8	8,59	46,26
pam	69	78,3	202	0,6	7,94	54,2
aam	66	68,1	201	0,77	7,91	62,1
pal	69,1	77,3	184	0,86	7,21	69,31
lig	66	78,1	173	1,3	6,8	76,11
tck	27,9	38,6	156	1,07	6,12	82,23
hp	74,3	71,5	135	0,78	5,28	87,51
wg	27,9	36,4	117	0,99	4,58	92,1
pamp	82,5	78,4	86,2	0,63	3,39	95,48
wid	81,4	77,2	66	0,84	2,59	98,07
ht	82,9	79,8	49,1	0,9	1,93	100

Table 15 (continued).

Groups Helgoland & Penn Cove: Average squared distance = 4017,72						
Variable	Helgoland Av.Value	Penn Cove Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
tck	76	38,6	1,47E+03	2,32	36,63	36,63
wg	64,9	36,4	867	1,95	21,59	58,22
aam	83,4	68,1	408	0,83	10,15	68,37
pal	90,9	77,3	254	1,04	6,33	74,7
mwid	82,6	72,3	204	0,77	5,08	79,78
pam	87,1	78,3	195	1,08	4,87	84,65
hp	81,2	71,5	181	0,7	4,51	89,15
ht	91,6	79,8	169	1,29	4,21	93,36
la	75,2	69,1	75,2	0,73	1,87	95,23
pamv	71,8	67,8	71,4	0,91	1,78	97,01
lig	83,9	78,1	53,2	0,96	1,32	98,33
pamp	80,1	78,4	33,9	0,73	0,84	99,18
wid	75,3	77,2	33,1	0,79	0,82	100
Groups Askö & Vigo: Average squared distance = 8481,14						
Variable	Askö Av.Value	Vigo Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
tck	27,9	82,7	3,22E+03	2,18	37,92	37,92
wg	27,9	81	2,94E+03	2,55	34,66	72,59
la	83,2	64,2	522	1,01	6,16	78,74
lig	66	84,2	387	1,25	4,57	83,31
aam	66	53,5	367	0,7	4,33	87,64
mwid	74,2	66,3	302	0,81	3,56	91,2
pal	69,1	76,2	180	0,82	2,12	93,32
hp	74,3	75,4	146	0,81	1,72	95,04
pam	69	70,7	111	0,62	1,3	96,35
pamp	82,5	80,6	94,8	0,66	1,12	97,46
wid	81,4	82,7	76,3	0,62	0,9	98,36
pamv	91,9	87,5	71,1	0,87	0,84	99,2
ht	82,9	88	67,7	0,72	0,8	100
Groups Helgoland & Vigo: Average squared distance = 3708,47						
Variable	Helgoland Av.Value	Vigo Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
aam	83,4	53,5	1,08E+03	1,25	29,17	29,17
wg	64,9	81	394	0,93	10,61	39,78
mwid	82,6	66,3	388	1,03	10,45	50,23
pam	87,1	70,7	379	1,2	10,23	60,46
pal	90,9	76,2	299	1,05	8,07	68,53
pamv	71,8	87,5	293	1,19	7,91	76,44
tck	76	82,7	281	0,99	7,59	84,02
la	75,2	64,2	197	1,03	5,32	89,34
hp	81,2	75,4	139	0,65	3,74	93,08
wid	75,3	82,7	109	0,67	2,95	96,03
pamp	80,1	80,6	53,5	0,69	1,44	97,47
lig	83,9	84,2	49,3	0,67	1,33	98,8
ht	91,6	88	44,4	0,78	1,2	100

Table 15 (continued).

Groups Penn Cove & Vigo: Average squared distance = 6028,52						
Variable	Penn Cove Av.Value	Vigo Av.Value	Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
tck	38,6	82,7	2,17E+03	1,83	35,92	35,92
wg	36,4	81	2,09E+03	2,26	34,69	70,61
pamv	67,8	87,5	437	1,56	7,24	77,85
aam	68,1	53,5	355	0,91	5,89	83,75
pam	78,3	70,7	179	0,65	2,97	86,71
mwid	72,3	66,3	154	0,84	2,56	89,27
la	69,1	64,2	114	0,9	1,9	91,17
hp	71,5	75,4	110	0,79	1,82	92,99
wid	77,2	82,7	96	0,64	1,59	94,58
ht	79,8	88	94,5	0,99	1,57	96,15
lig	78,1	84,2	85,5	0,67	1,42	97,57
pal	77,3	76,2	84	0,79	1,39	98,96
pamp	78,4	80,6	62,6	0,65	1,04	100

Analysed across all variables, the greatest distances were observed between Askö and Vigo, while smallest dissimilarities were found between Askö and Penn Cove – findings that are in perfect agreement with the results of the cluster analyses. Nevertheless, different to the dendrograms SIMPER outcomes suggested a closer relationship of Helgoland with Vigo than with Penn Cove or Askö, which is probably a consequence of the stringent reduction in data (from hybrid populations) and thus variance.

6.3 Shell morphology: genetic and salinity effects

To disclose associations between phenotype, genotype and habitat salinity Mantel tests were conducted applying two main approaches (2 markers/Vigo included vs. 5 markers/Vigo excluded). Regardless of differences in terms of quantity, both ways produced qualitatively identical outcomes (Table 17). While no links between genetic status, salinity regime and shell morphology were found in the western Baltic and Skagerrak (Table 16 & 17), highly significant correlations were obtained with the other sample sets, even if influences of one factor were removed. In turn, this means that the statistical effects in the Baltic data set were only due to the inclusion of Askö. Actually, this is what could have been expected, given the fact that phenotypes in Kiel and Tjärnö were more variable, while the other populations were characterized by distinct shell traits. Although r values were comparatively low in case of statistical significance, this does not mean that the correlations were biologically irrelevant, as the power of Mantel tests is usually reduced due to the transformation of sample data into distance matrices (Dutilleul et al. 2000; Legendre et al. 2000). Depending on the sample set used for the analyses, the relative importance of salinity and genetics in explaining the morphological identities varied, so that it is not possible to infer from these results which factor is of higher weight.

Table 16. Results of the simple Mantel test for Kiel, using five molecular markers. No significant genotype-phenotype relationships were observed.

Simple Mantel test	**Kiel**
Morphotype x Genotype	
p value	0,11
r value	0,05886
Confidence limits of r (95 %/99 %)	0,0800/0,1149

Table 17. Results of the partial Mantel tests. Either the influences of salinity (*Morphotype x Genotype*) or genetics (*Morphotype x Salinity*) were controlled. The effect of the *M. galloprovincialis* allele was considered, only if the two highly diagnostic markers EFbis and Glu-5' were used, but neglected otherwise (5 markers). Baltic + Species = data from both Baltic and reference populations (with or without Vigo); Baltic = only data from the Baltic/Skagerrak hybrid populations (Kiel, Askö, Tjärnö); Species = only data from the reference populations (with or without Vigo); outer Baltic = only data from Kiel and Tjärnö.

Partial Mantel tests	**Baltic + Species**	**Species**	**Baltic**	**Outer Baltic**
5 markers				
Morphotype x Genotype				
p value	**1e-04**	**1e-04**	**1e-04**	0,0553
r value	0,2146	1,072e-08	0,3416	0,07483
Confidence limits of r (95 %/99 %)	0,0727/0,1089	4,66e-10/9,38e-10	0,0853/0,1244	0,0771/0,1109
Morphotype x Salinity				
p value	**1e-04**	**1e-04**	**0,001**	0,9761
r value	0,378	1,072e-08	0,173	-0,1053
Confidence limits of r (95 %/99 %)	0,0694/0,0997	9,34e-10/1,88e-09	0,0919/0,1312	0,0933/0,1341
2 markers				
Morphotype x Genotype				
p value	**1e-04**	**1e-04**	-	-
r value	0,3309	0,5332	-	-
Confidence limits of r (95 %/99 %)	0,0621/0,0909	0,0543/0,0872	-	-
Morphotype x Salinity				
p value	**1e-04**	**1e-04**	-	-
r value	0,3881	0,1665	-	-
Confidence limits of r (95 %/99 %)	0,0520/0,0773	0,0628/0,0922	-	-

7 Discussion

7.1 The genetic structure of the Baltic hybrid zone with particular reference to the Kiel Fjord *Mytilus* population

Analysing variation at one mitochondrial and seven autosomal genes, this study reveals that blue mussels in the North Sea/Baltic transition zone are principally backcross hybrids of later generation (99 % in Kiel; 81 % in Tjärnö). Although a picture of noticeable gene flow between Baltic *M. trossulus* and *M. edulis* soon began to emerge after genotyping mussels at neutral nuclear and mitochondrial loci instead of allozymes (e.g. Borsa et al. 1999; Riginos et al. 2002; Quesada et al. 2003; Riginos & Cunningham 2005; Kijewski et al. 2006, 2011; Stuckas et al. 2009), it was not before the work by Stuckas et al. (2009) that movement of *M. trossulus* alleles into the gene pool of the Kiel Fjord population became apparent. Even so, these authors investigated a small number of individuals and few molecular markers, so that the amount of introgression might have been underestimated. This becomes clear, if one bears in mind that the high degree of hybridization seemed not to be solely caused by allele import at locus EFbis, given that hybrid indices were often greater than 1 – different to what could have been expected from recent allele frequency data (Stuckas et al. 2009; Kijewski et al. 2006, 2011). Besides, input of *M. galloprovincialis* gene variants was not considered and thus escaped detection in previous analyses.

Albeit unknown from the Kiel Fjord so far, occurrence of alien *M. galloprovincialis* alleles in the Baltic has recently been reported by Kijewski et al. (2006, 2011), who found a few introgressed individuals in the Danish Straits (Møn Island, Tårbæk) and along the Polish (Puck Bay, Gulf of Gdańsk) as well as Swedish (Askö) coasts. The origin of these hybrids, however, remains to be determined. At least two different explanations are possible: (1) natural invasion of mussels from the Atlantic *M. edulis*-*M. galloprovincialis* mosaic hybrid zone (see Skibinski et al. 1978; Costeau et al. 1991; Bierne et al. 2002, 2003a) into the Baltic Sea followed by repeated backcrossing with indigenous populations, possibly favoured by northward shifts of the secondary contact area due to climate change (Hilbish et al. 2012); (2) human-mediated immigration of *M. galloprovincialis* and their intergrades into Baltic populations via ship traffic (e.g. transport in ballast water or fouling of boat hulls) and hybridization with native mytilids. Given the high potential for interspecific mating within the *M. edulis* species complex and the scarcity of documented contact zones between all three species (Wonham 2004: Pudget Sound, Washington, USA; British Columbia, Canada; Beaumont et al. 2008: Loch Etive, Scotland), I rule out the option of simple admixture without local recombination here. Even if my data do not allow ultimate rejections of any of

these hypotheses, scenarios involving anthropogenic activities seem to be the most likely ones. Firstly, all findings of *M. galloprovincialis* alleles in the Baltic have been recorded in harbour-related areas with high cruise vessel or sailing yacht traffic. Secondly, intentional or accidental introductions of alien blue mussels have been shown to induce hybridization with native *Mytilus* species in other cases. One of the best examples is the import of Mediterranean *M. galloprovincialis* to multiple sites in the North Pacific for aquaculture purposes, which has resulted in intergradations with autochthonous mytilids (reviewed in Wonham 2004). Similarly, human-mediated, albeit unintended, invasions and gene introgression of this species have been reported from the southern coasts of Chile (Toro et al. 2005). Furthermore, commercial interests are known to have led to the successful establishment of *M. edulis* in British Columbia (Heath et al. 1995), while presumably unplanned introductions of North American *M. trossulus* have been proposed to explain the new occurrences of this species and its hybrids in Northern European waters (Väinölä & Strelkov 2011 and references therein). The low proportion of *M. galloprovincialis* alleles detected in the Kiel Fjord and other Baltic sites could indicate that the invasion was relatively recent or that mussels with predominately *M. galloprovincialis* background are not very competitive in the brackish, temperate waters of the Baltic Sea, which would explain why no pure individuals of this species were found. In fact, increasing evidence suggests that congeners of the *Mytilus edulis* species complex differ in their physiological abilities to cope with thermal as well as osmotic stress. While *M. trossulus* seems to be relatively well adapted to cold, oligohaline habitats, *M. galloprovincialis* is most tolerant of warmer temperatures and higher salinities (Hofmann & Somero 1996: protein denaturation; Braby & Somero 2006a: heart rate; Fields et al. 2006: enzyme kinetics; Evans & Somero 2010: protein phosphorylation; Tomanek & Zuzow 2010: proteomic responses to heat stress), as reflected by both their evolutionary histories and current biogeographical distributions (Vermeij 1991; Koehn 1991; Seed 1992; Sarver & Foltz 1993; Riginos & Cunningham 2005; Braby & Somero 2006b). Note, however, that only two molecular markers could reliably identify alleles specific to *M. galloprovincialis*, so that the true extent of gene flow might have been underestimated. This will also apply to the genome-wide level of introgressive hybridization between Baltic *M. trossulus* and *M. edulis*, in particular, if one takes into account that movement across a contact zone is usually more restricted for diagnostic than for polymorphic neutral loci (Brumfield et al. 2001). Therefore, it is questionable whether mussels in the Kiel Fjord actually retain a high fraction of *M. edulis* alleles as suggested by the population genetic analyses, although this would nicely agree with introgression patterns seen at mitochondrial loci (see below). In order to accurately assess the degree of hybridization in the Baltic Sea, further studies on the *Mytilus* hybrid zone should

thus consider development and application of additional markers such as (optimized) microsatellites (e.g. Presa et al. 2002; Varela et al. 2007; Lallias et al. 2009; Vidal et al. 2009; Ouagajjou et al. 2011) or the novel SNPs designed by Zbawicka et al. (2012), which could be especially advantageous in identifying the poorly differentiated species *M. edulis* and *M. galloprovincialis*. For this purpose, it will be necessary to use alternative reference populations, because the samples chosen in this study were obviously not completely allopatric. The two populations in Helgoland and Vigo seemed to be introgressed by *M. galloprovincialis* and *M. edulis* alleles, respectively – probably as a result of their geographical proximity to the outer edges of the *M. edulis*-*M. galloprovincialis* contact zone along the eastern Atlantic coast or due to anthropogenic invasions (Skibinski et al. 1978; Costeau et al. 1991; Luttikhuizen et al. 2002; Bierne et al. 2003a; Kijewski et al. 2011; Steinert et al. 2012). Besides, RFLP patterns potentially stemming from endonucleolysis of *M. trossulus* alleles (Heath et al. 1995) were found in both samples at locus PLIIa. Although this marker was not able to unambiguously discriminate between *M. trossulus* and *M. galloprovincialis*, presence of PLIIa *M. trossulus* alleles in the Helgoland population would not be surprising in view of slight gene introgression at other loci (Bierne et al. 2003b: EFbis, mac-1; Stuckas et al. 2009: EFbis, M7 Lysin), possibly linked to the recent detections of American foolish mussels in other North Sea locations (Väinölä & Strelkov 2011). However, to my knowledge this would be the first report of *M. trossulus* alleles in Spanish waters. Since evidence was only provided by one molecular marker and no detailed sequence analysis has been done so far, further research is needed to validate these findings and disentangle incomplete lineage sorting from introgressive hybridization and species-specific nucleotide polymorphisms with identical restriction sites.

As already noted by Stuckas et al. (2009), the fact that introgression was differential among genetic loci provides evidence for the existence of a semi-permeable barrier to gene flow and the weakness of reproductive isolation across the Baltic hybrid zone. While movement of *M. edulis* alleles into inner Baltic populations seemed to be comparatively relaxed for ITS, PLIIa and Glu-5', it was constrained for M7 Lysin, maternal D-loop, MAL-1 and especially EFbis – patterns that are in good agreement with allele frequency distributions obtained in previous studies (Riginos et al. 2002; Riginos & Cunningham 2005; Kijewski et al. 2006, 2011; Stuckas et al. 2009; Table 18). Such discordances between genes are likely to originate from several evolutionary forces (Schmidt et al. 2008; Stuckas et al. 2009). Whereas extensive asymmetric introgression as seen for ITS, PLIIa and Glu-5' may be best explained by past or ongoing geographical shifts in the position of the hybrid zone and stochastic

processes like genetic drift, differential selection regimes could be alternative mechanisms blocking interspecies gene flow for at least some of the other markers.

Table 18. *M. edulis* (E) allele frequencies in North Sea and Baltic Sea populations compared between different studies. n = total number of specimen. [a]Stuckas et al. (2009); [b]Riginos et al. (2002); [c]Riginos & Cunningham (2005); [d]Kijewski et al. (2006); [e]Kijewski et al. (2011).

	This study		**Previous studies**	
Locus	n	E allele frequency	n	E allele frequency
EFbis				
Helgoland	20	1	17	0,97[a]
Kattegat/Skagerrak	20	0,5 (Tjärnö)	20 32 40	0,525 (Tjärnö)[a] 0,2 (Tjärnö)[e] 0,18 (Egense)[d]
Western Baltic	100	0,205 (Kiel)	14 39	0,32 (Kiel)[a] 0,015 (Møn Island)[d]
Inner Baltic	20	0 (Askö)	15 37 46	0 (Askö)[a] 0,015 (Puck Bay)[d] 0,04 (Tvärminne)[d]
ITS				
Helgoland	20	1	19	1[a]
Kattegat/Skagerrak	20	0,975 (Tjärnö)	20/22 40	1 (Tjärnö)[ace] 0,975 (Egense)[d]
Western Baltic	100	0,93 (Kiel)	15 40	1 (Kiel)[a] 0,84 (Møn Island)[d]
Inner Baltic	20	0,725 (Askö)	27 15 20 60 37 45	0,7 (Hånko)[b] 0,8 (Askö)[a] 0,68 (Askö)[c] 0,66 (Askö)[e] 0,675 (Puck Bay)[d] 0,72 (Tvärminne)[d]
M7 Lysin				
Helgoland	19	1	10	0,95[a]
Tjärnö	17	1	10	0,9[a]
Kiel	96	0,875	10	0,85[a]
Askö	20	0,325	10	0,4[a]
Glu-5'				
Kattegat/Skagerrak	20	1 (Tjärnö)	20 35 40	0,95 (Tjärnö)[c] 0,97 (Tjärnö)[e] 0,96 (Egense)[d]
Western Baltic	100	0,9 (Kiel)	40	0,76 (Møn Island)[d]
Inner Baltic	20	0,575 (Askö)	28 20 56 40 49	0,75 (Hånko)[b] 0,75 (Askö)[c] 0,475 (Askö)[e] 0,57 (Puck Bay)[d] 0,565 (Tvärminne)[d]
MAL-1				
Tjärnö	19	1	20	1[c]
Inner Baltic	19	0,342 (Askö)	27 20	0,37 (Hånko)[b] 0,3 (Askö)[c]
PLIIa				
Tjärnö	19	1	20	0,95[c]
Inner Baltic	18	0,62 (Askö)	29 20	0,72 (Hånko)[b] 0,77 (Askö)[c]

For example, M7 Lysin – a protein with an important function in gamete recognition and dissolution of the egg vitelline coat (Takagi et al. 1994) – was shown to be under positive

selection pressures (Riginos & McDonald 2003; Stuckas et al. 2009), thereby potentially causing pre-zygotic isolation (Stuckas et al. 2009). In the case of the anonymous coding gene MAL-1, it remains to be shown whether natural selective forces are also acting on this locus, since no sequence information is available at the moment. However, even if it turned out to be neutrally evolving, pseudo-selection as result of linkage to a selected beneficial gene variant could be a possible scenario leading to the genetic structure seen for this marker. Apart from hybrid zone movements (Stuckas et al. 2009), genetic hitchhiking effects might also be plausible explanations for the prominent decline in *M. edulis* allele frequencies observed for EFbis (Bierne et al. 2003b). At least, indirect selection working at this locus was shown in other *Mytilus* hybrid zones (Faure et al. 2008).

The supposition that different evolutionary forces are shaping the hybrid zone is not contradicted by the observation that the genetic differentiation between populations followed a linear trend along the North Sea/Baltic transect, when analysed across a fraction of nuclear loci. Although positive relationships between genetic and geographic distance are often indicative of genetic drift (isolation by distance) (Wright 1943), in this case (1) the long range dispersal of blue mussel larvae, (2) the inclusion of M7 Lysin in the calculation, (3) the coincidence of ecological gradients (e.g. salinity) with locality separation and (4) the potential genetic hitchhiking scenarios discussed above do not allow ruling out simultaneous action of endo- and exogeneous selective pressures (Schmidt et al. 2008).

In contrast to the study by Stuckas et al. (2009), which found pervasive introgression of mother-derived ribosomal RNA into the Baltic Proper, my results indicate that gene flow is limited for the native maternal D-loop haplotype of *M. edulis* – a pattern consistent with the findings by Kijewski et al. (2006) and similar to that seen for patrilinear mtDNA. This is not surprising given the fact that gender-specific mitotypes and sex are co-inherited (though not causally linked) in *Mytilus* (DUI: doubly uniparental inheritance; Skibinski et al. 1994; Zouros et al. 1994; Kenchington et al. 2002; Kenchington et al. 2009; Zouros 2012), which points to co-evolutionary adaptations between cytoplasmic and nuclear factors (Saavedra et al. 1996; Riginos et al. 2004). While such a situation is likely to cause cyto-nuclear mismatch in foreign genetic backgrounds, compatibility constraints might be particularly strong for control regions, as these are suspected of having crucial functions in determining the male- or femaleness of mitochondrial genomes (Burzyński et al. 2003, 2006).

As a corollary, rearrangements in the displacement loops could have been important steps in the replacement of the original Baltic *M. trossulus* mtDNA by recombinant and masculinised *M. edulis* F genomes, relativizing deleterious interactions between mitochondrial and nuclear genes in partly introgressed *M. trossulus* (Burzyński et al. 2006).

Such asymmetries in gene flow are indeed commonly seen, if genotypes differ significantly in fitness (Barton & Hewitt 1985). In turn, the higher compatibility of derived *M. edulis* mtDNA compared to native *M. trossulus* mitogenomes could explain why hybridization between the two *Mytilus* species is more intense in the inner Baltic Sea than in the Canadian Maritimes. By contrast, outer Baltic populations retain the original *M. edulis* F and M genomes, which can be expected to superiorly fit the predominant *M. edulis* nuclear background than recombinant haplotypes. Interestingly, introgression of F_L polymorphisms was apparent at the entrance to the Kiel Fjord, while it seemed to be absent inside the basin, pointing to an equilibrium situation between genetic incompatibilities and dispersal from the inner Baltic Sea. In this context, the sheltered topology of the Fjord could further hamper the invasion of less fit, recombinant F haplotypes through interception of drifting larvae at the mouth, while the exposed nature of Wendtorf Marina could favour gene exchange with populations in the eastern Baltic, making for an insignificant differentiation from Askö.

In general, the narrowness and geographical marginality of the Baltic Sea entrance in conjunction with the strong environmental gradients (Voipio 1981; Bonsdorff & Pearson 1999; Leppäranta & Myrberg 2009) might have been conducive to the allele and haplotype frequency differences observed between populations inside and outside the estuary. As a consequence of their (partial) isolation, subpopulations that colonize peripheral and extreme habitats experience distinct selection pressures and stochastic events, thereby evolving differently than more central populations (Lesica & Allendorf 1995; Johannesson & André 2006). Apart from this, hybridization can sometimes increase divergence by triggering the generation of novel polymorphisms, which are often confined to hybrid individuals and reach high abundances in interbreeding populations (Barton & Hewitt 1985; Woodruff 1989; Bradley et al. 1993 and references therein). Considering the genetic variation observed at locus mac-1, my results indicate that such hybrid zone alleles exist in the Baltic as well, though it cannot be completely excluded that these are ancestral polymorphisms that have accidentally been lost in other populations. Whereas one length variant was exclusively found in the Baltic, two rare alleles appeared to be endemic to the Kiel Fjord and one to the Askö population, but none of them was shared with the reference taxa. Different hypotheses have been established to account for the origin and maintenance of new alleles in secondary contact zones, with hybridization-induced mutations and intragenic recombinations appearing as the most probable generative mechanisms (Woodruff 1989). In the case of infrequent, private polymorphisms Barton et al. (1983) proposed that they might be deleterious in nature, but would remain in the population through an equilibrium between production rate and selective removal. As mac-1 is a neutrally evolving locus, the balance hypothesis is not likely to apply

to the three endemic variants observed in Kiel and Askö, unless the first intron of the *Mytilus* actin gene has a hitherto unknown regulatory function (which is improbable given the large amount of length polymorphisms) or both alleles are linked to gene variants that are under purifying selection (which might be the case). Alternatively, I suggest that the low frequency of these alleles is caused by recent mutation or recombination events and is maintained simply as a consequence of the dominance of genotypes carrying more abundant alleles. On the other hand, the high numbers of the fourth hybrid zone polymorphism in Askö and the strong cline with respect to North Sea associated populations could indicate that this variant hitchhikes with a functional locus conferring adaptation to the harsh environment of the Baltic Sea. Nonetheless, a major caveat for the validation of these putatively unique alleles arises from the fact that only 20 individuals were analysed in each population outside the hybrid zone and that Atlantic allopatric *M. trossulus*, which are phylogenetically closer related to European lineages than Pacific mussels (Bierne et al. 2003b; Väinölä & Strelkov 2011), were not investigated. Moreover, results cannot be reasonably compared to earlier observations, as the lengths of mac-1 polymorphisms are seldom precisely listed in papers (last detailed publication of allele sizes by Daguin & Borsa 1999). However, even if variants with the same length were found inside and outside the Baltic hybrid zone, this would not necessarily mean that they have identical nucleotide sequences. Hence, future studies including additional *Mytilus* (reference) populations, larger sample sizes and sequencing techniques are required to confirm my findings.

In summary, the genetic analyses of *Mytilus* specimens presented here (1) corroborate the view that mussels of the eastern Baltic form a *M. trossulus*-like hybrid swarm (reviewed in Riginos & Cunningham 2005) and (2) shed light on a previously unrecognized amount of introgressive hybridization in populations of the outer Baltic (in particular Kiel Fjord). This genetic variability was partly mirrored by the shell morphologies of hybrid mytilids.

7.2 Blue mussel shell morphology: effects of hybridization, environmental stability and extreme salinities on phenotypic variability

While reference species as well as mussels from the inner Baltic exhibited population-specific shell phenotypes, *Mytilus* populations from Tjärnö and Kiel were morphologically not clearly identifiable, but characterized by a morpho-spectrum from *M. trossulus*-like to *M. edulis*-like forms. In the case of distinct morphotypes, the discrepancies in appearance seemed to be directly influenced by both genetics and habitat salinity. However, when talking about genetic effects, any results for mixed populations must be seen with caution, as no causative loci were

investigated. This combined with the highly variable environment in the Baltic transition zone might also explain why no genotype-phenotype relationships could be detected in Tjärnö and Kiel, even if they existed. That is, analyses of morphogenes and time-series measurements of salinity will be needed to identify causal links. On the other hand, plastic responses would of course blur any potential associations (see below for further discussion). Although the observed identity of semi-allopatric species appears plausible, given that these populations were genetically most differentiated, the fact that they were taken from ecologically distinct locations might have biased the outcomes. As demonstrated by Innes & Bates (1999), mussels being exposed to the same environmental conditions usually exhibit fewer differences in shell morphology. Later, Gardner & Thompson (2009) showed that geography can have a strong impact on morphometric trait variation both between and within species. Besides, in most cases the parameters with the highest discriminative power between species were shell thickness and weight. Since these characters are highly dependent on salinity and other ecological factors (Kautsky et al. 1990; Kossak 2006; Beaumont et al. 2008: salinity; Raubenheimer & Cook 1990; Akester & Martel 2000; Steffani & Branch 2003: wave exposure; Reimer & Harms-Ringdahl 2001: predation pressure), the importance of genetic components is disputable. Furthermore, I lacked the possibility to determine how old mussels were, so that a part of the morphological differences could result from dissimilar age structures and age-length relationships among populations, regardless of any corrections for size (see Seed 1973). Nevertheless, my results corroborate earlier studies showing that shell characteristics in the *Mytilus edulis* species complex are at least partly inherited. For instance, by means of reciprocal transplant experiments and inter-specific comparisons at various sites in Newfoundland Penney et al. (2007, 2008) showed that *M. edulis* has an intrinsically greater shell weight and thickness than *M. trossulus*. Similar findings were reported by Beaumont et al. (2008), who conducted morphometric and genetic analyses on cultured mussels from two sites of a western Scottish Loch. Moreover, a partial genetic basis of morphology might be evidenced by the fact that species could also be distinguished notwithstanding differences in shell stability. Consistent with previous studies (Sanjuan et al. 1990; McDonald et al. 1991), the length of the anterior adductor muscle scar was the most discriminative parameter between Atlantic *M. edulis* and *M. galloprovincialis* – species that should temporarily experience similar environmental conditions.

What remains to be explained is the observation that populations from the western and eastern Baltic Sea were so unlike in the degree of phenotypic diversity, considering that all of them consisted mainly of mussels from mixed ancestry. It seems probable that these outcomes reflect the contrasting amounts of environmental variations and the resulting

differences in phenotypic plasticity and/or selective regimes affecting diversity of morphological traits, though stochastic processes could also play a role.

Connecting the central Baltic Sea with the North Sea, the outer Baltic region forms a transition zone where dynamic estuarine flows lead to strong fluctuations in abiotic environmental factors (e.g. salinity, temperature, concentrations of nutrients and respiratory gases) (Bendtsen et al. 2009) – a situation that is likely to select for multiple polymorphisms and thus phenotypes in a population. Since North Sea *M. edulis* and Baltic *M. trossulus* seem to be locally adapted to their respective habitats (Riginos & Cunningham 2005; Johannesson & André 2006), oscillations between ecological conditions encountered by parental types could give hybrids a fitness advantage in the outer Baltic Sea, as already suggested by Gardner (1996). This might be either due to heterozygosity at causal loci, when novel combinations of species-specific alleles interact to generate a more vigorous hybrid phenotype (Birchler et al. 2006: heterosis via overdominance), or spatio-temporal superiority of different homozygous backcross genotypes (Arnold & Hodges 1995; see also Pamilo 1988). Considering the additive polygenic inheritance of many functional traits (Mather 1943), introgressive hybridization would therefore easily erode phenotypic differences between parental lineages and foster the formation of morphological continua, as often seen in secondary contact zones located in an ecologically intermediate habitat (Gardner 1996). In addition, phenotypic variation could further be increased by hybridogenetic mutations (Barton & Hewitt 1985; Woodruff 1989; Bradley et al. 1993; Seehausen 2004). On the other hand, as I have sampled across generations, the observed morphological variability might simply be a function of strong phenotypic plasticity, which is often adaptive in fluctuating environments (reviewed in Whitman & Agrawal 2009). Under this scenario, a small amount of positively selected plastic alleles could alter their expression (allelic sensitivity) or that of other genes and alleles (gene regulation) in response to environmental change (Via et al. 1995), which could produce a range of different phenotypes independent of genetic variation or heterozygote advantages. However, these alternatives need not be mutually exclusive, as increasing evidence suggests that hybridogenesis can augment both genetic variability (Barton & Hewitt 1985; Woodruff 1989; Bradley et al. 1993; Seehausen 2004) and plastic responses within a population (Silim et al. 2001; Whitman & Agrawal 2009). Depending on the costs and limits of phenotypic plasticity (DeWitt et al. 1998; Auld et al. 2010), it could for example be that hybrids are adaptively homozygous for a plastic regulatory allele and heterozygous at a morphogenetic locus that is under the control of the plasticity gene. Alternatively, mussels in the western Baltic might carry both *M. edulis* and *M. trossulus* alleles at a sensitive locus. Since either case would allow the respective individual to buffer environmental perturbations

and phenotype definitions were based on multiple shell characters, it is likely that there is more than one underlying mechanism to the observed pattern.

Interestingly, McDonald et al. (1991) found that mussels from the Kattegat are morphologically identical to *M. edulis*. This apparent contradiction to my observations might be explained by differences in genetic methods and statistical techniques applied in both studies. Based on allozyme surveys, McDonald et al. (1991) grouped these mytilids with pure *M. edulis* and used canonical variates analysis on log-transformed shell characters to examine the morphometric differentiation between the three *Mytilus* sibling species. This approach uses linear functions to maximize the distances among compared to those within samples. Combined with data transformations such a procedure might largely reduce the within-group variance, thereby potentially skewing the results. Besides, Innes & Bates (1999) pointed to a potential length bias in this study. However, further investigations will be needed to identify the true cause of these discrepancies.

Unlike the transition zone, the Baltic Proper is characterized by extreme and comparatively constant environmental conditions (e.g. nearly lethal salinities) (Schramm 1996; Kossak 2006), as exchange with the North Sea is hampered by shallow sills in the Danish Straits and dependent on particular, but rare meteorological as well as oceanographic events (Matthäus & Schinke 1994; Lass & Matthäus 1996; Schinke & Matthäus 1998). Furthermore, the composition of inflowing seawater increasingly approximates Baltic conditions during its passage through the transition zone. These circumstances might impose strong constraints on shell traits in local populations, directly or indirectly abrading the morphological variability that could be introduced by genetic mixing. The most striking feature of eastern Baltic mussels was their high shell frangibility, which is in concordance with other studies showing that decreases in salinity (below a certain threshold) relate to reduced shell stability as well as calcification rate (Malone & Dodd 1967; Almada-Villela 1984; Kautsky et al. 1990; Kossak 2006), possibly as a result of less availability of calcium (Schlieper 1971) and carbonate for biomineralization or energy allocation problems due to hypoosmotic stress (Tedengren & Kautsky 1986). Salinity effects alone, however, cannot account for the low shell strength of Baltic mytilids, because this character is partly maintained even when mussels are transplanted to more saline waters (Kautsky et al. 1990; Kossak 2006). Rather, the scarcity of predators in the Baltic might select against protective (and probably costly) phenotypic features (Kautsky et al. 1990), thereby eliminating variation at loci involved in morphogenesis despite extensive hybridization. Combined with the oligohalinity of the Baltic Proper, positive selection of particular *M. trossulus* alleles, which are known to code for weaker shells (Beaumont et al. 2008; Penney et al. 2007, 2008), would

be in line with the phenotypic identity of inner Baltic mytilids, the genetic effects observed in this study and the morphological similarity to allopatric *M. trossulus* (McDonald et al. 1991; this study). On the other hand, neutral evolutionary forces have a strong impact on marginal populations like those in the central Baltic Sea, where they seem to have caused diversity losses both at coding and non-coding loci (Johannesson & André 2006). Therefore, it appears equally likely that drift effects have led or at least contributed to the morphological confinement of Askö blue mussels. Variance in morphometric traits could additionally be limited by reductions in phenotypic plasticity, either by purifying selection on certain alleles, if it causes a fitness disadvantage in constant environments (DeWitt et al. 1998; Auld et al. 2010), or by random processes, if it is selectively neutral (Reimer & Harms-Ringdahl 2001).

Indeed, in contrast to their western conspecifics, mussels from the eastern Baltic seem to have forfeited a part of their ability to induce plastic reactions. As shown by Reimer & Harms-Ringdahl (2001), Baltic Proper mytilids can still respond defensively (e.g. reduced growth, increase in shell thickness and byssal attachment strength) to scents from predatory crabs, but – except for changes in byssus adhesion – show a weak or even absent reaction to those from starfish (e.g. no increase in adductor muscle size). Besides, reciprocal transplant and laboratory experiments by Kossak (2006) have found that *Mytilus* spp. from the outer Baltic Sea are able to sustain shell growth rates over a wide range of osmolarities, while those from the inner part exhibit a limited adaptability and grow relatively slowly even under optimal salinity conditions. Both studies have speculated that these contrasting patterns may in parts be related to differences in genetic makeup shaped by interactions with environmental factors. While mussels in the stable and predator-free eastern Baltic were proposed to have accumulated functional *M. trossulus* alleles that encode lower adaptive potential (Reimer & Harms-Ringdahl 2001), mytilids in the variable western Baltic and Skagerrak were suggested to have a higher adaptability by carrying *M. edulis* alleles (Reimer & Harms-Ringdahl 2001) or polymorphisms of both species at the causative loci (Kossak 2006). If the quality of phenotypic plasticity differs between species, the remaining ability of inner Baltic mussels to strengthen byssal attachment, for example, could be explained by neutral introgression of *M. edulis* alleles for Glu-5’ – the polyphenolic adhesive foot protein, which is involved in byssus production (Waite 1992). On the other hand, Helgoland mussels seem to be comparatively constrained in growth rates (Kossak 2006), reflecting the inevitability of physiological trade-offs. Moreover, as already stated by Reimer & Harms-Ringdahl (2001) it is not at all obvious why *M. trossulus* should be inferior in terms of plasticity compared to *M. edulis*, given the fact that both species occur in similar ecological habitats outside the Baltic Sea (see also Riginos & Cunningham 2005). Possibly, an overall reduced plastic capacity is a unique

feature of the Baltic *M. trossulus*, related to the species' evolutionary history in this extreme ecosystem. However, assuming that *M. edulis* alleles provide a better adaptation to conditions in the western Baltic and *M. trossulus* alleles confer a higher fitness in the eastern Baltic, the intensity of hybridization in this region is hard to understand. Evidently, many interesting questions concerning the functional relevance of hybridogenesis in the Baltic Sea are open to further research.

7.3 Future directions and implications

Although it seems probable that the contrasting degrees of morphological variability among populations result from differences in functional genetic diversity (i.e. level of heterozygosity, allelic richness) and/or phenotypic plasticity due to positive selection of species-specific alleles, further morphometric and genetic analyses are required to validate these assumptions. Firstly, future studies should increase the number as well as sizes of samples and preferentially use mussels from aquaculture farms, so that the variance within each population will be correctly approximated, whilst bias by age and length differences can be controlled. Secondly, it will be necessary to sequence candidate genes and check for signs of adaptive evolution (e.g. Eyre-Walker 2006: McDonald-Kreitman test), whereas surveys for heterozygote excesses or deficiencies at causal loci will be needed to identify hybridogenetic effects. Such investigations should be accompanied by laboratory as well as field studies. For example, in order to determine total genetic effects on shell morphology, common garden experiments with full-sib offspring from artificial crosses within and among species could be carried out. In this context, it would be interesting to see whether morphological variability in backcross hybrids might additionally be enhanced as a result of transgressive segregation, i.e. the production of extreme phenotypes due to the complementary action of parental genes in an additive fashion (Rieseberg et al. 1999, 2003; Seehausen 2004). Moreover, raising larvae of pure and mixed ancestry under a range of environmental conditions could illuminate the genetic basis of phenotypic plasticity and adaptive potential in the genus *Mytilus* (Figure 13). Similar approaches for growth rates have recently been done by Beaumont et al. (2004), who revealed negative heterotic effects in *M. edulis* x *M. galloprovincialis* veligers at different temperatures, though. In the field, fitness and plasticity assessments of reciprocally transplanted hybrid and parental genotypes could reveal the role of hybridization for mussel performance in natural Baltic ecotones and thus whether (bounded) hybrid superiority might be one mechanism maintaining the secondary contact zone (Moore & Koenig 1986; Arnold 1992; Arnold & Hodges 1995). Such studies should be combined with comparative transcriptome and qRT-PCR analyses to see whether species-specific alleles are differently

expressed in hybrids dependent on the environment. In this context, we already plan to establish reference and Baltic *Mytilus* transcriptome libraries via Illumina sequencing. After calculating phylogenetic trees, we will be able to assign orthologous genes to species and determine the amount of expressed *M. trossulus* alleles in hybrid mussels. Besides, while increasing transcriptomic and genomic information would provide a more realistic estimate of the level of gene flow between species, extended cline shape analyses with these data could help us to better infer the evolutionary forces acting in the hybrid zone and to identify genes that are important for ecological processes (see Schmidt et al. 2008 for a review).

Knowledge about the relative fitness and adaptability of hybrids compared to pure species will have significant ramifications for both evolutionary and ecological research. Not alone can such insights be valuable for understanding hybrid speciation, but they will also enable us to better comprehend and predict responses of Baltic blue mussels to environmental perturbations (e.g. global warming, habitat modification, ocean acidification) (IPCC Fourth Assessment Report 2007; Fabry et al. 2008; Doney et al. 2009). Since bivalves are important functional components of benthic communities, providing microhabitats for associated species and improving water quality (Ragnarsson & Raffaelli 1999; Kossak 2006 and references therein; OSPAR Report 2010), I anticipate that future interdisciplinary investigations on the *Mytilus edulis* complex will be crucial for elucidating impacts of global change not just on the species level, but on the ecosystem one as well.

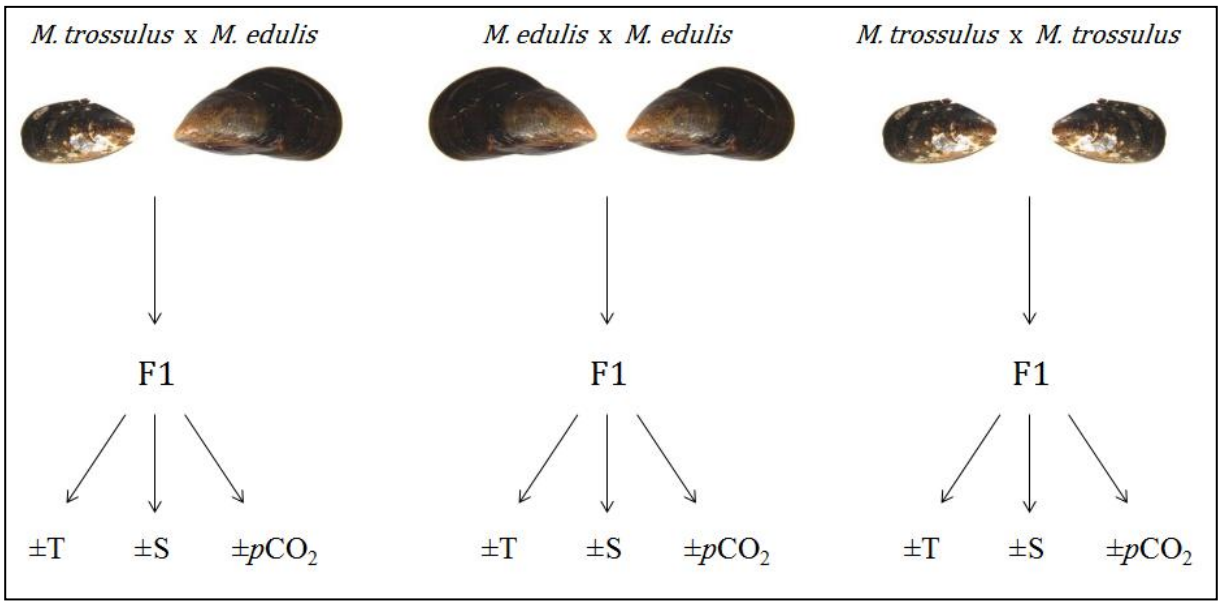

Figure 13. Potential laboratory cross experiments between *M. edulis* and *M. trossulus* to assess the effects of hybridization on phenotypic plasticity. Mussel pictures by H. Stuckas.

8 Acknowledgements

First of all, I want to thank Frank Melzner for offering me the opportunity to perform this study and for his helpful advice whenever questions arose.

Very special credits are given to Heiko Stuckas and Manuela Bartel for their excellent supervision during my time in Dresden. It is not only the remarkable support you happily provided in any situation inside and outside the lab, but also the positive work (and non-work) climate that I would like to stress here and that will make this stay unforgettable. Many thanks to a great team for a great time!

I am further grateful to Raffael Ernst – the helper in my hours of statistical problems.

No less appreciation goes to Christian Kehlmaier, Anke Müller, Anja Rauh and Ulli Panknin for lending much more than technical assistance in the Dresden and Kiel labs, respectively.

Generally, I want to acknowledge the working group 'Environmental Physiology' in Kiel for the good atmosphere at work. I really enjoyed the time with you!

Anja, Nathalie, Caro, Markus, Ute, Christiane, Monique and Annika – thank you for leading me into the diverse (Saxon) world outside the Senckenberg Natural History Collections.

I am also indebted to Thorsten Reusch, who did not insignificantly contribute to the fact that this thesis will not be the end of my scientific story.

Last but not least I want to thank my family for their apparently untiring attentiveness, interest and patience when I talked about things they did not completely understand. Hopefully, you have kept these qualities, as I am afraid you will hear about hybridization in mussels soon again...

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9.2 Images

1. http://www.imr.no/cano/__data/page/8516/Mytilus-edulis_TS_350.jpg
2. http://www.geomar.de/de/service/presse/geomar-bilder/
3. http://www.senckenberg.de/root/index.php?page_id=5229
4. www-lns.tf.uni-kiel.de/bilder/kiel003.jpg

10 Appendix

Supplement 1. Genotypic and haplotypic data for the population genetic analyses. E = *M. edulis* allele; T = *M. trossulus* allele; G = *M. galloprovincialis* allele; EG = *M. edulis/M. galloprovincialis* allele; GT = *M. galloprovincialis/M. trossulus* allele; FE = original *M. edulis/M. galloprovincialis* F haplotype; FL = recombinant *M. edulis/M. galloprovincialis* F haplotype; FT = original *M. trossulus* F haplotype; ? = missing data. In the case of mac-1 allele sizes are given. GEO = GEOMAR; SM = Museum of Ship Transport; FH = East Shore Harbour; MAR = Wendtorf Marina; SC = Penn Cove (*M. trossulus*); V = Vigo (*M. galloprovincialis*); H = Helgoland (*M. edulis*).

Mussel	ITS		EFbis		M7 Lysin		D-loop	PLIIa		Glu-5'		MAL-1		mac-1	
GEO1	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
GEO2	EG	EG	T	T	E	E	FE	EG	EG	E	T	EG	EG	361	361
GEO5	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
GEO6	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	363	363
GEO7	EG	EG	E	T	E	E	FE	EG	EG	E	T	EG	EG	363	363
GEO8	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
GEO10	EG	EG	T	T	E	E	FE	EG	EG	E	T	EG	EG	361	367
GEO11	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
GEO12	EG	EG	E	T	E	E	FE	EG	EG	E	T	EG	EG	369	359
GEO13	EG	EG	T	T	T	T	FE	EG	EG	E	E	EG	EG	369	349
GEO14	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	369	366
GEO23	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	369	364
GEO25	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	362	367
GEO26	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
GEO27	EG	EG	T	T	E	E	FE	EG	EG	E	T	EG	EG	361	363
GEO31	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
GEO35	EG	T	E	E	E	E	FE	EG	EG	E	T	EG	EG	363	363
GEO37	EG	EG	T	T	E	E	FE	GT	GT	E	E	EG	EG	363	363
GEO38	EG	EG	T	T	?	?	FE	EG	EG	E	E	EG	T	361	361
GEO40	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	367	367
SM4	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	364	393
SM10	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	361	364
SM11	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	364
SM12	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	369	369
SM13	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	393	393
SM14	EG	EG	T	T	E	E	FE	GT	GT	E	E	EG	EG	367	367
SM15	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	393
SM16	EG	EG	T	T	T	T	FE	EG	EG	E	E	EG	EG	361	361
SM17	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	393
SM19	EG	EG	E	T	E	T	FE	EG	EG	E	G	EG	EG	363	363
SM20	EG	EG	T	T	E	T	FE	EG	EG	E	T	EG	EG	363	363
SM24	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
SM25	EG	T	T	T	E	E	FE	EG	EG	E	T	EG	EG	362	362
SM26	EG	T	T	T	T	T	FE	EG	EG	E	E	EG	EG	361	366
SM27	EG	EG	T	T	E	E	FE	EG	EG	E	T	EG	EG	363	363
SM29	EG	T	T	T	E	T	FE	EG	EG	E	G	EG	EG	361	361
SM30	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
SM34	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
SM37	EG	EG	E	T	?	?	FE	EG	EG	E	T	EG	EG	358	361
SM40	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
FH1	EG	T	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	364
FH2	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	393
FH5	EG	EG	T	T	E	E	FE	GT	GT	E	E	EG	EG	361	393
FH6	EG	T	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
FH7	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	364
FH8	EG	EG	T	T	E	E	FE	EG	EG	E	T	EG	EG	361	363

Supplement 1 (continued).

Mussel	ITS		EFbis		M7 Lysin		D-loop	PLIIa		Glu-5'		MAL-1		mac-1	
FH10	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	368	368
FH11	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	363
FH12	EG	T	T	T	E	E	FE	EG	EG	E	T	EG	EG	364	393
FH13	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	364
FH15	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
FH24	EG	T	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	363
FH26	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	363
FH27	EG	EG	E	T	E	T	FE	EG	EG	E	T	EG	EG	363	363
FH31	EG	EG	E	T	E	T	FE	EG	EG	E	E	EG	EG	363	363
FH32	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	364	364
FH33	EG	EG	T	T	E	E	FE	EG	EG	E	T	EG	EG	369	364
FH34	EG	T	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	363
FH35	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	366	366
FH38	EG	EG	E	T	E	E	FE	GT	GT	E	E	EG	EG	361	361
Hörn2	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	366	363
Hörn3	EG	EG	T	T	T	T	FE	EG	EG	E	E	EG	EG	360	360
Hörn6	EG	T	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	364
Hörn8	EG	EG	T	T	E	E	FE	GT	GT	E	E	EG	EG	361	361
Hörn10	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	358	362
Hörn12	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
Hörn14	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	364
Hörn19	EG	T	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	363
Hörn20	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
Hörn24	EG	EG	T	T	?	?	FE	EG	EG	E	E	EG	EG	364	364
Hörn25	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	T	361	363
Hörn26	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	361	361
Hörn27	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
Hörn30	EG	EG	T	T	E	E	FE	GT	GT	E	E	EG	EG	363	363
Hörn31	EG	EG	E	T	E	T	FE	EG	EG	E	T	EG	EG	361	363
Hörn32	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
Hörn33	EG	EG	E	T	E	E	FE	GT	GT	E	E	EG	EG	361	361
Hörn34	EG	EG	E	T	E	E	FE	EG	EG	E	G	EG	EG	393	393
Hörn35	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	361	361
Hörn36	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	393
MAR1	EG	EG	T	T	E	E	FL	EG	EG	E	E	EG	EG	361	363
MAR2	EG	EG	T	T	E	E	FL	EG	EG	E	E	EG	EG	361	361
MAR3	EG	EG	E	T	E	T	FE	EG	EG	E	E	EG	EG	358	363
MAR7	EG	EG	T	T	E	T	FE	EG	EG	E	T	EG	EG	361	361
MAR8	EG	EG	E	E	?	?	FE	EG	EG	E	E	EG	EG	393	393
MAR10	EG	EG	T	T	E	E	FL	EG	EG	E	T	EG	EG	360	360
MAR11	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	358	364
MAR12	EG	EG	T	T	E	T	FE	GT	GT	E	E	EG	T	364	364
MAR13	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	363
MAR14	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	364	364
MAR15	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
MAR17	EG	T	E	T	E	E	FE	EG	EG	E	E	EG	EG	364	364
MAR26	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	367	363
MAR27	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	364	367
MAR28	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	EG	361	363
MAR29	EG	EG	T	T	E	E	FL	EG	EG	E	E	EG	EG	363	393
MAR30	EG	T	T	T	E	E	FE	GT	GT	E	E	EG	EG	361	361
MAR32	EG	T	E	T	E	E	FE	GT	GT	E	E	EG	EG	369	363
MAR33	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	364

Supplement 1 (continued).

Mussel	ITS		EFbis		M7 Lysin		D-loop	PLIIa		Glu-5'		MAL-1		mac-1	
MAR38	EG	EG	E	T	E	E	FE	GT	GT	E	E	EG	EG	361	393
SC1	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	606	606
SC2	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	606	606
SC3	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	588	600
SC5	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	631	631
SC6	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	604	600
SC8	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	611	608
SC9	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	368	600
SC10	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	604	604
SC11	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	605	605
SC12	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	604	602
SC13	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	604	633
SC14	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	606	606
SC16	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	605	605
SC17	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	600	604
SC18	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	605	605
SC19	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	606	606
SC20	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	371	371
SC21	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	604	604
SC22	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	602	606
SC23	T	T	T	T	T	T	FT	GT	GT	T	T	T	T	605	631
V51	EG	EG	G	G	E	E	FE	EG	EG	G	G	EG	EG	397	397
V52	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	364	397
V53	EG	EG	E	G	?	?	FE	GT	GT	G	G	EG	EG	397	397
V54	EG	EG	G	G	?	?	FE	EG	EG	G	G	EG	EG	363	397
V55	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	397	407
V56	EG	EG	G	G	?	?	FE	GT	GT	G	G	EG	EG	417	424
V58	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	397	397
V59	EG	EG	E	G	?	?	FE	GT	GT	G	G	EG	EG	363	363
V60	EG	EG	E	G	?	?	FE	GT	GT	G	G	EG	EG	397	397
V61	EG	EG	G	G	?	?	FE	GT	GT	G	G	EG	EG	397	397
V62	EG	EG	G	G	?	?	FE	GT	GT	G	G	EG	EG	397	397
V63	EG	EG	G	G	?	?	FE	EG	EG	G	G	EG	EG	424	424
V64	EG	EG	G	G	?	?	FE	GT	GT	G	G	EG	EG	363	424
V65	EG	EG	E	G	?	?	FE	GT	GT	G	G	EG	EG	364	397
V66	EG	EG	G	G	?	?	FE	GT	GT	G	G	EG	EG	397	397
V67	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	397	400
V68	EG	EG	E	G	?	?	FE	GT	GT	G	G	EG	EG	397	397
V69	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	397	397
V70	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	359	363
V71	EG	EG	G	G	E	E	FE	GT	GT	G	G	EG	EG	397	400
HF	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	354	361
H11	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	364	358
H12	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	360	360
H13	EG	EG	E	E	E	E	FE	GT	GT	E	E	EG	EG	363	367
H20	EG	EG	E	E	E	E	FE	GT	GT	E	G	EG	EG	363	363
H21	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	354	363
H22	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	367	393
H23	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	363
H24	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	364
H25	EG	EG	E	E	?	?	FE	EG	EG	E	E	EG	EG	361	361
H26	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	361
H27	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	363	363
H28	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	364

Supplement 1 (continued).

Mussel	ITS		EFbis		M7 Lysin		D-loop	PLIIa		Glu-5'		MAL-1		mac-1	
H29	EG	EG	E	E	E	E	FE	GT	GT	E	E	EG	EG	361	364
H30	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	363
H31	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	367
H32	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	363
H33	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	361
H34	EG	EG	E	E	E	E	FE	GT	GT	E	E	EG	EG	361	363
H35	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	361
Askö1	EG	T	T	T	T	T	FL	GT	GT	E	E	EG	T	361	361
Askö2	EG	EG	T	T	E	E	FL	GT	GT	E	E	EG	T	369	369
Askö5	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	T	369	369
Askö7	EG	EG	T	T	T	T	FL	GT	GT	E	E	EG	T	369	369
Askö8	EG	EG	T	T	T	T	FL	GT	GT	E	E	EG	T	369	352
AsköVI	EG	EG	T	T	E	T	FE	GT	GT	E	T	EG	T	368	368
Askö10	EG	T	T	T	E	T	FL	EG	EG	E	E	T	T	604	604
Askö13	EG	T	T	T	E	E	FL	EG	EG	T	T	T	T	369	369
Askö15	EG	EG	T	T	T	T	FE	EG	EG	E	T	EG	EG	369	369
AsköVIII	EG	EG	T	T	E	T	FE	EG	EG	E	E	EG	T	606	606
AsköB	EG	EG	T	T	E	T	FE	EG	EG	T	T	T	T	369	369
AsköD	EG	T	T	T	T	T	FL	EG	EG	T	T	EG	T	369	369
AsköE	EG	T	T	T	E	T	FE	?	?	T	T	?	?	369	369
AsköXIV	EG	T	T	T	E	T	FL	GT	GT	E	T	EG	T	361	604
AsköH	EG	T	T	T	E	T	FE	GT	GT	E	T	T	T	369	369
AsköIV	EG	EG	T	T	T	T	FL	GT	GT	T	T	T	T	363	369
AsköJ	EG	T	T	T	T	T	FL	GT	GT	E	E	T	T	606	606
AsköK	EG	T	T	T	?	?	FL	?	?	E	T	EG	T	369	369
AsköL	EG	T	T	T	E	T	FL	GT	GT	E	E	T	T	602	602
AsköN	EG	T	T	T	T	T	FL	GT	GT	T	T	EG	T	369	369
Tjärnö2	EG	EG	E	E	?	?	FE	EG	EG	E	E	EG	EG	363	393
Tjärnö3	EG	T	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	363
Tjärnö4	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
Tjärnö9	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	367	393
Tjärnö10	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	364
Tjärnö11	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
Tjärnö12	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	364	367
Tjärnö13	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	364	364
Tjärnö14	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	368	368
Tjärnö15	EG	EG	E	T	?	?	FE	EG	EG	E	E	EG	EG	361	364
Tjärnö16	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
Tjärnö17	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	361	361
Tjärnö19	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
Tjärnö20	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	361	364
Tjärnö21	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
Tjärnö23	EG	EG	T	T	E	E	FE	EG	EG	E	E	EG	EG	363	367
Tjärnö24	EG	EG	E	E	E	E	FE	EG	EG	E	E	EG	EG	361	361
TjärnöA	EG	EG	E	T	E	E	FE	EG	EG	E	E	EG	EG	363	363
TjärnöB	EG	EG	E	E	?	?	FE	EG	EG	E	E	EG	EG	364	364
TjärnöC	EG	EG	T	T	E	E	FE	?	?	E	E	?	?	361	363

Supplement 2. Raw data of the morphometric measurements. l = shell length; ht = shell height; wid = shell width; wg = shell weight; tck = shell thickness; aam = length of the anterior adductor muscle scar; pam = length of the posterior adductor muscle scar; pal = distance between pallial line and ventral shell margin midway along the shell; lig = distance between umbo and posterior end of ligament; pamp = distance between anterior end of posterior adductor muscle scar and posterior shell margin; pamv = distance between ventral edge of posterior adductor muscle scar and ventral shell margin; hp = length of hinge plate; la = ligamentary angle; mwid = point of maximum shell width. GEO = GEOMAR; SM = Museum of Ship Transport; FH = East Shore Harbour; MAR = Wendtorf Marina; SC = Penn Cove (*M. trossulus*); V = Vigo (*M. galloprovincialis*); H = Helgoland (*M. edulis*).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
GEO1	3,37	1,81	0,61	1,2398	0,033	0,32	0,5	0,27	1,7	0,98	1	0,365	55,00	0,65
GEO2	3,18	1,74	0,55	1,0188	0,033	0,46	0,5	0,19	1,55	1	0,9	0,435	56,67	0,65
GEO5	3,65	1,96	0,67	1,2035	0,032	0,33	0,53	0,22	1,62	1,08	1,13	0,35	59,67	0,63
GEO6	3,54	1,85	0,675	1,2915	0,030	0,4	0,53	0,22	1,62	1,01	1,04	0,35	60,67	0,65
GEO7	3,73	1,98	0,715	1,8764	0,044	0,38	0,54	0,25	1,89	1,12	1,02	0,38	51,00	0,64
GEO8	3,93	2,15	0,81	2,325	0,045	0,49	0,59	0,2	2,14	1,09	1,2	0,4	42,33	0,52
GEO10	3,3	1,82	0,605	1,2513	0,037	0,37	0,53	0,17	1,7	0,94	0,94	0,33	46,33	0,53
GEO11	3,7	1,75	0,675	0,9239	0,024	0,4	0,43	0,2	1,63	1,03	1,1	0,335	53,83	0,64
GEO12	3,9	1,97	0,86	2,2272	0,047	0,47	0,59	0,29	1,89	1,21	1	0,395	49,50	0,73
GEO13	3,76	2,1	0,75	2,308	0,048	0,36	0,61	0,26	2	1,23	1,03	0,405	52,33	0,65
GEO14	4,67	2,54	0,955	3,6081	0,056	0,44	0,83	0,35	2,5	1,6	1,3	0,455	46,83	0,65
GEO23	4,54	2,24	0,83	2,3453	0,035	0,49	0,59	0,26	2,25	1,25	1,35	0,445	51,50	0,72
GEO25	3,9	1,85	0,7	1,0783	0,023	0,36	0,47	0,22	1,6	1,2	1,18	0,4	56,33	0,75
GEO26	4,78	2,2	0,87	2,7596	0,043	0,49	0,65	0,33	2,3	1,35	1,19	0,515	50,50	0,83
GEO27	3,65	1,87	0,64	1,1616	0,027	0,33	0,44	0,22	1,8	1,03	1,08	0,41	61,67	0,65
GEO31	3,82	1,98	0,785	2,2479	0,048	0,37	0,65	0,26	2,23	1,26	1	0,325	42,33	0,53
GEO35	4,49	2,21	0,74	2,5763	0,056	0,36	0,6	0,28	2,39	1,36	1,2	0,48	48,17	0,76
GEO37	4,31	2,26	0,71	2,2315	0,041	0,51	0,6	0,26	2,15	1,16	1,32	0,46	59,67	0,89
GEO38	3,4	1,62	0,655	0,8325	0,025	0,34	0,47	0,22	1,57	0,97	1,05	0,39	56,00	0,62
GEO40	3,02	1,64	0,545	1,1736	0,046	0,29	0,48	0,21	1,65	0,93	0,95	0,315	53,00	0,45
SM4	3,83	2,02	0,795	1,624	0,029	0,5	0,62	0,25	2,09	1,11	1,1	0,41	56,17	0,8
SM10	3,51	1,74	0,645	1,4301	0,039	0,41	0,5	0,2	1,76	1,1	0,93	0,355	56,33	0,52
SM11	4,22	2,32	0,85	2,5403	0,046	0,48	0,62	0,36	2,1	1,26	1,12	0,345	63,83	0,85
SM12	4,29	2,03	0,755	2,0225	0,038	0,51	0,61	0,27	2,13	1,2	1,11	0,44	49,50	0,63
SM13	4,3	2,19	0,85	2,8629	0,055	0,58	0,65	0,29	2,4	1,31	1,08	0,465	52,17	0,71
SM14	4,25	2,14	0,795	2,4661	0,044	0,52	0,57	0,25	2,16	1,13	1	0,555	52,00	0,73
SM15	3,55	1,79	0,705	1,5262	0,040	0,44	0,5	0,27	1,78	1	0,9	0,41	51,17	0,62
SM16	2,87	1,6	0,655	0,973	0,031	0,29	0,46	0,21	1,5	0,97	0,82	0,365	55,17	0,56
SM17	3,66	2,02	0,685	1,5153	0,030	0,42	0,48	0,25	1,88	0,9	1,02	0,46	61,67	0,79

Supplement 2 (continued).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
SM19	3,29	1,67	0,66	1,2065	0,033	0,25	0,37	0,2	1,64	1	1,02	0,365	49,17	0,59
SM20	2,87	1,53	0,57	0,905	0,028	0,28	0,45	0,14	1,57	0,82	0,8	0,335	52,50	0,64
SM24	2,84	1,47	0,515	0,7222	0,025	0,29	0,42	0,2	1,43	0,9	0,75	0,35	50,67	0,5
SM25	3,1	1,62	0,635	1,1835	0,039	0,28	0,39	0,21	1,76	0,89	0,85	0,46	49,67	0,59
SM26	4,66	2,5	0,975	3,1088	0,042	0,49	0,75	0,33	2,39	1,5	1,32	0,515	53,17	0,93
SM27	3,23	1,57	0,645	1,1726	0,039	0,32	0,43	0,23	1,73	0,98	0,82	0,36	47,50	0,5
SM29	2,95	1,63	0,615	1,0616	0,032	0,4	0,43	0,22	1,63	0,78	0,81	0,335	53,67	0,58
SM30	2,28	1,17	0,415	0,3711	0,020	0,2	0,29	0,12	1,16	0,66	0,6	0,305	50,83	0,36
SM34	2,65	1,27	0,495	0,4925	0,021	0,27	0,43	0,15	1,35	0,82	0,66	0,305	50,67	0,46
SM37	2,5	1,35	0,44	0,5517	0,028	0,31	0,37	0,2	1,25	0,8	0,63	0,31	59,67	0,55
SM40	2,81	1,49	0,505	0,7577	0,029	0,32	0,36	0,17	1,49	0,81	0,79	0,35	52,50	0,5
FH1	3,67	2	0,845	1,4563	0,029	0,31	0,5	0,23	2	1,09	1,1	0,34	50,17	0,59
FH2	3,32	1,82	0,815	1,4449	0,031	0,45	0,5	0,3	1,5	1,05	1	0,315	57,83	0,5
FH5	4,05	2,04	0,82	1,7994	0,031	0,31	0,51	0,28	1,9	1,09	1,1	0,455	55,00	0,7
FH6	3,58	1,85	0,72	1,564	0,039	0,42	0,5	0,18	1,82	1,09	1	0,365	56,67	0,56
FH7	3,44	1,75	0,71	1,2641	0,033	0,38	0,5	0,21	1,72	0,95	0,93	0,32	52,17	0,6
FH8	3,84	2,1	0,88	2,0221	0,042	0,31	0,5	0,26	1,86	1,22	1,21	0,37	59,67	0,54
FH10	3,7	1,94	0,77	1,7122	0,043	0,43	0,5	0,25	1,91	1,04	1,1	0,37	48,83	0,6
FH11	3,64	1,82	0,725	1,4643	0,031	0,36	0,5	0,25	1,74	1,02	0,96	0,36	50,50	0,7
FH12	3,69	2,03	0,78	1,3397	0,025	0,31	0,4	0,22	1,8	1,19	1,19	0,405	55,33	0,7
FH13	3,5	1,9	0,76	1,1926	0,030	0,45	0,5	0,16	1,7	1,06	1,06	0,33	56,33	0,65
FH15	3,45	1,98	0,745	1,4771	0,036	0,35	0,42	0,25	1,84	0,98	1,02	0,41	54,00	0,71
FH24	2,68	1,43	0,555	0,6005	0,024	0,29	0,35	0,17	1,37	0,79	0,81	0,31	50,33	0,49
FH26	3,06	1,44	0,66	0,9098	0,031	0,37	0,35	0,26	1,55	0,9	0,81	0,355	49,83	0,5
FH27	3,11	1,59	0,575	0,8361	0,025	0,33	0,4	0,22	1,43	0,89	0,9	0,34	59,67	0,6
FH31	3,29	1,72	0,65	0,8765	0,022	0,38	0,5	0,23	1,56	1,04	0,97	0,36	52,17	0,53
FH32	3,35	1,7	0,63	1,1847	0,041	0,43	0,41	0,2	1,55	1,03	0,9	0,355	56,83	0,55
FH33	3,08	1,72	0,725	1,0285	0,029	0,31	0,4	0,24	1,82	0,85	0,83	0,345	52,50	0,49
FH34	2,91	1,43	0,58	0,7614	0,027	0,29	0,22	0,19	1,49	0,76	0,75	0,375	46,33	0,45
FH35	3,44	1,7	0,72	1,1054	0,025	0,36	0,43	0,19	1,63	0,86	0,96	0,325	55,67	0,58
FH38	2,82	1,49	0,595	0,8651	0,033	0,31	0,39	0,22	1,3	0,9	0,73	0,28	55,83	0,64
Hörn2	3,59	1,86	0,8	1,506	0,035	0,43	0,5	0,3	1,73	1	0,89	0,415	50,50	0,57

Supplement 2 (continued).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
Hörn3	3,05	1,68	0,655	1,1615	0,041	0,28	0,4	0,23	1,47	0,95	0,76	0,35	52,33	0,59
Hörn6	3,37	1,7	0,69	1,1365	0,026	0,44	0,47	0,24	1,71	1,09	0,99	0,29	47,17	0,51
Hörn8	3,1	1,71	0,66	0,9819	0,025	0,32	0,5	0,28	1,5	0,92	0,97	0,325	54,00	0,6
Hörn10	3,01	1,59	0,74	0,8602	0,026	0,36	0,36	0,24	1,69	0,79	0,79	0,3	47,17	0,55
Hörn12	3,69	1,92	0,785	1,5225	0,032	0,41	0,61	0,17	2	1,11	0,91	0,43	46,00	0,56
Hörn14	3,04	1,54	0,675	1,0559	0,027	0,33	0,45	0,27	1,55	0,94	0,75	0,35	49,50	0,5
Hörn19	3,05	1,57	0,575	0,7165	0,022	0,28	0,42	0,19	1,51	0,83	0,82	0,275	58,83	0,54
Hörn20	3,1	1,68	0,585	1,0495	0,049	0,4	0,51	0,2	1,57	1	0,87	0,33	53,17	0,49
Hörn24	3,24	1,71	0,68	1,1199	0,027	0,35	0,41	0,27	1,8	0,9	0,85	0,3	42,17	0,5
Hörn25	3,2	1,7	0,65	1,1723	0,029	0,33	0,42	0,23	1,66	0,89	0,9	0,32	53,50	0,51
Hörn26	3,22	1,61	0,74	1,1016	0,038	0,39	0,51	0,27	1,44	0,98	0,92	0,4	49,83	0,53
Hörn27	3,18	1,74	0,64	1,0098	0,027	0,36	0,43	0,25	1,62	0,9	0,94	0,335	53,67	0,57
Hörn30	3,19	1,73	0,685	0,9886	0,022	0,37	0,51	0,21	1,74	1	0,9	0,345	53,17	0,52
Hörn31	2,44	1,41	0,55	0,495	0,019	0,29	0,33	0,15	1,29	0,77	0,74	0,3	52,83	0,43
Hörn32	2,24	1,29	0,51	0,516	0,026	0,26	0,25	0,19	1,2	0,7	0,6	0,29	54,00	0,43
Hörn33	2,95	1,55	0,645	0,9598	0,031	0,38	0,46	0,23	1,64	0,9	0,73	0,31	57,67	0,5
Hörn34	3,29	1,75	0,65	1,1027	0,022	0,35	0,43	0,3	1,7	1,05	0,83	0,425	50,67	0,6
Hörn35	2,81	1,56	0,57	0,645	0,016	0,37	0,42	0,17	1,49	0,81	0,75	0,35	61,67	0,56
Hörn36	2,86	1,46	0,655	0,8322	0,030	0,34	0,39	0,24	1,57	0,85	0,78	0,305	44,00	0,49
MAR1	3,91	2,05	0,75	1,5653	0,036	0,44	0,53	0,25	1,85	1,13	1,2	0,465	58,50	0,76
MAR2	3,77	1,8	0,675	1,3169	0,032	0,43	0,53	0,21	1,85	1,15	1,03	0,375	42,17	0,5
MAR3	3,5	1,62	0,715	1,0898	0,030	0,33	0,48	0,2	1,8	1,09	0,82	0,29	47,00	0,43
MAR7	3,87	2,04	0,715	1,4007	0,027	0,43	0,45	0,23	1,83	1,2	1,19	0,495	56,83	0,69
MAR8	4,07	1,98	0,72	1,5647	0,028	0,4	0,64	0,3	1,89	1,38	1,15	0,43	58,50	0,59
MAR10	3,32	1,73	0,655	0,7939	0,020	0,32	0,42	0,19	1,61	0,91	1,13	0,355	51,17	0,46
MAR11	3,81	1,99	0,705	1,2138	0,031	0,49	0,58	0,23	1,66	1,2	1,2	0,375	59,67	0,66
MAR12	4,22	2,14	0,73	1,4807	0,028	0,35	0,57	0,29	2,2	1,35	1,26	0,32	55,00	0,6
MAR13	3,96	2,06	0,89	2,2027	0,045	0,48	0,52	0,3	1,87	1,27	1	0,42	55,17	0,64
MAR14	4,33	1,99	0,77	1,9025	0,036	0,4	0,63	0,23	1,93	1,23	1,16	0,5	47,50	0,65
MAR15	3,73	1,99	0,755	1,4963	0,028	0,49	0,64	0,24	1,85	1,25	1,02	0,48	52,67	0,72
MAR17	4,35	1,91	0,975	2,3112	0,041	0,39	0,69	0,31	2,14	1,3	1,05	0,46	40,00	0,65
MAR26	5,04	2,74	0,965	4,25232	0,061	0,42	0,71	0,35	2,65	1,56	1,58	0,56	57,00	0,74
MAR27	3,68	1,88	0,72	1,4714	0,038	0,39	0,5	0,2	1,93	1,17	1,19	0,355	51,67	0,67

Supplement 2 (continued).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
MAR28	4,1	2,08	0,765	1,8737	0,034	0,48	0,7	0,33	2,13	1,25	1,2	0,5	56,17	0,79
MAR29	3,95	1,91	0,74	1,2747	0,022	0,45	0,61	0,23	1,79	1,19	1,1	0,46	54,00	0,69
MAR30	4,1	2,22	0,845	2,811	0,064	0,38	0,68	0,3	2,24	1,29	1,15	0,4	48,67	0,61
MAR32	2,5	1,3	0,455	0,5093	0,028	0,36	0,39	0,16	1,2	0,79	0,69	0,37	58,33	0,49
MAR33	4,67	2,35	0,955	2,9979	0,043	0,46	0,65	0,29	2,53	1,3	1,39	0,395	48,17	0,73
MAR38	4,41	2,27	0,775	2,3147	0,039	0,5	0,6	0,27	2,13	1,24	1,25	0,41	49,33	0,59
SC1	3,2	1,59	0,615	0,7429	0,021	0,25	0,42	0,19	1,56	0,94	0,9	0,275	48,67	0,53
SC2	3,34	1,66	0,69	0,9401	0,023	0,3	0,42	0,23	1,7	0,92	0,83	0,32	49,67	0,54
SC3	3,32	1,53	0,63	0,8356	0,025	0,26	0,43	0,21	1,59	1,01	0,88	0,28	54,00	0,54
SC5	3,97	1,82	0,81	1,326	0,025	0,33	0,5	0,25	1,9	1,08	0,99	0,365	52,00	0,68
SC6	3,73	1,73	0,655	1,0691	0,026	0,2	0,51	0,225	1,86	1,13	0,86	0,315	50,50	0,54
SC8	2,76	1,27	0,525	0,5125	0,020	0,19	0,4	0,17	1,39	0,86	0,64	0,225	48,83	0,37
SC9	3,39	1,47	0,67	0,8663	0,025	0,27	0,5	0,18	1,71	1,01	0,72	0,32	44,33	0,46
SC10	3,5	1,74	0,615	1,1908	0,030	0,24	0,56	0,24	1,78	1,1	0,84	0,385	62,83	0,59
SC11	3,07	1,45	0,645	0,8467	0,027	0,23	0,4	0,2	1,62	0,93	0,69	0,32	41,67	0,46
SC12	3,1	1,46	0,645	0,8588	0,028	0,2	0,38	0,21	1,53	0,91	0,73	0,33	52,17	0,54
SC13	2,67	1,38	0,53	0,5749	0,021	0,18	0,47	0,17	1,39	0,85	0,66	0,235	50,83	0,52
SC14	2,65	1,32	0,525	0,6126	0,028	0,26	0,35	0,19	1,34	0,73	0,65	0,29	48,00	0,43
SC16	3,85	1,67	0,695	1,2395	0,031	0,34	0,52	0,26	1,78	1,16	0,92	0,36	54,17	0,55
SC17	3,6	1,59	0,635	1,0797	0,031	0,27	0,51	0,21	1,63	1,13	0,83	0,305	51,17	0,5
SC18	3,31	1,6	0,59	0,6634	0,021	0,3	0,47	0,22	1,61	1,01	0,83	0,29	51,50	0,51
SC19	3,45	1,55	0,645	1,0299	0,029	0,28	0,58	0,2	1,85	1,12	0,8	0,31	42,67	0,57
SC20	3,06	1,53	0,575	0,6743	0,018	0,25	0,42	0,21	1,54	0,87	0,74	0,33	50,17	0,55
SC21	3,21	1,72	0,7	1,3367	0,030	0,23	0,46	0,26	1,73	0,8	0,8	0,345	59,17	0,62
SC22	3,32	1,58	0,6	0,7579	0,020	0,26	0,44	0,2	1,62	0,96	0,89	0,29	53,83	0,57
SC23	2,96	1,5	0,6	0,6706	0,024	0,21	0,38	0,22	1,45	0,78	0,86	0,305	46,17	0,5
V51	3,46	1,68	0,7	2,2185	0,056	0,22	0,42	0,2	1,82	1,1	1,02	0,285	45,83	0,5
V52	3,9	2,03	0,79	2,8613	0,060	0,25	0,49	0,2	2,16	1,16	1,16	0,31	46,00	0,5
V53	4,42	2,2	0,9	3,8106	0,055	0,28	0,55	0,29	2,5	1,28	1,32	0,35	39,00	0,53
V54	3,73	1,93	0,695	2,3418	0,066	0,32	0,44	0,24	1,81	1,16	1,27	0,325	54,00	0,7
V55	4,28	2,12	0,895	3,5204	0,064	0,28	0,52	0,25	2,15	1,36	1,36	0,435	39,17	0,61
V56	3,66	1,9	0,82	2,8774	0,069	0,2	0,48	0,25	1,89	1,06	1,17	0,345	47,83	0,6

Supplement 2 (continued).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
V58	3,54	1,81	0,82	2,8092	0,063	0,2	0,45	0,2	1,93	1,03	1,18	0,345	51,67	0,49
V59	3,97	2,05	0,83	3,4152	0,073	0,25	0,44	0,22	2,18	1,1	1,18	0,415	42,67	0,52
V60	3,67	1,83	0,765	2,5415	0,047	0,13	0,4	0,22	2,04	0,98	1,1	0,4	42,83	0,5
V61	3,93	2,11	0,76	2,624	0,040	0,25	0,52	0,2	2,01	1,2	1,26	0,38	50,50	0,51
V62	3,5	1,8	0,695	2,168	0,053	0,19	0,41	0,25	1,95	1,11	1,2	0,4	52,67	0,54
V63	3,54	1,93	0,66	2,4103	0,060	0,2	0,48	0,21	1,91	1,12	1,12	0,315	44,83	0,53
V64	3,92	1,97	0,775	3,0092	0,073	0,29	0,43	0,28	2,04	1,12	1,2	0,38	46,17	0,52
V65	3,74	1,81	0,77	2,6826	0,061	0,22	0,6	0,24	1,81	1,26	1,13	0,335	46,50	0,41
V66	3,9	2	0,83	2,7979	0,062	0,23	0,49	0,24	1,85	1,16	1,32	0,4	57,33	0,63
V67	3,94	2,19	0,695	2,3235	0,057	0,21	0,58	0,23	1,88	1,4	1,35	0,35	53,50	0,55
V68	3,62	1,85	0,9	2,7571	0,058	0,2	0,48	0,25	2,28	0,95	1,07	0,285	37,00	0,42
V69	3,95	1,84	0,78	2,6352	0,041	0,28	0,44	0,25	2,28	1,12	1,16	0,32	39,67	0,53
V70	4,19	2,07	0,835	3,3049	0,069	0,28	0,49	0,22	2,17	1,27	1,35	0,385	43,17	0,54
V71	3,96	2,14	0,78	3,59	0,071	0,26	0,51	0,22	2,4	1,25	1,26	0,35	44,83	0,4
HF	3,74	2	0,715	2,1807	0,058	0,34	0,55	0,29	2,1	1,05	0,8	0,4	52,83	0,6
H11	5,57	2,73	1,1	8,5108	0,111	0,43	0,89	0,34	2,78	1,55	1,56	0,44	49,00	0,91
H12	6	2,77	1,115	7,226	0,082	0,45	0,85	0,37	2,99	1,7	1,5	0,475	49,00	0,95
H13	5,34	2,78	1,05	5,9574	0,074	0,64	0,69	0,4	2,78	1,47	1,52	0,5	54,17	0,86
H20	4,45	2,46	0,895	3,8997	0,069	0,42	0,71	0,3	2,42	1,33	1,16	0,39	54,67	0,84
H21	4,71	2,5	0,85	4,1329	0,059	0,46	0,8	0,36	2,38	1,4	1,25	0,51	58,50	0,9
H22	4,16	2,22	0,73	2,7012	0,056	0,37	0,6	0,31	2,16	1,19	1,05	0,385	59,67	0,75
H23	2,7	1,45	0,48	0,7853	0,038	0,23	0,46	0,21	1,45	0,85	0,64	0,285	56,67	0,51
H24	3,34	1,69	0,595	1,1634	0,035	0,38	0,51	0,2	1,64	0,98	0,79	0,385	59,17	0,58
H25	2,98	1,6	0,565	1,0973	0,046	0,33	0,48	0,21	1,66	0,85	0,82	0,34	53,50	0,6
H26	2,65	1,5	0,45	0,8631	0,042	0,21	0,35	0,21	1,35	0,75	0,65	0,275	53,33	0,52
H27	3,15	1,69	0,58	1,1445	0,041	0,27	0,48	0,23	1,62	0,95	0,85	0,33	55,33	0,62
H28	2,62	1,3	0,485	0,8134	0,038	0,26	0,43	0,2	1,3	0,84	0,61	0,365	49,83	0,47
H29	3,09	1,68	0,575	1,1043	0,041	0,32	0,47	0,25	1,62	0,98	0,82	0,32	55,50	0,5
H30	3,88	2,04	0,7	1,9251	0,048	0,32	0,53	0,31	2,05	1,1	1,04	0,36	54,50	0,63
H31	3,36	1,86	0,645	1,6831	0,058	0,34	0,51	0,23	1,74	1,05	0,92	0,33	55,67	0,57
H32	2,72	1,5	0,515	0,9848	0,044	0,29	0,44	0,21	1,5	0,91	0,74	0,3	50,33	0,51
H33	3,72	2	0,71	1,9409	0,049	0,33	0,62	0,25	1,99	1,15	0,96	0,37	51,50	0,71
H34	2,43	1,5	0,48	0,783	0,038	0,24	0,4	0,2	1,39	0,79	0,65	0,28	55,83	0,46

Supplement 2 (continued).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
H35	3,75	1,98	0,7	2,161	0,054	0,33	0,61	0,25	2	1,16	1	0,35	55,17	0,59
Askö1	2,93	1,48	0,71	0,6369	0,017	0,24	0,32	0,31	1,24	1,16	0,94	0,29	67,50	0,61
Askö2	2,6	1,38	0,56	0,4422	0,016	0,25	0,28	0,26	1,02	0,9	1	0,26	67,00	0,53
Askö5	3,5	1,49	0,65	0,6554	0,017	0,25	0,33	0,15	1,32	1,07	1,05	0,29	52,17	0,38
Askö7	3,11	1,65	0,675	0,6648	0,020	0,21	0,3	0,18	1,3	0,89	1,11	0,34	71,67	0,63
Askö8	2,76	1,5	0,615	0,5962	0,020	0,17	0,35	0,16	1,1	0,9	1,01	0,28	62,83	0,52
AsköVI	3,43	1,71	0,7	0,8704	0,021	0,19	0,46	0,17	1,56	1,05	1,16	0,355	52,50	0,43
Askö10	2,58	1,4	0,525	0,3518	0,016	0,15	0,34	0,15	1,14	0,89	0,94	0,255	67,17	0,49
Askö13	2,33	1,2	0,465	0,2588	0,013	0,23	0,24	0,12	1,06	0,73	0,72	0,235	49,67	0,33
Askö15	2,35	1,23	0,455	0,211	0,011	0,22	0,3	0,13	1	0,8	0,84	0,275	65,50	0,45
AsköVIII	2,84	1,46	0,55	0,3461	0,011	0,21	0,31	0,25	1,23	0,91	0,95	0,375	61,67	0,43
AsköB	2,59	1,39	0,56	0,3993	0,015	0,17	0,36	0,17	1,09	0,87	0,93	0,21	65,17	0,55
AsköD	2,45	1,34	0,515	0,2927	0,013	0,16	0,3	0,18	0,93	0,96	0,8	0,26	62,50	0,44
AsköE	2,5	1,19	0,51	0,2781	0,015	0,14	0,29	0,14	1,08	0,79	0,86	0,24	53,50	0,36
AsköXIV	3,03	1,5	0,625	0,477	0,014	0,24	0,35	0,15	1,3	0,76	1,06	0,37	69,67	0,56
AsköH	2,58	1,23	0,485	0,299	0,012	0,22	0,32	0,15	1,11	0,83	0,88	0,265	56,50	0,49
AsköIV	3,26	1,63	0,64	0,5801	0,017	0,24	0,43	0,21	1,3	1,13	1,05	0,315	51,00	0,57
AsköJ	2,48	1,3	0,55	0,351	0,014	0,17	0,32	0,17	1,19	0,81	0,83	0,315	67,33	0,6
AsköK	2,66	1,2	0,45	0,2574	0,011	0,21	0,32	0,15	1,03	0,84	0,84	0,235	54,33	0,41
AsköL	2,27	1,15	0,46	0,2987	0,015	0,16	0,29	0,17	1,06	0,72	0,75	0,275	57,67	0,43
AsköN	2,24	1,08	0,46	0,1959	0,010	0,18	0,28	0,13	0,96	0,67	0,7	0,2	55,00	0,36
Tjärnö2	3,9	1,8	0,74	1,3699	0,027	0,4	0,44	0,25	1,87	1,15	0,99	0,345	46,67	0,58
Tjärnö3	3,84	2,07	0,745	2,0349	0,047	0,44	0,7	0,25	2	1,18	1,05	0,43	57,00	0,76
Tjärnö4	3,96	1,89	0,795	2,2821	0,053	0,42	0,62	0,27	2	1,08	1	0,43	44,67	0,69
Tjärnö9	3,86	1,95	0,725	1,5175	0,032	0,42	0,57	0,26	1,95	1,13	1,05	0,39	53,67	0,64
Tjärnö10	3,83	1,93	0,75	1,7056	0,040	0,45	0,7	0,25	2,05	1,26	0,96	0,43	50,33	0,7
Tjärnö11	3,28	1,7	0,65	1,1429	0,031	0,35	0,5	0,21	1,69	1	0,84	0,405	54,33	0,61
Tjärnö12	4,09	2,19	0,815	1,8727	0,037	0,45	0,65	0,28	1,99	1,29	1,04	0,465	54,00	0,83
Tjärnö13	3,28	1,8	0,615	1,1879	0,035	0,34	0,51	0,26	1,57	0,99	0,99	0,41	59,33	0,64
Tjärnö14	3,86	1,8	0,685	1,3166	0,028	0,37	0,7	0,26	1,73	1,24	0,96	0,34	52,17	0,69
Tjärnö15	4,68	2,15	0,805	1,969	0,029	0,44	0,75	0,25	2,13	1,45	1,09	0,495	48,83	0,83
Tjärnö16	3,66	1,8	0,625	1,0353	0,022	0,44	0,59	0,25	1,64	1,27	0,93	0,395	53,67	0,75

Supplement 2 (continued).

Mussel	l [cm]	ht [cm]	wid [cm]	wg [g]	tck [cm]	aam [cm]	pam [cm]	pal [cm]	lig [cm]	pamp [cm]	pamv [cm]	hp [cm]	la [°]	mwid [cm]
Tjärnö17	3,56	1,88	0,7	1,3452	0,035	0,4	0,51	0,27	1,86	1,11	0,9	0,42	53,83	0,64
Tjärnö19	3,36	1,6	0,66	1,2189	0,034	0,32	0,51	0,26	1,71	1,08	0,79	0,34	50,83	0,58
Tjärnö20	3,73	1,92	0,71	1,8842	0,049	0,36	0,6	0,26	1,82	1,2	1,08	0,375	52,50	0,74
Tjärnö21	4,29	2,1	0,825	1,9944	0,033	0,37	0,58	0,24	2,14	1,13	1,08	0,43	51,67	0,73
Tjärnö23	3,63	1,77	0,66	1,2112	0,028	0,36	0,49	0,25	1,82	1,05	0,94	0,36	51,50	0,7
Tjärnö24	3,33	1,78	0,67	1,321	0,039	0,36	0,52	0,24	1,62	0,99	0,99	0,33	51,67	0,57
TjärnöA	3,56	1,79	0,685	1,369	0,035	0,39	0,46	0,28	1,77	1,05	0,95	0,375	53,17	0,64
TjärnöB	2,82	1,42	0,485	0,5985	0,023	0,32	0,46	0,22	1,35	0,95	0,72	0,32	57,33	0,59
TjärnöC	3,13	1,72	0,75	1,5111	0,048	0,45	0,5	0,26	1,8	0,9	0,84	0,415	44,67	0,6

Supplement 3. p values for population differentiation according to Weir & Cockerham (1984). Lower diagonal: nuclear loci (EFbis, Glu-5', M7 Lysin, mac-1). Upper diagonal: mitochondrial D-loop. Values that indicated significance after Bonferroni correction are marked in red. GEO = GEOMAR West Shore Campus, SM = Museum of Ship Transport, FH = East Shore Harbour, MAR = Wendtorf Marina, SC = Penn Cove, V = Vigo, H = Helgoland, AK = Askö, TJ = Tjärnö.

	GEO	SM	FH	Hörn	MAR	Kiel	SC	H	AK	TJ	V
GEO		0,99990	0,99990	0,99990	0,10524	-	0,00000	0,99990	0,00000	0,99990	-
SM	0,72676		0,99990	0,99990	0,10880	-	0,00000	0,99990	0,00010	0,99990	-
FH	0,61934	0,92912		0,99990	0,10761	-	0,00000	0,99990	0,00000	0,99990	-
Hörn	0,25522	0,27245	0,52183		0,10385	-	0,00000	0,99990	0,00000	0,99990	-
MAR	0,23829	0,44213	0,85694	0,65023		-	0,00000	0,10613	0,00822	0,10355	-
Kiel	-	-	-	-	-		0,00000	0,60123	0,00000	0,60875	-
SC	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000		0,00000	0,00000	0,00000	-
H	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000		0,00000	0,99990	-
AK	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000		0,00000	-
TJ	0,03485	0,00376	0,02386	0,00941	0,03643	0,00079	0,00000	0,00000	0,00000		-
V	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	

11 Erklärung (Statement)

Hiermit erkläre ich, dass ich die vorliegende Arbeit, abgesehen von der Beratung durch meine Betreuer, selbstständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Die eingereichte schriftliche Fassung der Arbeit entspricht der auf dem elektronischen Speichermedium. Weiterhin versichere ich, dass diese Arbeit noch nicht als Abschlussarbeit an anderer Stelle vorgelegen hat. Mit der Aufnahme meiner Masterarbeit in die Fachbibliothek des Helmholtz-Zentrums für Ozeanforschung sowie in die Universitätsbibliothek der Christian-Albrechts-Universität zu Kiel bin ich einverstanden.

I hereby declare that – apart from asking my supervisors' advice – I have completed the present thesis on my own and have not used other than the stated sources and aids. The written version corresponds to the electronic one. Furthermore, I assure that it has not been submitted as final assignment somewhere else. I agree on including my Master Thesis in the library of the Helmholtz Centre for Ocean Research as well as in the library of the Christian-Albrechts-Universität zu Kiel.

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Place, Date Signature