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# Invasion genetics of Pacific oyster *Crassostrea gigas* shaped by aquaculture stocking practices

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ABSTRACT

As a result of aquaculture activities Pacific oysters *Crassostrea gigas* (Thunberg, 1793) have invaded the European Wadden Sea. Using a variable noncoding mitochondrial marker, we show that the invaded range is the result of two independent invasions. Haplotype frequencies point towards two separate groups, one in the southern and the other in the northern Wadden Sea. We found virtually no genetic differentiation throughout the southern range and the putative source from British Columbia, Canada, suggesting that the Southern region can be considered as a closed population. In the North, mismatch distributions, haplotype ordination and isolation-by-distance analysis suggest a stronger, persistent impact of aquaculture on invasive populations. Due to the ongoing supply of new genetic material from hatchery production the northern invasive populations can therefore be considered as an open population highlighting the importance of aquaculture practice on the genetics of this keystone invader in the Wadden Sea.



## 1. Introduction

Originating from East Asia, Pacific oysters *Crassostrea gigas* (Thunberg, 1793) escaped from commercial oyster farms and successfully invaded a variety of coastal habitats worldwide (e.g. (Ruesink et al., 2005; Wrange et al., 2010). This also applies to the European Wadden Sea, where two invasions were reported. One originated from Pacific oysters that were imported from farms in British Columbia (Canada) into the Dutch Oosterschelde estuary between 1964 and 1982. Massive spatfalls during warm summers in 1976 and 1982 resulted in the establishment of a permanent population (Drinkwaard, 1998; Smaal et al., 2009). To the Wadden Sea, approximately 150 km north of the Oosterschelde estuary, Pacific oysters either arrived accidentally by mussel transports from the Oosterschelde or experimentally with imports to the island of Texel (western Wadden Sea) in 1978 (Smaal et al., 2009), which is also where the first wild Pacific oysters were encountered in 1983 (Bruins, 1983). From there the population spread east and arrived as far as the island of Baltrum in Germany by 1998 (Wehrmann et al., 2000) and rapidly spread further to Büsum across the Elbe estuary (Reise et al., 2005).

In the northern Wadden Sea off the island of Sylt, regular aquaculture with Pacific oysters commenced in 1986 with juvenile seed oysters imported from Britain every year and then grown to market size in the open water (Reise, 1998). Although it was thought that summer temperatures in the northern Wadden Sea are too low for reproduction, a successful north- and southward spread was soon observed (Diederich et al., 2005). Owing to high late summer temperatures in the early 2000s a strong expansion could be observed reaching population densities from 100 to 2000 individuals $m^{-2}$ (Nehls and Büttger, 2007; Nehring et al., 2009; Reise et al., 2005). Since wild *C. gigas* in the Wadden Sea is not commercially harvested, apparently not affected by predators, parasites and competitors to a large extent (Elsner et al., 2011; Kochmann et al., 2008; Krakau et al., 2006; Reise and van Beusekom, 2008), further population growth can be expected. This oyster invasion is perceived as the most severe impact an alien species so far had on the ecosystem of the Wadden Sea (Nehring et al., 2009), partly because mussel beds around low tide line became dominated by oysters with ecological consequences for native species (Diederich, 2005, 2006; Markert et al., 2010; Troost, 2010). For example, in mixed beds mussels are relegated by crabs to refuge positions at the bottom between the much larger oysters where growth is reduced (Eschweiler and Christensen, 2011).

Nothing is known about the genetic population structure and distinctness of the two invasions in the Wadden Sea. Such data is needed to clarify the origin and the specific demographic history of the invasive populations. It can also provide estimates for inocculum size and whether an invasion only happened once or on repeated occasions (Geller et al., 2010; Reusch et al., 2010; Roman and Darling, 2007). Such multiple introductions are usually cryptic and multiple invasions can only be revealed by genetic markers (e.g. invasion of green crab into West-Atlantic (Roman, 2006)).

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To test whether all Pacific oysters in the Wadden Sea originate from one or more genetic stocks of the global oyster trade, we constructed a mitochondrial phylogeography containing oysters from four northern and three southern sites throughout the Wadden Sea. To further evaluate the connectivity between aquaculture and invading populations (Petersen et al., 2010; Voisin et al., 2005) we also included specimen from the two putative aquaculture sources (i.e. Oosterschelde estuary and Sylt) as well as oysters from a naturalized population in British Columbia representative for the original stocking population of the Oosterschelde (Drinkwaard, 1998). Using this sampling scheme we can connect putative and known invasion routes with aquaculture sources and provide population genetic and demographic signatures of the most dramatic invasion process observed in the Dutch–German–Danish Wadden Sea. The results offer implications for further research and management practice.

## 2. Materials and methods

A total of 190 specimens of *C. gigas* were successfully analysed in this study. Oysters were collected between 2008 and 2010 from seven Wadden Sea sites and from one site in British Columbia representing wild invasive populations. These were complemented by commercially sold, farmed oysters from the Dutch Oosterschelde estuary and the Northern Waddensea (Dittmeyer's Austern Compagnie, List, Germany, Table 1). We took care to sample a comparable size/age spectrum between sites. DNA was extracted partially by using Qiagen Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions or by a quick extraction method, where small tissue pieces from the adductor muscle were prepared and dissolved in 40 µl Tris-buffer (10 mM Tris, pH 8.0) with 10% proteinase K for 15 min at 56 °C. This was followed by boiling for 10 min at 99 °C. Finally, samples were centrifuged for 1 min at 10,000 rpm and the supernatant was transferred to a new tube and stored at −20 °C. We used a noncoding region of the mitochondrial genome to have a selectively neutral and lineage-specific marker that offers a simple mutation model due to lack of recombination. This marker (major noncoding region, MNR) was found to be highly variable in Korean populations of *C. gigas* (Aranishi and Okimoto, 2005). Primers (Cg-mt-for TCACAAGTACATTTGTCTTCCA; Cg-mt-rev AACGTTGTAAGCGTCATGTAAT) were used for amplification in 50 µl reaction volume with: 3 µl of 1:10 diluted DNA extract, 0.2 mM of each dNTP, 0.2 µM of each primer, 1.5 mM of $MgCl_2$, and 2.5 U Taq polymerase (Qiagen; Hilden, Germany) in the buffer supplied by the manufacturer. The initial denaturation at 94 °C lasted 2 min, followed by 40 cycles with denaturation at 94 °C for 60 s, annealing at 61 °C for 60 s, and extension at 72 °C for 60 s. Successful amplification was confirmed by electrophoresis on 1% agarose gels. The fragments were purified using the QIAquick purification kit (Qiagen; Hilden, Germany). Unidirectional sequencing of PCR products was carried out by MWG Eurofins Biotech (Martinsried, Germany) or Starseq (Genterprises, Mainz, Germany). Twenty-three randomly chosen singleton haplotypes were verified by repeating DNA extraction, and PCR amplification, and were sequenced in reverse direction.

**Table 1**
Population details of *C. gigas* sampling.

| Sampling site (country) | Latitude longitude | Sampling date |
|---|---|---|
| Esbjerg (Denmark) | 55°01′54″ N 08°25′54″ E | April 2009 |
| Sylt North (Germany) | 55°02′31″ N 08°26′53″ E | June 2008 |
| Sylt Aquaculture (farmed) (Germany) | 54°59′38″ N 08°23′07″ E | April 2010 |
| Sylt South (Germany) | 54°47′33″ N 08°18′26″ E | June 2008 |
| Nordstrand (Germany) | 54°29′59″ N 08°48′51″ E | April 2009 |
| Büsum (Germany) | 54°07′40″ N 08°51′28″ E | April 2009 |
| Wilhelmshaven (Germany) | 53°31′03″ N 08°07′24″ E | March 2009 |
| Texel (Netherlands) | 53°00′09″ N 04°47′15″ E | August 2008 |
| Oosterschelde (farmed) (Netherlands) | 51°36′16″ N 03°54′28″ E | March 2009 |
| Cortes Island, British Columbia (Canada) | 50°05′29″ N 124°59′22″ W | September 2008 |

### 2.1. Data analyses

Our three aims for this data set were to describe the mitochondrial diversity, to assess population demography and estimate genetic differentiation between invasive and putative source populations.

### 2.2. Haplotype diversity

Quality of sequencing chromatograms was manually checked with the freeware CHROMAS Lite 2.01, before alignment using CLUSTAL W (Thompson et al., 1994) implemented in BIOEDIT (Hall, 1999). Sequence sets were defined with the software package DnaSP 5.0 (Rozas et al., 2003). We calculated standard indices of haplotype and nucleotide diversity using ARLEQUIN 3.0 (Excoffier et al., 2005) and a haplotype network was calculated and plotted with help of the *pegas* package in the R statistical environment (R Development Core Team, 2010).

### 2.3. Population demography

To investigate the genetic signatures of recent population demography we calculated neutrality test statistics of Tajima's D and Fu's F and mismatch distributions using ARLEQUIN (Excoffier et al., 2005). We fitted a population expansion model to obtain predicted values for the mismatch distribution and calculate population genetic parameters for population expansion (i.e. $\theta$ before and after the expansion and time since expansion $\tau$).

### 2.4. Genetic differentiation

To detect population clustering individual haplotypes were ordinated on population level using a discriminant correspondence analysis implemented in the *ade4* package (R Development Core Team, 2010). To avoid overweighting of rare haplotypes that do not contribute largely to population differentiation, we excluded all singletons from this analysis. We tested the resulting structure against 999 random permutations as implemented in the *randtest* routine in *ade4*. After detecting significant within group structure we conducted post-hoc pairwise discriminant correspondence analysis to single out which population pairs drive overall differences. Pairwise genetic differentiation ($F_{ST}$) was calculated using ARLEQUIN (Excoffier et al., 2005). Significance of pairwise genetic differentiation ($F_{ST}$) was assessed by comparing observed values to 500 random permutations. The influence of geographic distance on genetic differentiation (isolation by distance) was measured for all pairwise comparisons by correlating the pairwise genetic differentiation matrix ($F_{ST}/1-F_{ST}$) to the geographic distance matrix (measured as shortest water way) using Mantel tests implemented in the *ade4* package. Isolation-by-distance between aquaculture and invasive populations was assessed by linear regression.

## 3. Results

### 3.1. Haplotype diversity

In total, we found 76 mitochondrial haplotypes (GenBank accession numbers JF505202-JF505277) in 190 successfully sequenced individuals. Length of sequences in the alignment was 663 base pairs and 110 polymorphic sites could be detected including gaps.

Haplotype frequencies differed strongly between northern (Nordstrand, Sylt south, Sylt north, Esbjerg and Sylt aquaculture) and southern sites (Büsum, Wilhelmshaven, Texel and Oosterschelde, see Fig. 1). The northern group largely consisted of haplotypes ht01, ht02, ht04, ht06 and ht08, which were exclusively found at medium to high

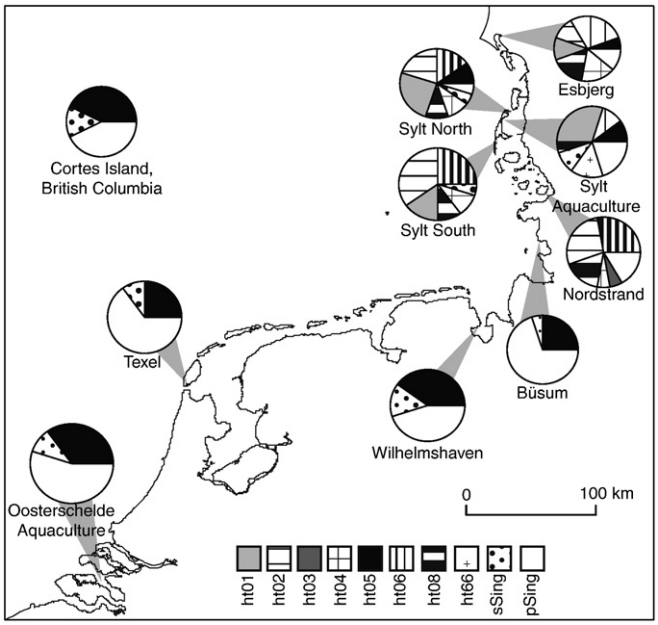


**Fig. 1.** Haplotype frequencies of invasive and source populations of *Crassostrea gigas* in the Waddensea. Only common haplotypes occurring more than three times in the data set are shown. Singletons shared between populations (sSing, dotted) and private singletons (pSing, white) have been pooled for graphical representation.

frequencies in northern populations and only very few singletons occurred. The southern group, on the other hand, was characterized by a single predominant haplotype (ht05) and large numbers of closely related singletons (Figs. 1, 2).

Samples from British Columbia also exhibited the characteristics of the southern group thereby confirming the supposed role as stocking source for the Oosterschelde. Haplotype ht05 was also the most likely ancestral haplotype in the minimum spanning network constructed from all sequences (Fig. 2). It occurred in all populations of the southern clade and in 3/5 populations of the northern group representing 18.9% of all haplotypes. The frequency of ht05 differed strongly between both groups. Only 4.8% of the northern oysters carried ht05 compared to 32.9% of oysters belonging to the southern group (Fig. 1). Most of the southern singletons differed from the basal type by only one mutation (25/55) while most of the main northern haplotypes differed by more mutational steps (9/17, see Fig. 2).

Not surprisingly, the higher genetic variance in the southern group was also reflected in a significantly higher number of haplotypes (Table 2, $F_{1,7}=48.051$, $P<0.001$). Since most of these haplotypes in the southern populations were actually singletons next to the common basal type ht05, haplotypic diversity (i.e. the likelihood of observing different haplotypes) did not differ significantly between northern and southern populations (Table 2, $F_{1,7}=2.820$, $P=0.137$). The larger genetic distance between haplotypes in the northern populations did however lead to higher estimates of nucleotide diversity (Table 2, $F_{1,7}=5.687$, $P=0.049$).

### 3.2. Population demography

Large negative values of Tajima's D and Fu's F in southern populations point to a stronger, probably longer lasting population expansion in the South than in the North, where no clear pattern indicative of population expansion or decline could be found (Table 2, Tajima's D: $F_{1,7}=81.375$, $P=<0.001$, Fu's F: $F_{1,7}=57.083$, $P=<0.001$). This is also reflected in the mismatch distribution of pairwise differences (Fig. 3). The observed distribution in the south is unimodal (Fig. 3B) and fits the expected population expansion model ($\tau=2.201$, $\theta_0=0.951$, $\theta_1=14.908$) very well (Sum of squared deviation $Ssd<0.001$, $P=0.95$). In the North, however, the mismatch distribution is multi-peaked (Fig. 3A). Although similar population expansion parameters were estimated as in the South ($\tau=4.584$, $\theta_0=0.003$, $\theta_1=15.417$) the observed data deviated significantly from predicted values ($Ssd=0.028$, $P=0.04$), indicating that a population expansion model does not represent the most likely demography. Haplotypes in northern populations were more divergent from each other than in the southern population (mean number of pairwise differences North = 3.735 vs. South = 3.031) probably reflecting temporal variation in genotypes used for stocking or crossing schemes within oyster hatcheries.

### 3.3. Genetic differentiation

The correspondence analysis supported grouping of northern and southern populations (P (based on 999 random permutations)< 0.001). The ordination based on non-singleton haplotypes revealed that the southern group clustered very closely with the basal haplotype (ht05) driving differentiation along axis 1 (Fig. 4). The Northern group differentiates mainly along axis 2 driven by frequencies of haplotypes common in the northern populations. Distance between northern populations along axis 2 in the ordination corresponded to geographic distance especially when considering distance from the

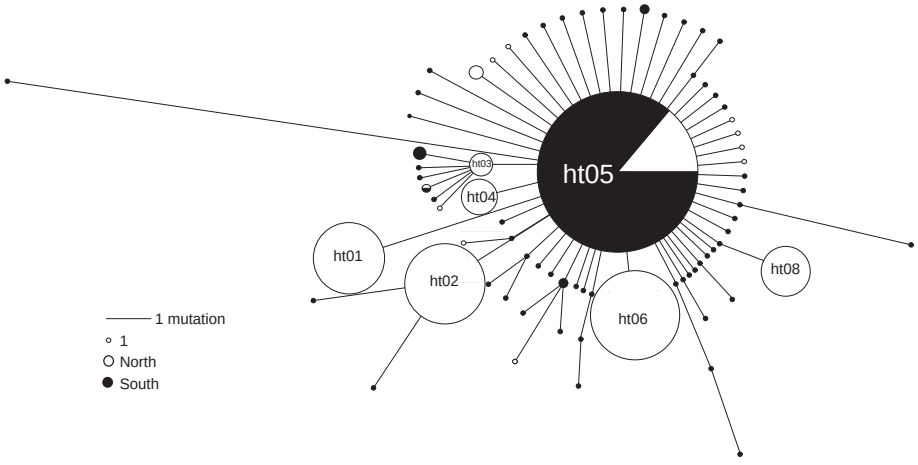


**Fig. 2.** Network of all 76 mitochondrial haplotypes found in Northern and Southern invasive as well as putative source populations. Pie charts give haplotype frequencies in Northern (white) and Southern populations (black) and branch length represents the number of mutations. Size of circles corresponds to haplotype frequency.

**Table 2**
Genetic diversity indices of *C. gigas* MNR mitochondrial DNA sequences.

| Population | | N | $HT_N$ | S | Hd | π | Tajima's D | Fu's FS |
|---|---|---|---|---|---|---|---|---|
| *North* | Esbjerg | 18 | 8 | 15 | 0.882 | 0.0051 | −0.983 | −0.838 |
| | Sylt North | 20 | 8 | 15 | 0.884 | 0.0057 | −0.424 | −0.214 |
| | Sylt Aquaculture | 20 | 10 | 16 | 0.890 | 0.0054 | −0.737 | −2.155 |
| | Sylt South | 20 | 6 | 12 | 0.811 | 0.0051 | −0.007 | 1.187 |
| | Nordstrand | 16 | 8 | 13 | 0.858 | 0.0044 | −0.777 | −1.609 |
| | *Mean* | | 8 | 14.2 | 0.865 | 0.0051 | −0.585 | −0.730 |
| *South* | Büsum | 20 | 16 | 32 | 0.947 | 0.0053 | −2.387 | −11.407 |
| | Willhelmshaven | 20 | 14 | 21 | 0.890 | 0.0034 | −2.324 | −11.041 |
| | Texel | 20 | 16 | 23 | 0.947 | 0.0038 | −2.361 | −14.625 |
| | Oosterschelde | 20 | 13 | 23 | 0.853 | 0.0039 | −2.273 | −7.813 |
| | *Mean* | | 14.75 | 24.75 | 0.909 | 0.0041 | −2.321 | −11.220 |
| | British Columbia | 14 | 7 | 9 | 0.773 | 0.0023 | −1.934 | −3.518 |
| | *Overall* | 190 | 76 | 110 | 0.929 | 0.0044 | −2.481 | −26.217 |

N = number of successfully sequenced individuals; $HT_N$ = number of haplotypes; S = polymorphic sites; Hd = haplotype diversity; π = nucleotide diversity.

putative source, i.e. Sylt aquaculture. Here, pairwise discriminant correspondence analyses were significant to the most distant populations (Sylt aquaculture — Sylt South: inertia = 0.066, p = 0.007; Sylt aquaculture — Nordstrand: inertia = 0.090, p = 0.001) but not to the closer populations (Sylt aquaculture — Sylt North: inertia = 0.039, p = 0.227; Sylt aquaculture — Esbjerg: inertia = 0.065, p = 0.050). Pairwise genetic distances ($F_{ST}$) supported the separation between northern and southern populations (Table 3). Within the southern group differentiation was very low even when including Oosterschelde aquaculture ranging from −0.008 to 0.001 and was not significant in any case representing a homogeneous population with little effect of genetic drift and/or high levels of gene flow. Values within the northern group were higher, ranging from −0.012 to 0.149, but were only significant between Sylt aquaculture and the most distant population in Nordstrand after Bonferroni correction (Table 3). Pairwise genetic differentiation was higher between aquaculture and invasive populations than within invasive populations ($F_{1,12} = 5.62$, P = 0.035) in both regions, but did not differ significantly between regions ($F_{1,12} = 3.01$, P = 0.108) despite on average seven times higher values in the Northern group (Fig. 5). Genetic differentiation (i.e. $F_{ST}/(1 - F_{ST})$) increased with distance as shortest possible waterway from the aquaculture site (Fig. 5, $F_{1,2} = 34.038$, P = 0.028) suggesting limited gene flow to distant populations and a continuous input of new genetic material from the farmed oyster seed into closeby invasive populations. While there was only a positive isolation-by-distance trend among all populations in the Northern group (Mantel test: r = 0.35, p (based on 1000 random permutations) = 0.13), isolation by distance was significant among populations in the Southern group (Mantel test: r = 0.76, p (based on 1000 random permutations) = 0.04). The slope of the correlation was however very small ($<10^{-4}$), and the maximal pairwise genetic differentiation of $F_{ST} = 0.001$ suggests that the effect size of geographic distance for limiting gene flow was small as well.

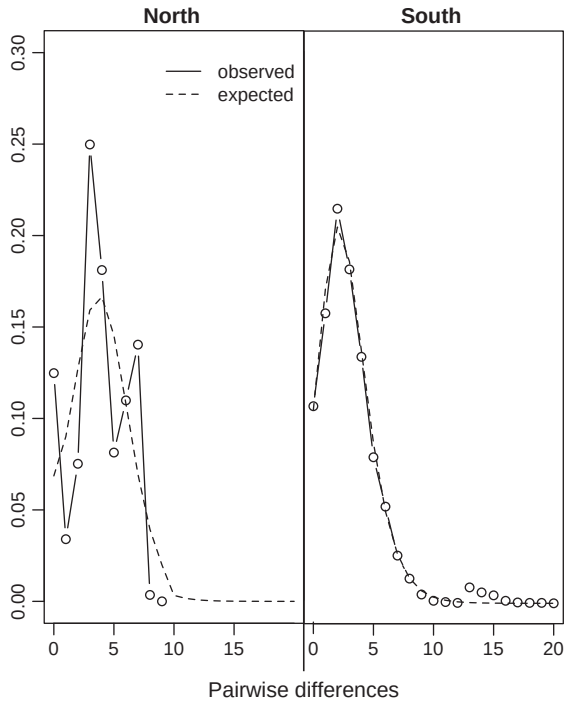


**Fig. 3.** Mismatch distribution observed in mitochondrial haplotypes of Northern (A) and Southern (B) invasive populations of Pacific oysters in the Wadden Sea. Dots and solid lines represent observed distributions and dashed lines show expected values under a demographic expansion model.

## 4. Discussion

With the help of a mitochondrial phylogeography we could demonstrate that two separate events led to invasion of Pacific oysters in the Wadden Sea, which confirms the historical reconstruction of the regional invasion process since the 1980s (Reise et al., 2005). Haplotype composition assigned populations into a Northern and a Southern group (Figs. 1, 4). Both invasions could be traced back to potential aquaculture sources (i.e. Oosterschelde and Sylt, respectively) because samples obtained from commercial farms fell into the same respective clusters (Fig. 4). Aquaculture activity can potentially lead to large inocculum sizes (Roman and Darling, 2007), especially in highly fecund species like Pacific oysters. It is therefore not surprising that all invasive populations in the Wadden Sea displayed a high genetic diversity (Table 2) and obviously did not lose genetic variability during repeated founder events, which is often observed in cultured shellfish (Petersen et al., 2010) and cultured algae (Voisin et al., 2005).

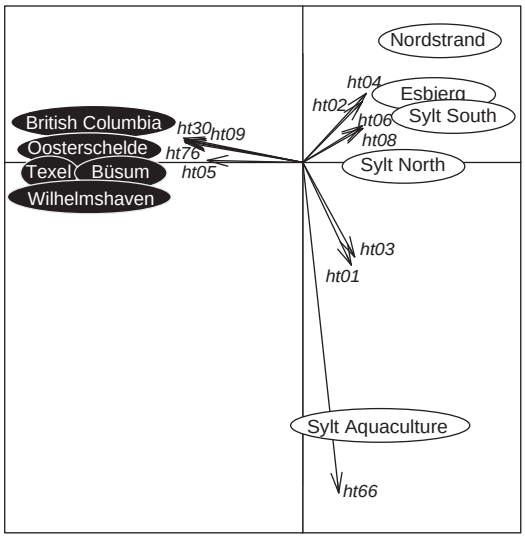


**Fig. 4.** Ordination of invasive and source population based on common mtDNA haplotypes. Ellipses represent population centroids and arrows show the canonical weights of haplotypes occurring at least twice in the data set. Southern populations are shown in black while Northern populations are shown in white. Axis 1 differentiates Northern from Southern populations mainly driven by high frequencies of ht05 in the south, while axis 2 differentiates between Northern sites reflecting the distance to the aquaculture source.

**Table 3**
Pairwise genetic differentiation ($F_{ST}$) between all population pairs.

| | E | SN | Saq | SS | N | B | W | T | O | BC |
|---|---|---|---|---|---|---|---|---|---|---|
| E | - | | | | | | | | | |
| SN | −0.012 | - | | | | | | | | |
| Saq | 0.049 | 0.002 | - | | | | | | | |
| SS | −0.008 | −0.021 | 0.075 | - | | | | | | |
| N | 0.006 | 0.050 | 0.149 (*) | −0.012 | - | | | | | |
| B | 0.043 (*) | 0.071 (*) | 0.087 (*) | 0.085 (*) | 0.078 (*) | - | | | | |
| W | 0.055 (*) | 0.080 | 0.094 | 0.109 (*) | 0.111 (*) | −0.005 | - | | | |
| T | 0.061 (*) | 0.086 (*) | 0.099 | 0.109 (*) | 0.107 (*) | −0.005 | −0.008 | - | | |
| O | 0.064 (*) | 0.095 (*) | 0.108 (*) | 0.113 (*) | 0.107 (*) | 0.001 | −0.002 | −0.007 | - | |
| BC | 0.057 | 0.084 | 0.105 | 0.111 | 0.113 | −0.007 | −0.012 | −0.019 | −0.017 | - |

(*) = significant after Bonferroni correction, E = Esbjerg, SN = Sylt North, Saq = Sylt Aquaculture, SS = Sylt South, N = Nordstrand, B = Büsum, W = Willhelmshaven, T = Texel, O = Oosterschelde, BC = British Columbia.

### 4.1. Southern group

Interestingly, the Southern group also clusters with the putative source population from British Columbia and thereby reflects the documented invasion route from B.C. via aquaculture into the Oosterschelde and from there to Texel and the Southern Wadden Sea (Drinkwaard, 1998; Troost, 2010). This group was dominated by a common basal haplotype (ht05, Fig. 2) accompanied by several closely related singleton sequences. The resulting star-shaped phylogeny is characteristic of expanding populations, which is also reflected in large negative values of Tajima's D and Fu's F (Table 2) as well as a unimodal mismatch distribution (Fig. 3B). Interestingly, we could not detect any significant genetic differentiation even between the most distantly located populations (i.e. Texel and Büsum) despite an overall significant but very weak isolation by distance pattern. Since Büsum reflects the current frontier of the Southern expansion, this is somewhat surprising as this population must have gone through several colonization events during the expansion (Brandt et al., 2008). Haplotype diversity was however as large as on Texel (Table 2) indicating that sequential sites were colonized by a large inocculum stemming from undifferentiated populations with little importance of genetic drift or several rounds of colonization from a common source (Drake et al., 2005). Since recruitment is usually stronger during warm years (Diederich et al., 2005) large inocculum sizes are likely and thus genetic drift during colonization might not have lead to a substantial loss of genetic variability or differentiation between populations. For marine bivalve populations relying on external fertilization such mass settlements may be the rule rather than the exception as population persistence can only be guaranteed by high initial densities preventing extensive dilution of gametes.

### 4.2. Northern group

In contrast, the Northern group was characterized by several medium to high frequency haplotypes, which could not be found at the base of the phylogeny (Fig. 2). This indicates that oyster seed used for aquaculture on Sylt most likely originated from crosses of divergent lines not containing the basal haplotype and thereby artificially amplifying haplotype frequencies of distantly related haplotypes. Crossing of divergent lines decreases inbreeding depression, which is common in Pacific oysters due to high genetic load (Launey and Hedgecock, 2001). Selection for higher growth rates by oyster breeders might have been facilitated by outbreeding to reduce genetic load of produced spat and increase yield (Hedgecock et al., 1995; Hedgecock and Davis, 2007). Seed oysters used for aquaculture in the Northern Wadden Sea presumably came from breeders on the British Isles. And although aquaculture practice there was also based on oysters from British Columbia (Syvret et al., 2008) neither aquaculture oysters nor invasive populations resembled oysters from British Columbia (Fig. 5), indicating that the breeders must maintain a genetically distinct brood stock for spat production.

The Northern populations were characterized by on average seven times higher values of pairwise genetic differentiation within their range than the Southern population (Fig. 5), which was mainly driven by high values differentiating aquaculture from invasive populations. A similar trend could be observed in the Southern group although on a much smaller scale (Fig. 5). Large values of genetic differentiation observed between Northern Wadden Sea populations and their putative aquaculture source might indicate that genotypes used for regular, yearly stocking changed since the first successful spatfalls in the 1990s. For example, haplotype ht66 only occurred at intermediate

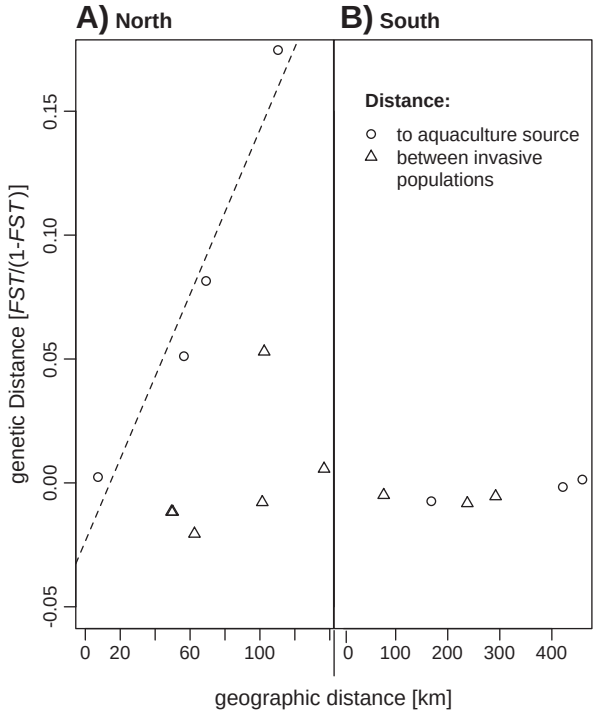


**Fig. 5.** Isolation by distance plots for the Northern (A) and Southern (B) groups of Pacific oysters in the Wadden Sea. Triangles show pairwise geographical and genetic distances between invasive and aquaculture sites (i.e. Sylt Aquaculture in the North and Oosterschelde in the South) and circles show distances between invasded sites. No significant differentiation could be observed in the Southern groups, whereas there was a tendency for isolation by distance in the Northern group, which was mainly driven by a significant relationship between aquaculture and invaded sites (regression line in A).

frequency of 15% in the northern aquaculture oysters and could not be observed in any wild population (Figs. 2, 4) suggesting that this haplotype might have been introduced by recent stocking events. Nevertheless, the differentiation along axis 2 of the haplotype ordination (Fig. 4) as well as the significant increase of genetic differentiation ($F_{ST}$) with distance between aquaculture and naturalized populations suggests that the current farm broodstock still has an impact on the surrounding wild populations in the Northern Wadden Sea. Similar phenomena are unlikely in the Southern Wadden Sea since no commercially produced spat was introduced since the 1970s. That genetic drift alone causes the observed patterns in the Northern group seems unlikely because of their magnitude in comparison to the Southern Wadden Sea. Furthermore, no isolation by distance pattern could be observed between naturalized populations indicating that gene flow is sufficient or genetic drift is too weak to cause genetic differentiation on the observed time scales. Such persisting input of new genetic material can also lead to different signatures of population expansion. While the southern populations show clear signs of expanding populations (i.e. star-shaped phylogeny, large negative values of Fu's F owing to input of new mutations), the northern populations lack such a signature. Also the observed multi-peaked mismatch distribution suggests that the northern oyster population has been stable for some time (Fig. 3). There is however no doubt that the northern Wadden sea is also characterized by similarly high population growth rates and range expansion as in the south (Diederich et al., 2005). However, multi-peaked mismatch distributions can also arise from admixture between divergent mitochondrial lineages (Simon-Bouhet et al., 2006) suggesting that input of divergent mitochondrial lineages from farmed oysters formed the initial starting material and also happened over time. This suggests that the population genetic demographic patterns of invasive populations are influenced to a large degree by the genetic starting material and the temporal sequence in which new genetic material is disseminated.

### 4.3. Implications and conclusions

Strong discrepancy between genetic differentiation measured with mitochondrial markers and genetic differentiation measured with nuclear markers has previously been observed for marine species (Larmuseau et al., 2010) demonstrating that processes like sex biased dispersal can influence differentiation estimates. Although mitochondrial discontinuities have been reported for American oysters *C. virginica* (Reeb and Avise, 1990) selectively neutral autosomal markers are unlikely to reveal different patterns of genetic differentiation, because oysters are hermaphrodites making sex-biased dispersal unlikely for Pacific oysters. Since the two invasions now potentially form a secondary contact zone, admixture of the gene pools and directional introgression could also influence the adaptive and invasive potential of Pacific oysters in the Wadden Sea. In this context it is highly interesting that Southern populations encountered strong selection by summer mortality with adult mortalities>60% (Watermann et al., 2008) from which Northern populations have been spared so far. Northern populations did however suffer from temperature dependent winter mortality during the last years (Büttger et al., in press). It will be a very interesting avenue of research to investigate how these previous divergent selection events as well as genetic admixture will impact patterns of directed introgression in the face of hard selection by biotic and abiotic conditions encountered in the face rapidly adapting competitors, predators and parasites as well as of advancing global warming. Therefore, it will be interesting to investigate oyster invasions with autosomal markers to identify genomic regions under selection during invasion.

In conclusion, our genetic data can reconstruct the demographic history of invasive populations of the Pacific oyster in the Wadden Sea and highlights the importance of invasion genetics to understand biological invasions in general (Geller et al., 2010; Lee, 2002). While both invasions happened during a similar time frame they nevertheless differ strongly from each other in terms of genetic diversity and differentiation patterns. These differences arise primarily from two decisive reasons. First of all, broodstock production for Northern aquaculture uses strains containing different and divergent mitochondrial lineages thereby altering the starting material for invasion. Secondly, and more importantly, we present three lines of evidence that aquaculture practice in the North has persisting implications on the genetic make-up of invasive populations. The choice of broodstock used in oyster hatcheries influenced the population genetic/demographic parameters (Table 2, Fig. 3) and haplotype frequencies as well as genetic differentiation between the farm and invasive populations suggesting a continuous influence of imported oyster seed on invasive populations. Therefore, the Northern populations close to the farm can be characterized as open populations with a changing input of new genetic material arising from changing broodstock strains, while the southern population seems to be closed in terms of input of new genetic material thereby solely relying on mutation as source for new genetic material. This demonstrates two aspects in which aquaculture practice can influence the characteristics of biological invasions by a) determining the starting material as well as b) providing continuous input into naturalized populations, thus resembling repeated invasions and admixture from genetically diverse sources (Kelly et al., 2006; Simon-Bouhet et al., 2006). Repeated genetic impact of aquaculture has been demonstrated for natural populations (McGinnity et al., 1997), but is actually scarce for invasive populations derived from aquaculture sources as demonstrated here for the case of Pacific oysters. Environmental and fisheries management will be confronted with the question whether the current practice of repeated, ongoing oyster introductions may carry an intolerable risk.

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