Diensten van de Eerste Minister
Federale diensten voor wetenschappelijke, technische en culturele aangelegenheden

Tweede plan voor wetenschappelijke ondersteuning van een beleid gericht op duurzame ontwikkeling

Deel II: "Global change, Ecosystemen en Biodiversiteit"

Netwerkovereenkomst EV/02/25A

**HOGERE TROFISCHE NIVEAUS IN DE ZUIDELIJKE NOORDZEE “TROPHOS”**

Universiteit Gent
Sektie Mariene Biologie
- Institut voor Natuurbhouh
- Katholieke Universiteit Leuven
Laboratorium voor Aquatische Ecologie

WETENSCHAPPELIJK VERSLAG voor de periode van 01/02/2002-31/01/2003
Food web interactions at the Belgian Continental Shelf

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Introduction

Based on results obtained in a previous OSTC-programme (Functional and structural biodiversity of North Sea ecosystems), the existence of two different food webs on the Belgian Continental Shelf (BCS) was hypothesized. A comparison of the response of nematode communities at an “open” sea station (Station 330) and a coastal ‘accumulation” station (Station 115 bis) (Fig. 1) to the deposition of phytodetritus during a spring phytoplankton bloom revealed completely different reactions in terms of nematode densities, vertical distribution and diversity (Steyaert et al. subm, Vanaverbeke et al., in prep).

Figure 1 Map of the Belgian continental shelf with indication of the sampling stations

Within the framework of TROPHOS, we aim to unravel the differences in the benthic food webs at these two target locations. In a first step, all biota and structuring variables
at both stations are examined in order to quantify and understand the differences in primary and secondary production at both locations at the BCS.

**General sampling scheme**

Both stations are visited monthly with *RV Belgica* or Zeeleeuw and sampled according to the scheme presented in Fig. 2. The concentration and organic matter content of suspended particulate matter (SPM) will provide necessary insight into trophic status of the stations, whilst their stable isotope ratios (carbon and nitrogen) together with phytopigment concentrations will help track changes and blooms of primary producers. Similarly, the isotope ratios and pigment concentrations of surface sediment is analysed to follow organic matter transfer to the sediment (benthic-pelagic coupling). The amount of incoming organic matter channelled into benthic fauna will be traced through quantification of the biomass, standing stock and nitrogen and carbon isotope signatures of major benthic components (micro-, meio- and macro-fauna). Bulk remineralization of organic matter is followed by monthly sediment oxygen consumption (SOC) measurements.

![General sampling scheme](image-link)
Preliminary results

The sampling campaigns, initiated in September 2002, have been very successful and analyses on the way. Here we present some preliminary data obtained in November 2002 during a cruise with the RV Zeeleeuw.

Sediment oxygen consumption (SOC) is a good indicator of total benthic activity and in accordance with the hypothesis, station 115 bis is characterized by higher metabolic activity. SOC at station 115 bis is more than twice that found at the “open” sea station (Fig. 3), reflecting a larger input of reactive organic matter to the former station.

![Figure 3: Sediment Oxygen Consumption at Station 115 bis and Station 330 (data from November 2002)](image)

Similarly, surficial sediment bacterial biomass was also significantly higher at station 115 bis compared to station 330 (Fig. 4). Trends in SOC and bacterial biomass highlight the large differences in trophic status of the target locations.

![Figure 4. Bacterial biomass at Station 115bis and Station 330 (data from November 2002)](image)
These measurements confirm the already observed differences between both locations during the spring phytoplankton bloom in 1999 (Steyaert et al. subm., Vanaverbeke et al. in prep). Using chl \( a \) concentrations in the sediment as a proxy for the labile organic matter pool in the sediment (Boon & Duineveld 1998), it became clear that the amount of mineralisable organic matter at Station 115bis was considerably higher. This results in a higher bacterial biomass as observed in the November measurements of 2002.
Evolution, foraging behaviour and reproductive output of coastal breeders at Zeebrugge

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Institute of Nature Conservation

The creation of new land in the outer harbour of Zeebrugge in 1985 attracted large number of coastal breeding birds. Each species shows its own specific evolution in breeding numbers since the creation of new suitable nesting habitat. As a pioneer species the population of Little Tern initially increased up to a maximum of 425 pairs in 1997. Because of habitat loss and succession of the vegetation numbers decreased afterwards. The Common Tern population shows a gradual increase up to 2446 pairs in 2002, while the number of Sandwich Terns strongly fluctuated. The latter species shows peaks in occurrence in 1993 and 2000. The reasons for these strong fluctuations are not clear. All three species of gulls (Black-headed Gull, Herring Gull and Lesser Black-backed Gull) show a strong increase up to 2001. The Lesser Black-backed Gull further increased in 2002, but the number of Black-headed and Herring Gull slightly decreased, probably because of competition for nesting habitat with Lesser Black-backed Gull. For all species the outer harbour of Zeebrugge is by far the most important site within Flanders. Also at the international level, the numbers of Little Tern, Common Tern, Sandwich Tern and Lesser Black-backed Gull are extremely important (1.9% - 3.9% of the total geographical population!). European law protects all the three tern species because their populations are vulnerable for extinction. So, the Zeebrugge harbour has a unique and internationally important ornithological value, but unfortunately this is not translated into any protection of the breeding sites at the national level.

Being highly specialised piscivorous birds, terns are often used as bio-indicators for example to predict the abundance of specific species of fish or the presence of toxicants in the coastal marine environment. To serve as bio-indicator one must, however, know precisely how fluctuations in the marine environment translate into changes in the
biology of the investigated species. This requires the monitoring of a range of parameters for several years. For this reason the reproductive output of Common Terns at Zeebrugge is monitored since 1997 by enclosing a part of the colony with chicken wire to prevent the chicks to walk away from the study site. Up to and including 2001 clutch size (i.e. the average number of eggs per nest), hatching success (i.e. the proportion of eggs that actually hatched) and fledging success (i.e. the proportion of hatched eggs that actually fledge) of the Zeebrugge Common Terns was high. This resulted in a high reproductive output averaging 1.2 fledglings/pair during the period 1997-2001. Such output is by far sufficient to maintain a stable population and is high when compared to foreign colonies. The high reproductive success suggests a high availability of food at Zeebrugge. Surprisingly in 2002 none of the parents were able to fledge a chick. Clutch size and hatching success in 2002 were comparable to those in earlier years, but chick mortality was exceptional high. Data on food composition, food intake rate and growth of the chicks were gathered, but were not yet analysed. However, we got the impression that a combination of food shortage during the chick rearing period and high predation rates by Herring and Lesser Black-backed Gull caused the failure of the 2002-breeding season. Poor breeding success was also recorded in nearby colonies in the Dutch Delta area, suggesting a lack of clupeids in the entire southern North Sea. After all, clupeids are the major food source for Common Tern chicks.

Earlier research on terns in The Netherlands suggests that foraging trip duration (i.e. the time it takes a parent from leaving the colony until it returns with a fish) is a good indicator for the amount food available to the terns. In 2001 and 2002 foraging trip duration was measured at the Zeebrugge Sandwich Tern colony. It appears that the time a parent is absent from the nest is positively related to the size of the prey it brings back to colony (Fig. 1). Compared to Europe’s largest colony, which is established at the isle of Griend in The Netherlands, it took parents at Zeebrugge less time to return with a fish. At Zeebrugge in particular small clupeids were brought to nest in a relatively short time. Even compared to a colony at Hirsholm (Denmark) where the availability of clupeids appeared to be very high, foraging trip duration of small clupeids was even shorter at Zeebrugge. In other words: at Zeebrugge there seems to be a superabundance of small
clupeids. There were no large differences in foraging trip duration between 2001 and 2002, suggesting that the availability of clupeids did not decline in 2002. This seems to be in contradiction with our above conclusions that for Common Terns the availability of clupeids has drastically decreased in 2002. There is, however, a major difference in the timing of breeding season between Common and Sandwich Terns, which can explain this seemingly contrast. The latter species hatches most chicks during the last week of May, while most Common Tern chicks hatch around the second half of June.

Although we have no evidence for this, it seems that in 2002 the population of small clupeids collapsed somewhere in June when Sandwich Tern chicks did no longer depend on these small prey. On the other hand, Common Tern chicks heavily depend on small clupeids from hatching until fledging. In the next period we will further analyse data gathered in 2002 on chick growth will be linked to the rate of food transport to the chicks. During the coming breeding season also new data will be collected. This will lead to a better understanding of the local food situation and fluctuations therein.
Peer reviewed publications:


Life History and dispersal of selected key species

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Introduction

Although the North Sea is a relatively small basin, its hydrodynamical range varies from fully mixed to stratified, with tidal regimes up to 6 m. Tidally induced currents and wind stress generate a complex pattern of local currents. Marine invertebrates and fish, inhabiting this environment, are characterized by a high potential for dispersal. However, the realized dispersal depends on how they behaviourally deal with the current dynamics they experience. Here we report on how dispersal patterns of a mysid and two fishes, all common inhabitants of the North Sea, are reflected in their life-history and in their genetic variation.

Material and methods

Mesopodopsis:

Specimens of Mesopodopsis slabberi were collected from 5 Northeastern Atlantic (Westerschelde, Belgian Continental Shelf, Seine, Mondego, Guadalquivir) and one Mediterranean population (Ebro). Samples from each location were collected with a hand net or a hyperbenthic sledge (mesh size 1 mm). After collection, the samples were stored in ethanol (70 – 95%) or aceton at 4°C. Ten specimens were analysed from each location. DNA was extracted using a modified CTAB protocol (Kocher et al. 1989). Small aliquots of extracted nucleic acids (1 µl) were used as template for polymerase chain reaction
amplification (PCR) of the mitochondrial cytochrome oxidase I gene (COI). COI amplifications used the universal primers CO1490F (5’-GGT CAA CAA ATC ATA AAG ATA TTG G -3’) and CO2498R (5’-TAA ACT TCA GGG TGA CCA AAA AAT CA -3’) (Folmer et al. 1994). Two species specific primers were designed to amplify the COI gene (520bp) of *Mesopodopsis slabberi*, COMSF (5’-GTA CTT TGC TTT TGG AGC CTG-3’) and COMSR (5’-AGG TGC TGG TAT AGA ATA GGG-3’). The following thermocycle profile was used: denaturation of template DNA at 95°C for 2 min, followed by a step-down PCR of 4 cycles (1 min at 95°C, annealing at 58°C for 80 s, extension at 72°C for 70 s), followed by 35 cycles of 1 min at 95°C, 80 s at 54°C and 70 s at 72°C, followed by a final extension of 5 min at 74°C. PCR products were purified with exonuclease I (10 U µl⁻¹; Amersham) and shrimp alkaline phosphatase (1 U µl⁻¹; Amersham). Purified products were cycle sequenced using BigDye Terminator Mix (PE Applied Biosystems) and electrophoresed on a Perkin-Almer ABI Prism 377 DNA sequencer. An additional five specimens of *M. wooldridgei* were sequenced for use as a close outgroup in the phylogenetic analyses. Alignment of the data was produced with the Clustal X program (Version 1.74, Thompson et al. 1997). Phylogenetic relationships between the haplotype sequences were investigated by building a tree with the Neighbour Joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) algorithm in PAUP 4.0b8 (Swofford 1998). Molecular diversity values at the inter and intrapopulation level were calculated using ARLEQUIN 2.0 (Schneider et al. 2000).

*Solea solea:*

Twenty samples of each about 30 adult fish were collected along the shores of the North-eastern Atlantic Ocean and the Mediterranean Sea in the period 1997-2000. They were genotyped with six microsatellite loci and Single Strand Conformational Polymorphism at the ND5/6 locus. Allelic variation was assayed at six microsatellite loci, *F8-I, F8-II, F8-III, F8-IV, F13 and F14* described by Iyengar *et al* (2002). A total of 19 populations were assayed with an average sampling size of 25 individuals per population. DNA samples were extracted from fin clips using a Proteinase K extraction protocol.
All PCR reactions were preceded by an initial denaturation step of 3 min at 95°C followed by 35 cycles extension steps: 30s at 95°C, 30s at the annealing temperature (61°C for F13; 56°C for F8-I and F8-II, 60°C for F8-III and F8-IV and 58°C for F14) and 30s at 72°C. A final elongation step of 7 min at 72°C was performed. PCR products were diluted with 5 µl (1:3) of stop-loading solution (Formamide 99% + Bromophenol blue) and were separated by electrophoresis on 25 cm, 6% polyacrylamide gels and detected on an automatic sequencer (LI-COR, model 4200) using the software E-seq ver. 2.00 (LI-COR Inc., 2001). Products were scored using the software Gene ImagIR ver. 4.03 (Scanalytics Inc., 2001).

Results

Mesopodopsis:

A total of 38 haplotypes were observed for the mitochondrial COI gene of *Mesopodopsis slabberi*. Haplotype diversity was in general very high, but didn’t significantly differ between the different locations. As shown in Fig. 1, a strong geographic structure could be observed in the distribution and genealogical relationships between the haplotypes. The centre of the minimum spanning network (Fig. 1) consists of haplotypes observed in the ‘northern’ Atlantic populations (Seine, Westerschelde and Belgian Continental Shelf). Haplotypes from the southern and northern Atlantic populations are largely divergent. A clear genetic break could be observed between the Atlantic and Mediterranean populations. Also the pairwise Fst values between populations (Table 1) reflect this strong phylogeographic structure of *M. slabberi*. 
Figure 1. Minimum spanning network among haplotypes of *Mesopodopsis slabberi*. Lines crossing branches represent minimum observed number of nucleotide differences between haplotypes. The area of each circle is representative of the frequency with which the haplotypes occurred in the total sample. Circles are shaded according to their geographic occurrence.

<table>
<thead>
<tr>
<th>Fst</th>
<th>Westerschelde</th>
<th>Seine</th>
<th>Mondego</th>
<th>Guadalquivir</th>
<th>Ebro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Westerschelde</td>
<td>-</td>
<td>-0.018</td>
<td>0</td>
<td>0</td>
<td>0.307</td>
</tr>
<tr>
<td>Seine</td>
<td>0.016</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0.313</td>
</tr>
<tr>
<td>Mondego</td>
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<td>0.691</td>
<td>-</td>
<td>0</td>
<td>0.428</td>
</tr>
<tr>
<td>Guadalquivir</td>
<td>0.161</td>
<td>0.195</td>
<td>0.449</td>
<td>-</td>
<td>0.313</td>
</tr>
<tr>
<td>Ebro</td>
<td>0.953</td>
<td>0.967</td>
<td>0.979</td>
<td>0.918</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1 Matrix of pairwise Fst values between populations of *Mesopodopsis slabberi*. Values are calculated from haplotypic frequencies (above the diagonal) and from genetic divergence data (Tajima & Nei, 1984) (below the diagonal). Italic values are not significant (P>0.05)
*Solea solea*:

Nineteen samples of about 25 Dover sole each were collected along the Eastern Atlantic Ocean and in the Mediterranean Sea. They were analysed with 6 microsatellite loci, whose number of loci varied from 9 to 26 (Table 2). Fst and Gst values varied from 0.039 to 0.113 and 0.070 to 0.140 respectively.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number of alleles</th>
<th>Fst</th>
<th>Gst</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8-I</td>
<td>9</td>
<td>0.11298</td>
<td>0.1398</td>
</tr>
<tr>
<td>F8-II</td>
<td>14</td>
<td>0.03869</td>
<td>0.0702</td>
</tr>
<tr>
<td>F8-III</td>
<td>11</td>
<td>0.09663</td>
<td>0.1127</td>
</tr>
<tr>
<td>F8-IV</td>
<td>17</td>
<td>0.06848</td>
<td>0.1006</td>
</tr>
<tr>
<td>F13</td>
<td>26</td>
<td>0.08466</td>
<td>0.0999</td>
</tr>
<tr>
<td>F14</td>
<td>19</td>
<td>0.07931</td>
<td>0.1013</td>
</tr>
<tr>
<td>Multi-locus</td>
<td>16</td>
<td>0.07695</td>
<td>0.1018</td>
</tr>
</tbody>
</table>

Table 2: Overview of the number of alleles, Fst and Gst values by locus of Dover sole.

Typically for many marine organisms, haplotype and microsatellite diversity were high. The highest haplotype diversity was observed in western Mediterranean Sea, where the most ancestral population seems to live.

Genetic differences between populations were limited (microsatellite multi-locus $G_{ST} = 0.10$), with the Venetian sample differing the most from the others. Nevertheless, structuring of the populations was clear; with an Atlantic and a Mediterranean group (Fig. 2).
Figure 2. Factor analysis of the microsatellite genotypes of 19 populations of *Solea solea*

Neighbouring populations differed slightly from each other (Fig. 3). A pattern of isolation-by-distance indeed characterises the Dover sole, which is sufficient to erase the genetic discreteness of populations and to maintain the differentiation of phenotypic stocks.

Figure 3. Plot of genetic distance versus geographical distance of Dover sole

*Pomatoschistus minutus*: cfr. submitted manuscript C. Pampoulie et al. (appendix)
Mesopodopsis:

Variation of the mtDNA cytochrome oxidase I gene revealed remarkable levels of genetic structuring in *Mesopodopsis slabberi* along the NE Atlantic and Mediterranean Sea. A considerable amount of variation was observed along the NE Atlantic, the northern Atlantic populations (Westerschelde, BCS and Seine) seemed to be more closely related then the southern Atlantic populations (Mondego and Guadalquivir). This pattern could be concordant with the relatively recent contractions and expansions of populations during the Pleistocene glaciations (Hewitt 1996, 2000). Changing climates and their associated glacial cycles over the past 2.4 My have periodically fragmented many species into widely separated refugia. Restriction of distributions to small refugia during glacial episodes and resulting constraints on population size might lead to loss of allelic variation. At the end of the last glaciation (18,000 BP) the warming climate and the retreat of the glaciers led to the rapid migration of species out of refugial areas as they spread into previously unavailable or unsuitable habitats. Hence populations could exhibit reduced genetic variability in areas glaciated during the Pleistocene (Avise 2000, Hewitt 1996, 2000, Wares 2002).

A long-term interruption of gene flow (Fst=0.918) was detected between the Atlantic and Mediterranean *M. slabberi* lineage, reflecting the strong genetic discontinuity found between the lineages and suggestion that current lineages undergo no genetic exchange. The level of divergence between both lineages suggests that the presence of historical geological barriers that prevented gene flow between the Mediterranean and Atlantic basin. Since the Pleistocene the history of the Mediterranean can be seen as a succession of glacial and interglacial periods with associated regressions and transgressions (Blanc, 1968). It is possible that during one of the regressions the Mediterranean and Atlantic populations of *M. slabberi* seperated. Beside the possible contribution of the history and hydrographic barriers, evolutionary processes such as genetic drift and founder effect, and/or selection, may have produced the observed genetic differentiation between the Atlantic and Mediterranean basins.
**Solea solea:**

Marine organisms are highly mobile, tend to have huge spawning populations, have a high fertility and are not so much restricted by physical obstructions. Dover sole is no exception to that typical description. However, despite their mobility mark/recapture data show that they are reasonably faithful to their spawning ground; only the occasional straggler visits a neighboring spawning ground. Also, spawns seem skewed by fertility-at-age and individual fertility rates. Finally, the shortage of suitable spawning habitat seriously restricts the distribution of sole. These limitations make that the potentially high dispersal rate is not necessarily realized.

There is a clear separation between the Mediterranean and Atlantic populations, which has been recorded for many other species, including sand goby, tuna, sea bass, mussel and *Mesopodopsis*. What differentiates the species is the timing when this happened. Some separated during the Mediterranean salinity crisis some 5.2 mA ago, others as recently as the Late Pleistocene. This will be clarified later on with the help of haplotypic variation at the ND5/6 locus.

In a previous genetic study Kotoulas et al. (1995) showed that isolation-by-distance characterized the Dover sole populations. Our study confirms these results and provides stronger results by the nature of the sampling coverage, the markers used and the quality of the analysis. Dover sole shows from the most eastern sample (Greece) via the Strait of Gibraltar up to the North Sea a gradual change in allele frequency. Populations in the English Channel and the North Sea seem to carry testimony to a late colonization of the area after reflooding in the Holocene.

Our findings have implications for fisheries in that stock structure is not so clear in the northern range and much more obvious in the central and southern range. It also shows that there is lots of exchange among stocks, which should act as a buffer against local overfishing. Chronic overfishing throughout the range (which is currently the case) may threaten long-term survival.

**Pomatoschistus minutus:** zie ingediend manuscript C. Pampoulie et al (appendix)
References

ANNEX

Subtle genetic differentiation in a high gene flow marine species

(Pomatoschistus minutus) living in a dynamic environment

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Keywords: Allozymes; microsatellites; allele shift; gene flow; sand goby; North Atlantic.

Running head-title: genetic structure in sand goby populations

Word count: 5,012
We assessed genetic differentiation and diversity in 15 populations of sand goby (*Pomatoschistus minutus*) within the Southern Bight of the North Sea, using seven microsatellite and thirteen allozyme loci. While no significant differentiation was observed with allozymes, microsatellite loci revealed a moderate level of differentiation (overall $F_{ST}=0.026$; overall $R_{ST}=0.058$). The hierarchical analysis of molecular variance revealed a significant differentiation ($p<0.01$) between Oosterschelde, Westerschelde, coastal and offshore samples with microsatellites but not with allozymes. This structure was confirmed by multidimensional scaling analysis based on Nei and $\bar{D}^2$ genetic distances. The comparison of the different estimators ($F_{ST}$ and $R_{ST}$) of differentiation lead us to conclude that this genetic structure was mainly due to an historical event, namely colonisation of the estuary during its establishment, and to restricted actual gene flow. Therefore, using all of these loci, we found that the 15 natural populations of the sand goby can be clustered into two different breeding units, namely the Oosterschelde and coastal populations. Despite this clear distinction, there were indications of an actual complex dynamic local structure that would bear further examination to understand the processes involved in the differentiation of these two breeding units.
INTRODUCTION

One of the most interesting challenges in evolutionary biology is to assess the processes responsible for genetic differentiation of distantly or closely related populations without any obvious barriers. In general, genetic drift and adaptation to local conditions are known to counteract gene flow, but recently other factors such as complex mating systems (Ross, 2001), sex-biased gene flow (Scribner et al., 2001) and chaotic processes (Huisman & Weissing, 2000) were shown to influence the genetic structure of populations. Given the potential association of high dispersal capabilities and barriers to gene flow of adults or larvae of marine species, it remains unclear to understand how divergence can arise and be maintained in the marine environment. Marine organisms with a pelagic larval stage have a high potential for gene flow resulting in a lower degree of genetic differentiation than freshwater or anadromous species (Ward et al., 1994; DeWoody and Avise, 2000). Indeed, many marine species show little or no intraspecific genetic differentiation even on a scale of thousands of kilometres (Suk et al., 1996; Lundy et al., 1999). However, the marine environment, although physically less structured than freshwater or terrestrial ecosystems, is not homogeneous. Fronts, local and global oceanic current patterns, bottom topography and climatic barriers restrict the dispersal of pelagic larvae and adults and promote genetic differentiation within populations (Sinclair, 1988; Bowen and Grant, 1997; Lessios et al., 1999). In recent years this has been confirmed by high resolution genetic markers such as microsatellites, which have yielded evidence for a subtle but significant genetic structure within marine species.
(Ruzzante et al, 1998; Lemaire et al, 2000; Wirth and Bernatchez, 2001). However, most studies have been carried out on a large geographic scale of hundreds or thousands of kilometres on species migrating over vast distances. Thus, little information is available as to whether small-scale systems of larval retention in the ocean exist and influence dispersal.

The Belgian Continental Shelf, situated in the Southern Bight of the North Sea, is characterised by a combination of sand banks and gullies which are continuously being swept by strong tidal currents (De Moor and Lanckneus, 1990). A long-shore and inshore/off-shore gradient under the influence of the Schelde estuary is reflected in a number of abiotic factors (Nihoul and Hecq, 1984). Consequently, biotic factors vary according to distance from the coast (Govaere et al, 1980; Offringa et al, 1996; Dewicke, 2001). These physical and biological differences might structure local fish populations (Stepien et al, 2001; Rocha et al, 2002).

This paper focuses on the small-scale genetic differentiation of a marine fish species, the sand goby Pomatoschistus minutus. It is one of the most common fishes along the area and it plays an important role in the food web as predator of benthos (Hamerlynck and Cattrysse, 1994) and prey for larger fish (Doornbos and Twisk, 1987). This annual species reproduces from May to July (Fonds, 1973) along the coasts of Northwestern Europe. Males build a nest and attract females to obtain eggs (Lindström, 1992) that they defend until the hatching of the larvae. The larvae are pelagic for 4 to 6 weeks and adopt a demersal life-style after metamorphosis. Adults are thought to have poor
swimming abilities, yet they carry out a spawning migration (Fonds, 1973; Pampoulie et al, 1999). Given the dispersal capabilities of this species, we might expect only slight genetic differentiation among populations on the scale of the Southern Bight of the North Sea. On the other hand, the geomorphology of the shelf and the presence of small-scale gyre systems might limit dispersal and promote small-scale inter-population differentiation.

Microsatellites are frequently used to detect subtle population structure on a small geographic scale as they evolve very quickly and are considered representative of the whole genome because of their independence and assumption of neutrality (Goldstein and Schlötterer, 1999). Allozymes on the other hand, have a lower resolution when discerning population differentiation but might be more subjected to historical events and to selective pressure as shown in numerous studies (Mitton, 1997). A combination of these two markers might enable us to gain more knowledge on genetic structure among sand gobies in the Southern Bight of the North Sea.

The scope of this study is to:

1) examine whether any evidence exists for genetic differentiation of a small marine fish species with an extended pelagic larval phase at a relatively small geographic scale.

2) assess the pattern of differentiation exhibited by two markers with a different rate of evolution to discriminate between long and short term evolution.

Materials and Methods
Sampling on the Belgian Continental Shelf was carried out with the oceanographic research vessels R/V ‘Belgica’ and R/V ‘Zeeleeuw’ along an inshore/offshore gradient on the Coastal Banks (Sb, Ht), the Flemish Banks (K), the Westerschelde, and the offshore banks (Of1, Of2) (Figure 1). In addition, four samples were taken in the Oosterschelde (The Netherlands) for microsatellite analysis only. The latter area consists of a unique marine environment and is used as a nursery area for fish from the adjacent North Sea. Although partly separated from the sea by a dike system, about 80% of the inflow passes through, thus conserving the exchanges between the ecosystems (Hamerlynck and Hostens, 1994). One distant population has been sampled in Texel (Tx; The Netherlands) as outgroup.

Fishes were either frozen in dry ice or liquid nitrogen immediately after capture and kept in a –80°C freezer until analysis. Gobies were identified morphologically on the basis of the dermal papillae of the head according to Miller (1986), and biochemically according to Wallis and Beardmore (1984a,b).

**Allozyme electrophoresis**

Allelic variation was assayed for 9 populations at 8 enzymes coding for 13 loci, namely Creatine kinase (CK-1*, EC 2.7.3.2), Lactate dehydrogenase (LDH-A*, EC 1.1.1.27; LDH-B*, EC 1.1.1.27; LDH-C*, EC 1.1.1.27), Malate dehydrogenase (MDH-1*, EC 1.1.1.37; MDH-2*, EC 1.1.1.37), Phosphoglucomutase (PGM-1*, EC 5.4.2.2; PGM-2*, EC 5.4.2.2), Glucose phosphate isomerase (GPI-1*, EC 5.3.1.9; GPI-2*, EC 5.3.1.9), Glutamate oxaloacetate transferase (GOT*, EC
2.6.1.1), Adenylate kinase \( \text{AK}^* \), EC 2.7.4.3) and Fumarate hydratase \( \text{FH}^* \), EC 4.2.1.2).

Liver, eye and muscle tissues were dissected and ground in distilled water. The samples were subjected to cellulose acetate allozyme electrophoresis (Richardson et al, 1986) using two continuous buffer systems: Tris-maleate (pH 7.8) and Tris-glycine (pH 8.8) as described by Hebert and Beaton (1989) with modification. Loci were stained according to recipes described by Hebert and Beaton (1989) and Richardson et al (1986). The fastest migrating locus was designated 1 or A according to the nomenclature of Shaklee et al (1990).

**Microsatellite amplification**

Allelic variation was assayed at seven microsatellite loci, \( P\text{min-01} \), \( P\text{min-05} \) and \( P\text{min-10} \) described by Jones et al (2001a, b), and \( P\text{min-06} \), \( P\text{min-07} \), \( P\text{min-08} \) and \( P\text{min-11} \) (see sequences in Table 2). A total of 15 populations were assayed with an average sampling size of 50 individuals per population (Table 1). DNA samples were extracted from fin clips using a Chelex (Biorad, 10%) extraction protocol (Walsh et al, 1991).

For all primers sets used, PCR was conducted in a 10 µl reaction volumes containing specific amounts of primers and MgCl\(_2\) ranging respectively from 10 to 20 mM and 0.6 to 2 mM. All PCR reactions were preceded by an initial denaturation step of 2 min at 95°C followed by 25 cycles extension steps: 1 min at 95°C, 1 min at the annealing temperature (60°C for \( P\text{min-01} \); 62°C for \( P\text{min-05} \) and \( P\text{min-10} \), 54°C for \( P\text{min-06} \), 57°C for \( P\text{min-07} \), 56°C for \( P\text{min-08} \) and 60°C
for *Pmin-11*) and 1 min at 72°C. A final elongation step of 3 min at 72°C was performed.

PCR products were diluted with 5 µl (1:3) of stop-loading solution (Formamide 99%+Bromophenol blue) and were electrophoresed on 25 cm, 6% polyacrylamide gels and detected on an automatic sequencer (LI-COR, model 4200) using the software E-seq ver. 2.00 (LI-COR Inc., 2001). Products were scored using the software Gene ImagIR ver. 4.03 (Scanalytics Inc., 2001) several times to avoid scoring errors. Suspect individuals were deleted from the analysis.

**Genetic data analysis**

Allele frequencies, observed (*H*_O) and unbiased expected heterozygosity (*H*_E) were calculated in GENETIX ver. 4.0 (Belkhir *et al.*, 1999). Hardy-Weinberg equilibrium (HWE) was calculated and tested for significance in GENEPOP version 3.1 (Raymond and Rousset, 1995). Significance levels were adjusted with a sequential Bonferroni test (Rice, 1989). Wright’s single-locus *F*-statistics (Wright, 1969) were calculated from allele frequencies of all loci examined for each population according to Weir and Cockerham (1984) in GENETIX (θ). For the microsatellite loci, differentiation between populations was also quantified using the analogue *rho* of the *R*<sub>ST</sub> of Slatkin (1995) following Goodman (1997) using the computer program RSTCALC (Goodman, 1997) and assuming the Stepwise Mutation Model (SMM; Kimura and Otha, 1978). Standard deviations of single-locus *F*<sub>ST</sub> values were obtained by jack-knifing over all populations according to Weir (1990). Significance of multilocus *F*<sub>ST</sub> and *R*<sub>ST</sub> was assessed with permutation tests (1000 replicates). Pairwise genetic distances corrected for bias in
sampling (Nei, 1978) was calculated in GENETIX assuming genetic drift-mutation equilibrium and a constant population size over time for both allozyme and microsatellite loci. For microsatellite loci, genetic distance between populations was also assessed by the specific distance $\hat{d}_m^2$ of Goldstein et al (1995) obtained with RSTCALC. A graphical representation of $R_{ST}$ values was computed using the neighbour joining algorithm (Saitou and Nei, 1987) in the NEIGHBOUR program inferred in the PHYLIP software package (Felsenstein, 1995) in conjunction with TREEVIEW (Page, 1996). Genetic linkage disequilibrium between locus pairs was estimated according to Weir and Cockerham (1979) and tested on contingency tables under the null hypothesis of independence. A microsatellite multilocus estimate of the effective number of migrants ($Nm$) according to Slatkin’s private allele model (1985) was assessed with GENEPOP. The effective number of migrants was also assessed according to the “Island model” (Wright, 1969). When significant genetic differentiation was observed between populations, a Mantel test (Mantel, 1967) was performed in GENETIX to test for isolation-by-distance. An Analysis of Molecular Variance (AMOVA) was carried out in ARLEQUIN version 2.0 (Schneider et al, 2000) to assess hierarchical partitioning among and within populations of genetic variability. We carried out a principal component analysis on the allele frequencies and a multidimensional scaling (MDS) approach on pairwise genetic distance (Nei, 1978; Goldstein et al, 1995) using respectively PCA-GEN version 1.2.1 (Goudet, 1999) and Statistica 5.1 (Statsoft Inc, 1997).

Results

Allozymes
Hardy-Weinberg Equilibrium

Nine out of 13 scored allozyme loci were polymorphic in the nine populations analysed (LDH-A*, LDH-B*, LDH-C*, MDH-1*, PGM-1*, PGM-2*, GPI-1*, GPI-2* and GOT*; Table 3). The observed heterozygosity averaged over all loci ranged from 0.09 to 0.11. No inter-population differences in mean heterozygosity, number of alleles per locus or levels of polymorphism were observed (Table 3).

All polymorphic loci were in HW equilibrium after Bonferroni correction with the exception of LDH-C*, where a strong heterozygote deficit across all populations examined was observed, independent of sample size (ranging from 30 to 200 individuals). Neither a trend nor gradient in allele frequencies across sampling sites was observed at this locus. A Mantel test failed to show any correlation between Nei’s (1978) genetic distances and geographic distances (p>0.05 under null hypothesis after 1000 permutations).

Population structure

As no clear differentiation was observed between all populations separately, samples were grouped according to the season (summer and winter) to assess temporal variation and variation of the F-estimates. The multilocus $F_{ST}$ value (0.01) for ‘summer-autumn’ populations (Of1, Os, K1, W1 and Ra1) was significant (p<0.05), which was entirely due to a differentiation at locus LDH-C* ($F_{ST}=0.028$, p<0.05). Excluding LDH-C*, the multilocus $F_{ST}$ was only 0.003 (not significant). Exact tests confirmed the differentiation at LDH-C* (p=0.0001). The ‘winter’ populations (Ra3, Of2, Sb and K2) were less differentiated ($F_{ST}=0.005$)
than the ‘summer’ populations ($F_{ST}$ not significant). No differentiation at locus $LDH-C^*$ was observed in this group.

Pair-wise genetic distances (Nei, 1978) calculated over all loci between the populations of sand goby were not significant. AMOVA revealed that within-population effects explained all the observed variation. Temporal variation in allele frequencies was assessed by comparing samples taken at approximately the same site in two different seasons. K2 was compared with K1, Sb with Ht and Oh with Of2 (Figure 1). Exact tests for allelic homogeneity (Raymond and Rousset, 1995a) showed no differences.

Neither the hierarchical analysis of molecular variance (Table 4) nor the PCA-GEN (data not shown) analysis showed consistent differentiation between the samples while the MDS revealed the presence of slight differentiation between offshore, Westerschelde and coastal populations (Figure 2a).

**Microsatellite loci**

Hardy-Weinberg Equilibrium

Although the seven microsatellite loci studied exhibited a high level of polymorphism (Table 2; $P_{(0.95)}=1$), two of them can be considered as highly polymorphic ($Pmin-01$ and $Pmin-05$) while three are moderately polymorphic ($Pmin-06$, $Pmin-08$ and $Pmin-10$) and two slightly polymorphic ($Pmin-07$ and $Pmin-11$) compared to values found in literature. The number of alleles per locus across all populations ranged from 8 ($Pmin-11$) to 88 ($Pmin-05$). Observed
heterozygosity averaged over all loci ranged from 0.62 to 0.76 in the 15 populations and tended to be lower than the expected heterozygosity. Genotypic proportions in 55 of 105 exact tests were out of HWE (Table 5). In particular, at Pmin-10 no HWE was observed in any population excepted for K1. The overall excess of homozygotes for all loci combined, as quantified by the correlation of alleles within individuals (FIS) was 0.163 (Table 6). Based on permutation tests (1000 replicates), the FIS values were significant for six out of seven loci (0.001<p<0.01).

Exact tests for linkage-disequilibrium yielded several significant values (0.01<p<0.05) involving several pairs of loci without any consistency, thus suggesting that the results were not due to physical linkage of the marker loci. No linkage disequilibrium was observed between allozyme and microsatellite loci in the three common sampling sites (Of1, K1 and W1).

Population structure

The partitioning of genetic variance among and within the 15 populations as estimated by F-statistics showed a mean FST value of 0.026 and a FIS of 0.163 while R-statistics showed a mean RST value of 0.058 and a RIS of 0.197 (Table 6). Pair-wise comparisons between populations exhibited significant FST values following sequential Bonferroni adjustment, while RST values were not all significant (Table 7). The highest FST and RST values were observed between the off-shore populations and the other populations. The total observed differentiation was essentially due to Pmin-06, Pmin-07 and Pmin-10 for FST and
to $P_{min-06}$ and $P_{min-07}$ for $R_{ST}$ (Table 7). Without these three loci the mean $F_{ST}$, $F_{IT}$ and $F_{IS}$ were respectively 0.011, 0.133 and 0.120 while $R_{ST}$ values were respectively 0.0271, 0.156 and 0.143. Nei’s (1978) and $\hat{\mu}^2$ (Goldstein et al, 1995) distances exhibited significant values between all pairs of populations (Table 8). All pair-wise comparison remained significant after Bonferroni correction. The highest values were observed between offshore populations and the others, and to a lesser extent between Oosterschelde and coastal populations.

Based on Slatkin’s private allele model (1985), the corrected number of migrants ($Nm$) was 7.07 while it varied between 2.7 and 24.6 based on Wright (1969).

A Mantel test failed to show any correlation between Nei’s (1978) and $\hat{\mu}^2$ genetic distances and geographic distances ($p>0.05$ under null hypothesis after 1000 permutations).

Based on Nei’s (1978) genetic distances, MDS clearly clustered the populations of the coastal area (K1, K2, Zs, Ra8, Ot1, Np) (except Ot2) and also the sample from Texel (Tx), the populations of the Oosterschelde (Oc7, Oc15, Oc37, Oc 21), the populations of the Westerschelde (W1, W2) and separated the offshore populations (Of1) from these three groups (Figure 2b; correlation values on axis 1 and 2: 0.311). Based on the $\hat{\mu}^2$ distance of Goldstein et al. (1995), MDS clearly clustered the Oosterschelde populations (except Oc21), the populations of the coastal area and separated the offshore population from these two groups (Figure 3; correlation values on axis 1 and 2: 0.1511). The principal
component analysis carried out on the allele frequencies using PCA-GEN confirmed the presence of three clusters (with the exception of Ot2 which did not cluster with the coastal populations) as Westerschelde populations clustered with the coastal populations. (total inertia: 0.21; percent inertia per axis: 1:21.48, 2:13.72; $F_{ST}$ per axis: 1:0.008, $p=0.045$, 2:0.005, $p=0.046$; global $F_{ST}=0.035$; data not shown). The obtained phenogram using the neighbour joining tree based on the $R_{ST}$ values was concordant and indicated a separation between estuarine and coastal populations (Figure 4). Inter-annual temporal variation within sites (K and Ot) showed to be non-significant (see Figure 4).

The AMOVA analysis revealed a weak but highly significant interregional pattern of genetic structure of the Belgian population of sand gobies which was composed of four different groups, namely the offshore, the Oosterschelde, the Westerschelde and the coastal populations (Table 4).

**DISCUSSION**

**Genetic diversity of sand goby populations**

The degree of heterozygosity as assayed with allozymes was comparable to values found by Ward *et al* (1994) for marine species and other gobioid fishes (Suk *et al*, 1996; Geertjes *et al*, 2001). Wallis and Beardmore (1984a) found slightly lower values in the sand goby but studied more loci of which a fairly large proportion was monomorphic, thus decreasing overall values of heterozygosity. Polymorphism at most allozyme loci was comparable to the results of Wallis and
Beardmore (1984a), with the exception of the highly polymorphic \( LDH-C^* \), which was completely monomorphic in the former study, probably due to the use of different electrophoresis techniques.

The genetic diversity assessed as the expected level of heterozygosity \( H_E \) exhibited a wide range for the microsatellite loci (0.157-0.976) and were generally comparable to other marine fish species (García de León et al., 1997; Ruzzante et al., 1998; De Innocentiis et al., 2001) except for loci \( Pmin-07 \) and \( Pmin-11 \). On the contrary, the polymorphism of the loci \( Pmin-01 \) (86 alleles), \( Pmin-05 \) (88 alleles) and \( Pmin-10 \) (45 alleles) is higher than in any other species studied (DeWoody and Avise, 2000). This high polymorphism could be explained by the complex mating system (Ross, 2001) of the studied species, in which males can breed with several females during a season and defend nest where 6 females have laid eggs (Jones et al., 2001a,b).

**Hardy-Weinberg equilibrium**

Most microsatellite loci and the allozymatic \( LDH-C^* \) locus clearly show a deficit in heterozygotes. This pattern is not unusual in populations of marine organisms as shown in numerous studies (Smith, 1987; García de León et al., 1997; De Innocentiis et al., 2001), and could find its origin in scoring errors, Wahlund effect (Wahlund, 1928), inbreeding, the occurrence of null alleles, assortative mating or selection against heterozygotes.

The Wahlund effect should result in significant \( F_{IS} \) (or \( R_{IS} \) for microsatellites) values at more than one allozyme locus, as drift causing population structuring
should affect all polymorphic loci equally (Pogson et al, 1995). For microsatellites this hypothesis can be rejected as well, because the level of differentiation observed between samples ($F_{ST}=0.026; R_{ST}=0.058$) was by far smaller than the mean $F_{IS}$ value ($F_{IS}=0.163; R_{IS}=0.197$) (García de León et al, 1997). Moreover, when populations were grouped together following the AMOVA defined structure, the overall $F_{IS}$ value did not decrease, confirming that the Wahlund effect can be rejected.

Inbreeding in gobies is highly improbable, mainly because this phenomenon is usually observed when populations are of limited size and isolated (Hartl, 1988).

For microsatellite loci, we do not favour the hypothesis of null alleles because all $F_{IS}$ estimates were positive, significant and relatively consistent across loci ($F_{IS}=0.165$ to $0.330; R_{IS}=0.072$ to $0.0451$). It seems highly improbable that all these loci exhibited null alleles with such a constant frequency. On the contrary, we do not reject this hypothesis for the $LDH-C^*$ locus even if selective pressure could also be a possible explanation for heterozygote deficit in allozyme loci (Jollivet et al, 1995; Allegrucci et al, 1997). On the contrary, selection against heterozygotes has never been proven using microsatellite loci which are considered representative of the whole genome.

Assortative mating has been suggested in Pomatoschistus species closely related to the sand goby but has never been empirically demonstrated (Lindström, pers.com.). Nevertheless, we believe that the complex mating system of this species involving sexual selection by both sexes is mainly
responsible for the high $F_{IS}$ values we observed (see Ross 2001).

**Spatial patterns of differentiation**

Allozyme electrophoresis yielded no significant population structure except at locus LDH-C*. However, this locus deviates strongly from Hardy-Weinberg proportions in all samples and thus caution is warranted when interpreting results. Indeed, AMOVA results showed that all of the observed variation in sand goby is due to within-population effects and no spatial structure is observed, suggesting balancing selection as a homogenizing force (Pogson et al, 1995).

On the other hand, our results at microsatellite loci lead us to conclude that the null hypothesis, namely that panmixia occurs among populations of sand goby, could be rejected. Estimates of fixation indices were mostly significant between pairs of populations leading to a highly significant overall $F_{ST}$ of 0.026 and $R_{ST}$ of 0.058. In marine fish, such as dusky grouper, the observed level of differentiation detected was 0.0179 (De Innocentiis et al, 2001), while it amounted to 0.007 in European sea bass (García de León et al, 1997), and 0.0084 in cod (Ruzzante et al, 1998). Higher pair-wise $F_{ST}$ (0.044-0.151) values were found for the silverside fish (Beheregaray and Sunnucks, 2001) suggesting the existence of a ‘divergence-with-gene-flow’ system.

In general our study suggests the existence of two spatially separated breeding units, namely the Oosterschelde and coastal area, while other populations are more related to one or the other unit. The comparison of our data
with the work of Beheregaray and Sunnucks (2001) leads us to conclude that the structure found in sand goby populations is more complex than the suggested four sub-units detected in the AMOVA analysis. The fixation indices, showing either high level of differentiation with $R$-statistics (6%, SE=0.002) or moderate level of differentiation with $F$-statistics (3%, SE=0.042), suggest that differences in allele sizes between populations were far more pronounced than differences in allele frequencies (see review of Balloux and Lugon-Moulin (2002) for the significance of both fixation indices), and especially between the Oosterschelde and coastal populations ($F_{ST}=0.008$ and $R_{ST}=0.022$). Nonetheless, gene flow ($7.07<Nm<24.6$) appears to be sufficiently large to swamp any potential for large genetic differences detectable with $F_{ST}$ estimates within the Southern Bight of the North Sea even if a small differentiation was put forward with our data. Moreover, estimates of gene flow and genetic distances based on $F_{ST}$ assume that population structure has been stable for sufficient time to allow an equilibrium between drift and migration. As a consequence, small $F_{ST}$ values can be observed due to high migration rates in the past years, despite little or no current gene flow. Under a strict SMM, $R_{ST}$ will be more sensitive to historical events and to restricted or pronounced gene flow (Balloux and Lugon-Moulin, 2002). The observed $R_{ST}$ value (6% of the differentiation) suggests that the allele shift between the Oosterschelde and coastal populations is large enough to be explained by isolation of these two populations during the formation of the Holocene coast, including the estuary some 700 years ago. Thus, we assume that several processes such as historical events (colonisation of the
Oosterschelde by coastal populations) and to a lesser extent restricted actual gene flow due to the geomorphology of the studied area, contributed to the observed differentiation.

**Congruence between allozyme and microsatellite loci**

The variation of fixation indices among types of markers is one of the most powerful methods for examining whether natural selection has played a role in the observed genetic divergence (see Allendorf and Seeb, 2000). Consequently, discrepancy between allozyme and microsatellite markers has been described in several marine species (Lemaire *et al*, 2000; De Innocentiis *et al*, 2001). Various results have been observed, showing either differentiation in one or both markers, attributed either to selection or stochastic events (drift-gene flow) (De Innocentiis *et al*, 2001; McLean and Taylor, 2001). In our data set, microsatellite exhibited higher $F_{ST}$ values than allozyme, suggesting that the highest mutation rate in microsatellite has increased allele frequency divergence among populations (Allendorf and Seeb, 2000). In addition, none of the alleles scored occur at high frequency in one population and are absent in the others, as would be observed if new mutations drift to high frequencies within their population of origin before being distributed to others by migration (Allendorf and Seeb, 2000). Thus, our results suggest that the observed differentiation was mainly due to drift and restricted gene flow between the distinct breeding populations (coastal and Oosterschelde groups) and the other samples, but not to any type of selection (see allozymes).
A model for the spatial pattern of sand goby in the Southern Bight of the North Sea

In the Southern Bight of the North Sea, sand gobies breed along the coast and disperse either during the larval or adult phase (Fonds, 1973). During summer time, breeding units were essentially observed on the east coast in June-July (Dewicke, 2001) while they were observed on the west coast in July-August (Pampoulie, pers. obs.; Ot1 and Ot2 sampling sites) suggesting a temporal variation in the location of the breeding sites. Moreover, a breeding population is known to occur in the Oosterschelde but not in the Westerschelde. Unfortunately, the ecological literature focused on the coastal populations (Fonds, 1973). So far, we assume that the high level of differentiation observed between the Oosterschelde and coastal populations may be the result of historical events and drift between two breeding sub-units (the mean pair-wise $F_{ST}$ value between these two groups is 0.008 while the $R_{ST}$ value is 0.022). Hence, the population structure is quite similar to the one observed by Beheregarey and Sunnucks (2001), namely a divergence-with-gene-flow-system between the Oosterschelde and coastal area (Figure 5). However, we do not have any precise ecological information about these two putative breeding units to assess whether ecological data would be consistent with genetic data. Marine juveniles of the sand goby are known to undertake migration from the coastal area and the Oosterschelde to the Westerschelde, supposedly to avoid the high level of predation and to find a high amount of food (Maes et al, 1998). This may result in the pooling of genotypes.
from both breeding units with subsequent low pair-wise differentiation. In addition, the offshore populations seemed to be more related to the coastal populations (see Figure 4) suggesting a coastal origin.

To conclude, based on microsatellite loci, our study clearly revealed the existence of two breeding units presumably connected with a low amount of migrants, consistent with a divergence-with-gene-flow system. This differentiation is partly attributable to a combination of older historical events, restricted actual gene flow and the complex mating system of sand gobies. Additional temporal (summer and winter) investigations should be done in order to increase the knowledge on the ecology and genetics of the two distinct breeding units (coastal area and Oosterschelde) and to assess the spatio-temporal stability and dynamics of both units.

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References


De Innocentiis S, Sola L, Cataudella S, Bentzen P (2001) Allozyme and
microsatellite loci provide discordant estimates of population differentiation in the endangered dusky grouper (*Epinephelus marginatus*) within the Mediterranean Sea. *Molecular Ecology*, **10**, 2163-2175.


Lessios HA, Kessing BD, Robertson DR, Paulay G (1999) Phylogeography of the


Rocha LA, Bass AL, Robertson R & Bowen BW (2002) Adult habitat preferences,
larval dispersal, and the comparative phylogeography of three Atlantic


Ruzzante DE, Taggart CT, Cook D (1998) A nuclear DNA basis for shelf and
bank scale population structure in northwest Atlantic cod (*Gadus morhua*):

reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406-
425.

Scanalytics (2001) Gene ImagIR version 4.03, Genotyping and DNA fragment
analysis software. LI-COR Incorporation, USA.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN ver 2.0: A software for
population genetic analysis. Genetics and Biometry Laboratory, University
of Geneva, Geneva

Scribner KT, Petersen MR, Fields RL et al. (2001) Sex-biased gene flow in
spectacular eiders (Anatidae): inferences from molecular markers with
contrasting modes of inheritance. Evolution, **55**, 2105-2115.

Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature
for protein-coding loci in fish. *Transactions of the American Fisheries Society*,
**119**, 2-15.

Sinclair M (1988) Marine populations: an essay on population regulation and
Wahlund S (1928) The combination of populations and the appearance of correlation examined from the standpoint of the study of heredity. Hereditas, 11, 65-106.
Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques, 10, 506-513.


