Panmixia in Zoarces viviparus: implications for environmental monitoring studies

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In this study, the genetic population structure of the eelpout Zoarces viviparus was investigated by using microsatellites. Samples were collected at 10 sites in the Baltic Sea, covering a distance of c. 90 km. Ten newly developed microsatellite loci were used to infer the population structure. No global spatial genetic differentiation was found (global $F_{ST} = 0.0001; D_{est} = -0.0003$), indicating strong gene flow at this scale, nor any clear pattern of isolation by distance. The results suggest that gene flow among the studied populations of Z. viviparus is stronger than usually thought, which might be caused by environmental homogeneity. This is important for planning and evaluating monitoring activities in this species and for the interpretation of ecotoxicological studies. Strong migration might lead to wrong conclusions concerning the pollution in a given area. Therefore, reference stations should be placed at a larger distance than presently practiced.

Key words: Baltic Sea; bioindicator; eelpout; gene flow; microsatellites; population genetics.

INTRODUCTION

The eelpout Zoarces viviparus (L. 1758) is a widespread species in the coastal waters of the North Sea and Baltic Sea and has been used extensively in environmental monitoring studies around the Baltic Sea. This is mainly caused by two important traits: (1) the species is viviparous and (2) it is supposed to be philopatric (Schmidt, 1917). Due to its viviparous behaviour, reproductive impairments can easily be linked to the mother fish and hence also to the environment. This has extensively been used in ecotoxicological and environmental studies, where characters such as reproductive success, endocrine disruption and biomarker have been used to monitor effects of pollution and to link individual effects to a pollution source (Vetemaa et al., 1997; Frenzilli et al., 2004; Gercken et al., 2006; Napierska & Podolska, 2006).

The first indications of site fidelity in the species originate from a morphological study by Schmidt (1917), who found two different races of Z. viviparus in a
Danish fjord at a distance of c. 35 km. Christiansen & Frydenberg (1974) were the first who presented a molecular study of the geographical pattern of the species using allozymes. Parallel allele frequency clines were found in two loci, starting in the Kattegat, decreasing in the belts and reaching a minimum in the Baltic Sea. Since then, several allozyme studies have been conducted, investigating the genetic structure in *Z. viviparus*, confirming these results (Hjorth & Simonsen, 1975; Christiansen et al., 1976, 1981, 1984, 1988; Simonsen & Strand, 2010). As phenotypic markers, such as allozymes, often are under selection, it still remains unknown at what geographical scale neutral gene flow occurs in *Z. viviparus*. Apart from the morphological and genetic studies some mark–recapture studies have also been performed in order to analyse the mobility of the species. Christiansen & Frydenberg (1974) reported a recapture rate of only 10% in Danish waters, suggesting a substantial migration of the fish. On the other hand, Jacobsson et al. (1992) had a higher recapture rate (c. 30%) in a shallow area on the Swedish west coast, indicating fairly limited migration. Hence, the field studies of the migration of *Z. viviparus* do not give a clear picture of the mobility of the species.

The assumption of philopatry has strong implications, and falsifying this may strongly influence the interpretation of results from monitoring studies, where uncontaminated spatial reference sites are needed to assess the effect of pollution. Reference stations have commonly been located c. 3 to 100 km away from the potential pollution source (Vetemaa et al., 1997; Gercken et al., 2006; Napierska & Podolska, 2006). If *Z. viviparus* is not as stationary as assumed, results from the ongoing monitoring activities are not as easy to interpret. Both a discrepancy between reproductive impairments and biomarker responses at contaminated and reference sites and a lack thereof could be explained by other causes than the potential pollution source investigated.

The aim of this study is to investigate gene flow and the hypothesis of philopatry of *Z. viviparus* by analysing the genetic population structure in a relatively small geographical area in the Baltic Sea proper (i.e. excluding the Gulf of Bothnia, Gulf of Finland, and Gulf of Riga), using selectively near-neutral microsatellite markers. This is the first microsatellite study in this species. Due to the high mutation rate of microsatellites and their non-coding nature, they usually provide a better resolution than allozymes when investigating spatial genetic patterns. Microsatellites have also proven to be the most statistically powerful marker under neutral expectations (André et al., 2011). The study was conducted in an archipelago, where the environment is assumed to be comparatively homogenous regarding salinity, temperature, pollution and exposure to water currents. The results presented will be of use for planning future monitoring studies and to interpret pollution measured in the field, as well as understanding genetic patterns and gene flow in a viviparous fish species.

**MATERIALS AND METHODS**

**COLLECTION OF FISH**

A total of 355 *Z. viviparus* individuals were collected at 10 different locations in an outer archipelago area in or near environmental protected areas in the Baltic Sea (Fig. 1 and Table I). Sampling was performed during 1 week of fishing in August 2010 using fyke nets. The distance between sampling sites ranged from 0-24 to 86-3 km. Total length (*L*<sub>T</sub>; mm)
and mass (g) were measured in all fish and a subsample were sex and age determined (Table SI, supporting information). Muscle tissue was taken for DNA extraction and subsequent microsatellite analysis.

DNA EXTRCTIONS, AMPLIFICATION AND SEQUENCING

The DNA was extracted from ethanol preserved muscular tissue using the Chelex protocol described by Walsh *et al.* (1991). Ten microsatellite markers, newly developed for this species, were scored (Molecular Ecology Resources Primer Development Consortium *et al.*, 2012).

The loci were co-amplified in two multiplex PCR reactions with c. 50 ng of template DNA. Primers were end-labelled with fluorescent dyes to enable co-migration in the same capillary during electrophoresis, *i.e.* loci labelled with the same dye had non-overlapping size ranges.
Table I. Summary statistics by sampling location for the 10 microsatellite loci studied from Zoarces viviparus from south-west Sweden

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>G</th>
<th>A</th>
<th>F_{IS}</th>
<th>H_{O}</th>
<th>H_{E}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>0.730</td>
<td>9.749</td>
<td>0.027</td>
<td>0.710</td>
<td>0.730</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>0.731</td>
<td>10.044</td>
<td>-0.008</td>
<td>0.729</td>
<td>0.723</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>0.742</td>
<td>10.034</td>
<td>-0.007</td>
<td>0.728</td>
<td>0.723</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.747</td>
<td>10.596</td>
<td>0.035</td>
<td>0.715</td>
<td>0.721</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>0.741</td>
<td>10.675</td>
<td>0.006</td>
<td>0.742</td>
<td>0.747</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>0.723</td>
<td>9.706</td>
<td>-0.02</td>
<td>0.756</td>
<td>0.742</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>0.723</td>
<td>10.552</td>
<td>0.016</td>
<td>0.720</td>
<td>0.731</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>0.733</td>
<td>10.172</td>
<td>0.046*</td>
<td>0.670</td>
<td>0.733</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>0.765</td>
<td>10.877</td>
<td>0.028</td>
<td>0.743</td>
<td>0.764</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>0.756</td>
<td>10.527</td>
<td>-0.011</td>
<td>0.765</td>
<td>0.756</td>
</tr>
</tbody>
</table>

*P < 0.05. n, number of fish; G, gene diversity; A, allelic richness; F_{IS}, deviation from Hardy–Weinberg proportions (Weir & Cockerham, 1984); H_{O}, observed level of heterozygosity; H_{E}, expected level of heterozygosity.

Uniform signal intensity among loci was achieved by adjusting primer concentrations. For multiplex one 10 μM primer solutions were used (forward and reverse) c. 15 μl C01 (NED), 2 μl A01 (VIC), 40 μl B08 (PET), 6 μl D10 (6FAM) and 30 μl B10 (6FAM). For the second multiplex 15 μl B12 (PET), 5 μl H03 (6FAM), 4 μl E10 (6FAM), 45 μl F03 (PET), 2.5 μl D01 (VIC) was used and added water up to 410 μl total volume for 100 reactions. For each reaction 4 μl of the primer mix, 4 μl Type-it Multiplex PCR Master Mix (Qiagen; www.qiagen.com) and 0.6 μl template DNA was used.

The PCR amplification was initiated with a denaturation step at 95°C for 5 min followed by 27 cycles of 30 s at 95°C, 90 s at 56°C and 30 s at 72°C. The process was terminated with a 20 min elongation step at 60°C. Electrophoresis and size determination of alleles was made with the Liz 500 sizer on an ABI Prism 310 Genetic Analyzer (Applied Biosystems; www.appliedbiosystems.com) according to the manufacturer’s recommendations. The genotyping was done with the programme Genotyper 3.7 (Applied Biosystems) and GeneScan 3.7 (Applied Biosystems) using the global southern method.

Statistical Treatments

Reliability of the genotypic data and potential errors, such as allelic dropouts, stuttering or null alleles, were analysed using Micro-Checker 2.2.1 (Van Oosterhout et al., 2004). Linkage disequilibrium (LD) between all pairs of loci was tested in Genepop 3.3 (Raymond & Rousset, 1995). For each locus, number of repeats, number of alleles and departure from Hardy–Weinberg equilibrium (HWE) estimated as heterozygote deficiency, F_{IS}, were calculated. For each location, gene diversity was determined and allelic richness, based on minimum sample size of 29 diploid individuals, was estimated using FSTAT 2.9.3 (Goudet, 2001). Expected and observed heterozygosities were estimated with Genepop 3.3 (Raymond & Rousset, 1995). Departure from HWE per populations was calculated as F_{IS} with FSTAT 2.9.3 (Goudet, 2001). 2000 randomization tests were performed to determine the proportion of randomizations that gave a larger or smaller F_{IS}-value than the observed.

Population Differentiation and Power

Genetic differentiation among all locations (global F_{ST}) and pairs of locations was estimated by F_{ST}-values, obtained by Genepop 3.3 (Raymond & Rousset, 1995), after the method of Weir & Cockerham (1984). To assess how the genetic variation was partitioned among loci, a locus-by-locus AMOVA was performed using the programme Arlequin 3.5.1.2 (Schneider © 2012 The Authors Journal of Fish Biology © 2012 The Fisheries Society of the British Isles, Journal of Fish Biology 2012, 80, 2302–2316
et al., 2000). As the traditional \( F_{ST} \) may have undesirable attributes in some situations when estimated from highly polymorphic markers such as microsatellites this might lead to that low values are misinterpreted as low genetic differentiation (Jost, 2008). Therefore, an alternative measure \( D_{est} \) was calculated, based on the effective number of alleles resulting in a more meaningful measure of differentiation (Heller & Siegismund, 2009; Jost, 2009; Ryman & Leimar, 2009), using the package DEMEtics in R 2.11 (Gerlach et al., 2010). The power for detecting population differentiation for the set of marker loci was evaluated using a simulation process incorporated in POWSIM (Ryman & Palm, 2006).

**Spatial genetic patterns**

The association between genetic similarity and geographical distance among locations as evaluated through the regression of \( F_{ST}(1 - F_{ST})^{-1} \) on the logarithm of geographical distance (Rousset, 1997). The geographical distance between any two sites was calculated as the Euclidian distance using ArcGIS 9.1 (ESRI; www.esri.com). Mantel’s test was used to test the correlation \( (r^2) \) between genetic and Euclidian distance using Genepop 3.3 (3000 permutations) (Raymond & Rousset, 1995). PCAgen (Goudet, 1999) was further used to visualise genetic patterns among locations.

To decide the genetic patch size, which would give further information regarding the spatial scale migration—gene flow takes place, spatial autocorrelation among pairs of individuals at different geographical distances (Sokal & Wartenberg, 1983; Epperson, 1995; Epperson & Li, 1996; Smouse & Peakall, 1999) was calculated as Moran’s \( I \) in the software SPAGeDi (Hardy & Vekemans, 2002). For the analysis a grouping of the material into 10 distance intervals was done, yielding on average 497 individual pairs per distance interval. This alternative gave a reasonable balance between an equal distribution of the number of individual pairs within an interval and a decreased random noise. Moran’s \( I \) takes values between \(-1\) and \(1\) where positive values indicate individuals being more genetically similar than the average and negative values that two individuals are less similar than two randomly chosen individuals in the data set. This approach is effective in situations where the individuals are more or less continuously distributed without obvious population boundaries (Exeler et al., 2008). This technique also provides an estimate of the distance below which the correlation between pairs of genotypes is positive (patch size, Smouse & Peakall, 1999).

**RESULTS**

**GENOTYPING ERRORS AND SUMMARY STATISTICS**

No scoring errors such as stuttering, allele dropout or presence of null alleles was identified in Micro-Checker. LD was detected in four out of 45 locus pairs across populations, which is a few more than expected by chance (8.9%). No LD was locus by population specific. All markers were polymorphic and allelic richness over loci ranged from 9.71 to 10.89 per location (Table I). Additionally, gene diversity, observed and expected heterozygosities were similar among locations (Table I). Out of 100 \( F_{IS} \)-values for separate locus-location combination, eight indicated significant deviation from HWE \( (P < 0.05) \), but none of these remained significant after applying Bonferroni correction. Combined \( F_{IS} \)-values from all loci revealed significant heterozygote deficiencies at location 8 \( (P < 0.05) \) (Table I), which is non-significant when applying Bonferroni correction. If still true, the heterozygote deficiency could be due to null alleles, population subdivision or inbreeding. Since no null alleles were suggested in the Micro-Checker analysis, this is ruled out from potential explanations. No departure from HWE was found to be explained by heterozygote excess \( (P > 0.05) \).
POPULATION DIFFERENTIATION

Consistently, very low values of genetic differentiation were found (global $F_{ST} = 0.0001$, $P > 0.05$; corresponding to $D_{est} = -0.0003$, $P > 0.05$). Pair-wise comparisons revealed no population differentiation, both for the $F_{ST}$ and $D_{est}$ estimates (Table II). The few significant cases of pair-wise $F_{ST}$ values were only supported by one of the 10 loci, as revealed in the locus-by-locus AMOVA (locus D01, $F_{ST} = 0.0211$, $P < 0.05$; Table III).

The markers were highly variable (Table IV) and the number of alleles, their frequency distribution and sample size of the data yielded sufficient statistical power for assessing genetic heterogeneity. According to the POWSIM simulation for these 10 loci and 353 individuals, power is high also at quite small levels of true divergence ($F_{ST}$). A true $F_{ST}$ of 0.002 would be detected with a probability of c. 95%. The chance of missing a pattern of significant global $F_{ST}$-value is thus low considering the loci and sample size.

SPATIAL GENETIC PATTERNS

No clear significant pattern of isolation by distance was found ($r^2 = 0.105$, Mantel $P > 0.05$; Fig. 2). Despite the low genetic differentiation, there seem to be some weak patterns within the sample as PCA1 and 2 in the PCAgen analyses explained nearly half of the total genetic variance (42.9%) among populations (Fig. 3). The PC2 appear to slightly reflect a north–south gradient and explained 18.1% of the total variation, while the explanation behind PC1 is more unclear.

The highest Moran’s $I$ values were found among sites that were situated geographically close to each other, but all values were very close to zero, and there was no significant decrease with increasing geographical distance ($r = 0.485$, $P > 0.05$;
Table II. Pair-wise $F_{ST}$ and $D_{est}$ over 10 microsatellite loci from Zoarces viviparus and 10 locations off south-west Sweden (see Fig. 1) with significance levels. $D_{est}$ above diagonal and $F_{ST}$ below diagonal.

<table>
<thead>
<tr>
<th>Location</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>-0.0048</td>
<td>-0.0208</td>
<td>-0.0260</td>
<td>-0.0131</td>
<td>-0.0071</td>
<td>-0.0188</td>
<td>-0.0046</td>
<td>-0.0115</td>
<td>-0.0187</td>
</tr>
<tr>
<td>2</td>
<td>-0.0021</td>
<td>—</td>
<td>-0.0005</td>
<td>-0.0002</td>
<td>0.0112</td>
<td>-0.0013</td>
<td>0.0065</td>
<td>0.0165</td>
<td>0.0245</td>
<td>-0.0118</td>
</tr>
<tr>
<td>3</td>
<td>-0.0027</td>
<td>-0.0005</td>
<td>—</td>
<td>-0.0309</td>
<td>-0.0003</td>
<td>-0.0066</td>
<td>-0.0312</td>
<td>0.0104</td>
<td>0.0086</td>
<td>0.0123</td>
</tr>
<tr>
<td>4</td>
<td>0.0039</td>
<td>-0.0023</td>
<td>0.0034</td>
<td>—</td>
<td>-0.0094</td>
<td>-0.0058</td>
<td>-0.0242</td>
<td>0.0171</td>
<td>0.0102</td>
<td>0.0141</td>
</tr>
<tr>
<td>5</td>
<td>0.0054</td>
<td>-0.0017</td>
<td>0.0020</td>
<td>0.0051</td>
<td>—</td>
<td>-0.0117</td>
<td>0.0002</td>
<td>-0.0150</td>
<td>-0.0068</td>
<td>-0.0071</td>
</tr>
<tr>
<td>6</td>
<td>-0.0015</td>
<td>-0.0006</td>
<td>0.0032*</td>
<td>0.0001</td>
<td>-0.0008</td>
<td>—</td>
<td>-0.0085</td>
<td>-0.0135</td>
<td>0.0048</td>
<td>-0.0054</td>
</tr>
<tr>
<td>7</td>
<td>0.0020</td>
<td>-0.0024</td>
<td>0.0017*</td>
<td>-0.0066</td>
<td>-0.0048</td>
<td>-0.0000</td>
<td>—</td>
<td>0.0031</td>
<td>0.0042</td>
<td>0.0036</td>
</tr>
<tr>
<td>8</td>
<td>-0.0044</td>
<td>-0.0025</td>
<td>0.0036*</td>
<td>0.0017</td>
<td>0.0034</td>
<td>-0.0014</td>
<td>-0.0010</td>
<td>—</td>
<td>-0.0077</td>
<td>-0.0017</td>
</tr>
<tr>
<td>9</td>
<td>0.0002</td>
<td>0.0020*</td>
<td>0.0018*</td>
<td>-0.0009</td>
<td>0.0007</td>
<td>-0.0031</td>
<td>-0.0012</td>
<td>0.0001</td>
<td>—</td>
<td>-0.0166</td>
</tr>
<tr>
<td>10</td>
<td>0.0025</td>
<td>0.0041</td>
<td>0.0052*</td>
<td>0.0056</td>
<td>0.0059</td>
<td>-0.0010</td>
<td>0.0021</td>
<td>-0.0013</td>
<td>-0.0019</td>
<td>—</td>
</tr>
</tbody>
</table>

* $P < 0.05$. 
Table III. Locus by locus AMOVA (Arlequin, Schneider et al., 2000) among locations $F_{ST}$ and $P$ values for samples of *Zoarces viviparus* from south-west Sweden

<table>
<thead>
<tr>
<th>Locus</th>
<th>$F_{ST}$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>0.0026</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>H03</td>
<td>−0.0002</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>C01</td>
<td>−0.0000</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>A01</td>
<td>$&lt;$0.0000</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>B08</td>
<td>−0.0026</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>E10</td>
<td>−0.0021</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>D10</td>
<td>0.0022</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>F03</td>
<td>−0.0016</td>
<td>$&gt;$0.05</td>
</tr>
<tr>
<td>D01</td>
<td>0.0211</td>
<td>$&lt;$0.01</td>
</tr>
<tr>
<td>B10</td>
<td>−0.0034</td>
<td>$&gt;$0.05</td>
</tr>
</tbody>
</table>

Fig. 4). The first time the correlogram intercepted with the x-axis was at 4.6 km, but the s.d. was large. Hence, no clear value of spatial genetic unit can be concluded at this scale, in corroboration with the low population structuring that was found.

**DISCUSSION**

The data achieved revealed a clear absence of genetic differentiation among *Z. viviparus* populations at distances up to 90 km in the Baltic Sea. This suggests that gene flow is strong, questioning the philopatric behaviour at this geographical scale. Taken into account that *Z. viviparus* is viviparous and lacks a free living larvae phase, genetic differentiation even at these short distances should easily be obtained. In contrast, studies on marine fish species with free living larvae have revealed population structure also at local scales (Knutsen et al., 2003; Adams et al., 2006; Jorde et al., 2007; Bergek et al., 2010).

Table IV. Summary statistics for microsatellite loci for *Zoarces viviparus* from south-west Sweden

<table>
<thead>
<tr>
<th>Locus</th>
<th>$n_R$</th>
<th>$n_A$</th>
<th>$H$</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>2</td>
<td>26</td>
<td>0.788</td>
<td>0.017</td>
</tr>
<tr>
<td>H03</td>
<td>1</td>
<td>4</td>
<td>0.450</td>
<td>0.038</td>
</tr>
<tr>
<td>C01</td>
<td>2</td>
<td>29</td>
<td>0.811</td>
<td>−0.004</td>
</tr>
<tr>
<td>A01</td>
<td>3</td>
<td>28</td>
<td>0.885</td>
<td>0.016</td>
</tr>
<tr>
<td>B08</td>
<td>1</td>
<td>29</td>
<td>0.852</td>
<td>0.037*</td>
</tr>
<tr>
<td>E10</td>
<td>2</td>
<td>8</td>
<td>0.693</td>
<td>0.065*</td>
</tr>
<tr>
<td>D10</td>
<td>2</td>
<td>17</td>
<td>0.831</td>
<td>0.021</td>
</tr>
<tr>
<td>F03</td>
<td>2</td>
<td>14</td>
<td>0.857</td>
<td>−0.031</td>
</tr>
<tr>
<td>D01</td>
<td>2</td>
<td>11</td>
<td>0.248</td>
<td>−0.012</td>
</tr>
<tr>
<td>B10</td>
<td>2</td>
<td>30</td>
<td>0.895</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* $P < 0.05$. $n_R$, number of repeats; $n_A$, number of alleles; $H$, heterozygote deficiency; $F_{IS}$ values, with significance levels obtained from AMOVA (Arlequin, Schneider et al., 2000).
The salinity in the study area is fairly homogenous (<5; Fiskeriverket, 2010). In addition, the sampling sites were all situated in the outer part of the archipelago, so that temperature, exposure to water currents and pollution should be similar. Earlier genetic studies of *Z. viviparus* populations that supported philopatry have been conducted in fjords, where salinity, depth and temperature vary over greater distances. Indeed, based on the present study, conclusions of the degree of differentiation in more heterogeneous environments, such as the Danish fjords or the differentiation between North Sea and Baltic Sea cannot be drawn. Using allozymes, Simonsen & Strand (2010) found strong congruence in the genetic composition of populations sampled 36–38 years ago by Frydenberg *et al.* (1973) in Kattegatt and western Baltic Sea. This suggests either low migration rates and stable population structure over c. 40 years or an ongoing selective pressure. Variability in temperature, salinity and oxygen may cause gradients in fitness resulting in some allele frequency clines, as in the studies conducted in Kattegatt and in the Danish fjords. Different prerequisites in the environment might hence very well lead to spatial genetic structuring. To investigate this further, it would be of great interest to apply the near-neutral microsatellite markers to the populations in Kattegatt and Danish fjords. Olsen *et al.* (2002) found a change in population distribution of *Z. viviparus* in Mariager Fjord, where genetic data obtained in studies from the 1970s were re-examined 1998. The population differentiation found in the 1970s had disappeared in the 1990s, which was thought to be related to eutrophication and oxygen depletion happening during that time. This suggests a complex relationship between migration pattern of *Z. viviparus* and the environment. The environment and type of genetic marker might thus show different pictures regarding the level of gene flow and migration. Microsatellites are believed to be selectively nearly neutral and they are also more variable than allozymes. Hence, the 10 markers used in this study should reveal a genetic structure if present, especially since the statistical power for the set of markers and number of individuals was high. The absence of population structure at this geographical scale in the Baltic Sea is therefore unambiguous.

Fig. 3. Ordination of *Zoarces viviparus* samples (see Fig. 1) from the first two principal components in the PCAGen (Goudet, 1999) analysis. The principal components explain 24.8 and 18.1% of the total variation, respectively.
isolation-by-distance pattern, however, suggests that this might become significant when populations from a larger area are included. The spatial scale of panmixia should receive more attention in the future by sampling larger areas. The few significant pair-wise comparisons of population differentiation were only supported by one locus (D01). It cannot be ruled out that this locus is under indirect selection, due to genetic hitchhiking.

Jacobsson et al. (1992) studied the annual migration of *Z. viviparus* outside a power plant station on the Swedish west coast. At 2–4 m depths, highest catch of *Z. viviparus* were noted at temperatures of 4–12°C, while the catches > 14°C were low. This might suggest that *Z. viviparus* may move from the shallow waters towards deeper areas during the warmer summer months. As spawning is expected to take place in August and September (Rasmussen et al., 2006), fish possibly congregate in the deep water. This would enable mixing of individuals from different areas and explain the panmixia of *Z. viviparus* populations found in this study.

Genetically structured populations arise when gene flow between groups of individuals is reduced due to geographical or behavioural barriers, making it possible for random genetic drift to act separately. For example, in herring *Clupea harengus* L. 1758, environmental variables such as salinity and temperature create barriers for dispersal between populations (Bekkevold et al., 2005; Jørgensen et al., 2005). In perch *Perca fluviatilis* L. 1758, stretches of deep waters have been shown to hinder dispersal and gene flow among individuals, both at large (Olsson et al., 2011) and small spatial scales (Bergek et al., 2010). Also ocean currents have been shown to create genetic differences among closely located cod *Gadus morhua* L. 1758 populations (Knutsen et al., 2003). Different environmental conditions could hence give rise to population structure. The lack of structure in this study suggests the environment to be homogeneous in the area. The results should not be a consequence of...
cohort or sex since fish from a number of \( L_T \) classes and ages was used. Hence, both females and males from all ages seem to mix, giving rise to the extensive gene flow in the area.

Many monitoring studies using \( Z. \) viviparus as biomarker have used reference stations within a distance of 100 km (Sandström et al., 1996; Sandström, 1997; Svedäng & Grotell, 1997; Vetemaa et al., 1997; Fagerholm, 2006; Napierska & Podolska, 2006; L. Förlin, pers. comm.). This study implies that the distance needs to be reconsidered and further research concerning the philopatry of the species are needed. Uncontaminated reference sites need to be situated in a similar environment as the polluted sites, but as far away from the potential hazardous source as possible. A lack of effect on reproduction or biomarker response might indicate a strong exchange of individuals among the reference station and the area of pollution. This was considered by Napierska & Podolska (2006) when studying the effects of contaminants on physiological biochemical variables of female \( Z. \) viviparus in Poland. The highest number of malformed fry was found at a station that was supposed to be uncontaminated and far away from pollution sources (Napierska & Podolska, 2006). This reference location was situated \( c. \) 40 km away from coastal line and the polluted sites. The results might hence be a cause of individuals mixing in these areas. In another study, Larsson & Förlin (2002) showed male biased populations located 1-7 and 1-2 km from a pulp mill. This indicates that the endocrine disrupters believed to originate from this pulp mill alone will affect close coastal waters or that the fish is migrating between areas at these distances. The findings achieved in this study suggest alternative explanations to these kinds of results, and that fish sampled at a reference site could very well have been in contact with contaminated waters. This has large implications for how to interpret contaminant and biomarker measurements and that lack of pollution patterns does not imply that there is no negative effect of the pollution source. Instead, it might just be explained by the individuals sampled originated further away from the source which hence have not been negatively influenced. Rather than searching for ecologically similar environments for reference sites, it might be more relevant to situate reference sites as far away from potential pollution sources as possible. To be able to resolve and specify a minimum distance required in biomarker studies, the scale of migration needs further exploration.

It has to be mentioned that very few migrants per generations are needed to achieve genetic panmixia. If \( N \) is effective population size and \( m \) the migration rate per generation populations are expected to diverge due to random genetic drift if \( Nm < 1 \) and to remain alike if \( Nm > 1 \) (Wright, 1941; Kimura & Maruyama, 1971). This means that a fairly large number of individuals may stay within the same area even if no population structure is seen. Since estimates of effective population size remains unknown (lack of temporal data), how many individuals are actually moving within this area is a matter of speculation. The very low \( F_{ST} \) values and relatively high relatedness suggests a substantial proportion of individuals to be mixing each generation. To be able to evaluate the effect this might have on monitoring studies, i.e. how many individuals that are truly stationary, the sampling of temporal data and estimates of effective population sizes should receive more attention.

In conclusion, this study has revealed considerable gene flow and an absence of a genetic divergence on distances up to 90 km in the Baltic Sea. The absence of spatial
genetic structuring may explain why some monitoring studies have not been able to show reproductive impairment or biomarker differences near pollution sources. Both a pattern and a lack of pattern could reflect something else than investigated if the fish are migrating between the areas.

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SUPPORTING INFORMATION

Supporting Information may be found in the online version of this paper:
Table SI. Biometric data for the sampled fish used in this study. Total length ($L_T$), mass and a subsample were aged and sex determined.

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