Ecological Indicators 27 (2013) 116–122

Contents lists available at SciVerse ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Strong isolation-by-distance in the absence of genetic population structure in the eelpout (*Zoarces viviparus*, Linnaeus 1758)

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ARTICLE INFO

Article history:
Received 4 July 2012
Received in revised form
30 November 2012
Accepted 2 December 2012

Keywords:
Bioindicator
Gene flow
Microsatellite
Panmixia
Population genetics

ABSTRACT

The eelpout (*Zoarces viviparus*) is a benthic marine fish, which has long been assumed to be philopatric. This made it particularly suitable for environmental monitoring programs as it allows matching its content of pollutants to the area of catch. However, a recent small scale genetic study in the Baltic Proper suggested that gene-flow might be stronger than generally believed and may occur frequently up to a distance of at least 90 km. As challenging the assumption of philopatry might have strong implications for environmental monitoring studies, we tested the hypothesis of philopatry at a larger geographical scale using ten microsatellite loci. A total of 220 individuals sampled from eight locations covering almost the entire geographic range of the species was genotyped. Our results show that genetic diversity decreases from the North Sea to the northeastern Baltic Sea. No strong population structuring was found, but a highly significant isolation-by-distance pattern was detected, suggesting a stepwise migration pattern among populations. Thus, the hypothesis of long-distance migration can be falsified. It is more likely that only limited gene flow exists among adjacent populations without any barriers between them. Our results suggest that dispersal in the eelpout is weak enough to retain this species as an important bioindicator. However, we suggest that reference stations should be placed in an appropriate distance to avoid misleading results from migrating individuals. We conclude that a more precise knowledge on migration rates of the eelpout is required in order to get more reliable insights into the potential area over which the concentration of environmental pollutants is integrated.

1. Introduction

During recent decades, marine environmental monitoring has become an important tool for the evaluation of human impacts on marine ecosystems (cf. Rüdel et al., 2003, 2010; Hedman et al., 2011). Monitoring schemes and Environmental Specimen Banks are used to validate chemical restrictions, to investigate the spatial and temporal distribution of chemicals and to establish sample archives for future retrospective analyses of substances that are not yet identified or characterized as harmful for the environment. The requirements that a monitoring organism has to fulfill can vary depending on whether the accumulation of substances or their direct effects shall be analyzed. In the first case the organism needs to be tolerant to harmful substances, while in the second case it has to be sensitive. However, in both cases organisms should be relatively stationary to ensure a direct link between the geographical location of the sampling site and the measured compound concentrations (Butler et al., 1971; Haug et al., 1974; Phillips, 1977). On the other hand, the mobility of animals provides a spatial integrated information about chemical pollutions. Thus, the required strength of philopatry depends on the spatial scale of a given monitoring study. Therefore, the exact knowledge of the mobility of bioindicators is a prerequisite for the correct interpretation of monitoring data.

The eelpout, *Zoarces viviparus* Linneaeus, 1858, is a marine benthic teleost fish, which occurs from north-eastern France, the western and northern parts of the British Isles, throughout the coastal regions of the Baltic Sea and along the Norwegian coastal line up to the White Sea (Muus and Dahlstrøm, 1974). It is one of the few viviparous marine fish species, and therefore particularly suitable to analyze both the accumulation of chemical substances and their effects on the larvae. Hence, it is broadly used as a model organism in environmental monitoring programs

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http://dx.doi.org/10.1016/j.ecolind.2012.12.001

(Jacobsson et al., 1986; Schladot et al., 1997; Rüdel et al., 2010; Hedman et al., 2011). The biology of the eelpout is quite well investigated (e.g. Kristofferson and Oikari, 1975), particularly the maternal–fetal trophic relationship has been studied in detail by Korsgaard and Andersen (1985) and Korsgaard (1986, 1997). This knowledge and the assumption that the eelpout lives relatively stationary (Schmidt, 1917; Hedman et al., 2011) provided the basis for numerous field studies on eelpout as an environmental indicator of contaminant effects in coastal areas (e.g. Larsson and Förlin, 2002; Strand et al., 2004; Ronisz et al., 2005; Gercken et al., 2006; Sturve et al., 2005; Tairova et al., 2012).

The assumption that *Z. viviparus* is philopatric is based mainly on older morphological (Schmidt, 1917) and allozyme studies (e.g. Frydenberg et al., 1973; Christiansen and Frydenberg, 1974; Hjorth and Simonsen, 1975; Christiansen et al., 1976, 1981, 1984, 1988). Some of these results have been confirmed recently (Lajus et al., 2003; Simonsen and Strand, 2010). However, other recent studies have questioned these findings, e.g. the strong genetic and morphological differentiation found in a Danish fjord system has not been confirmed (Olsen et al., 2002). This was explained by a hypoxia/anoxia event leading to a major fish kill one year before sampling, or by major changes in population dynamics (e.g. Christiansen et al., 1976). However, it has been criticized that parts of the examined allozyme loci may be under natural selection (e.g. Bergek et al., 2012). In fact, early allozyme studies in the eelpout have shown that some loci are under strong selection (e.g. Christiansen and Frydenberg, 1974). Thus, a neutral molecular marker system might provide more reliable insights into the genetic population structure of the eelpout.

A first microsatellite analysis of populations in the Baltic Proper showed that no genetic population structure occurs at a geographical scale of about 90 km (Bergek et al., 2012). These findings challenge the validity of ecotoxicological studies, in many of which the distance between reference sites and study sites was less than 90 km (e.g. Napierska and Podolska, 2006). However, it has also been argued that the missing population structure along the Swedish coast might be caused by environmental homogeneity (Bergek et al., 2012), while stronger genetic population structure might be found at a larger geographical scale or in regions with stronger geographic structure (e.g. Danish Fjord systems). Furthermore, Bergek et al. (2012) found a weak indication of isolation-by-distance and argued that this pattern might become significant at a larger spatial scale.

The aim of the present study was to investigate population differentiation among eelpout populations at a large geographical scale covering nearly the entire distributional range of the species. We used selectively near-neutral microsatellite markers to test the assumption of philopatry of this species. The following hypotheses were tested: (1) *Long-distance dispersal* (LDD): if LDD occurs regularly, we expect to find no genetic structure, no isolation by distance (IBD) and no spatial changes in genetic diversity across the species' range (Bialozyt et al., 2006). (2) *Stepping stone model* (SSM): under a SSM we would expect no strong genetic structure, but IBD and a decrease in genetic diversity toward the leading edge of the distribution. (3) *Philopatry*: if the species is completely philopatric, we expect a strong genetic population structure and a decrease in genetic diversity toward the northern edge of the range.

2. Materials and methods

2.1. Sampling and study sites

Muscle tissue of 220 individuals was collected from eight sampling sites in the North Sea and the Baltic Sea (Fig. 1 and Table 1). Sampling was conducted in June 2009 (Treimani (TM)), in November 2009 (Frederiksværk (FV) and Agersø (AG)), during June and July 2010 (Jadebusen (JB), Meldorf Bay (MB) and Darßer Ort (DO)) and in August 2010 (Kvädöfjärden (KJ)). Samples from the Gulf of Finland (GF) were collected in June 2010. Eelpouts from JB and MB were caught using trawl nets; all others were caught using fyke nets. Tissue samples were stored separately at −20 °C or ethanol (KJ and TM) until extraction of deoxyribonucleic acid (DNA). Except for GF, all sampling sites are integrated in present or past environmental monitoring programs or ecotoxicological studies utilizing the eelpout as monitoring organism (see Hedman et al., 2011).

2.2. DNA extractions, amplification and genotyping

A small piece of muscle tissue was cut out of each sample with a sterile scalpel. DNA extraction was performed using QIAGEN DNeasy Blood & Tissue Kit according to the

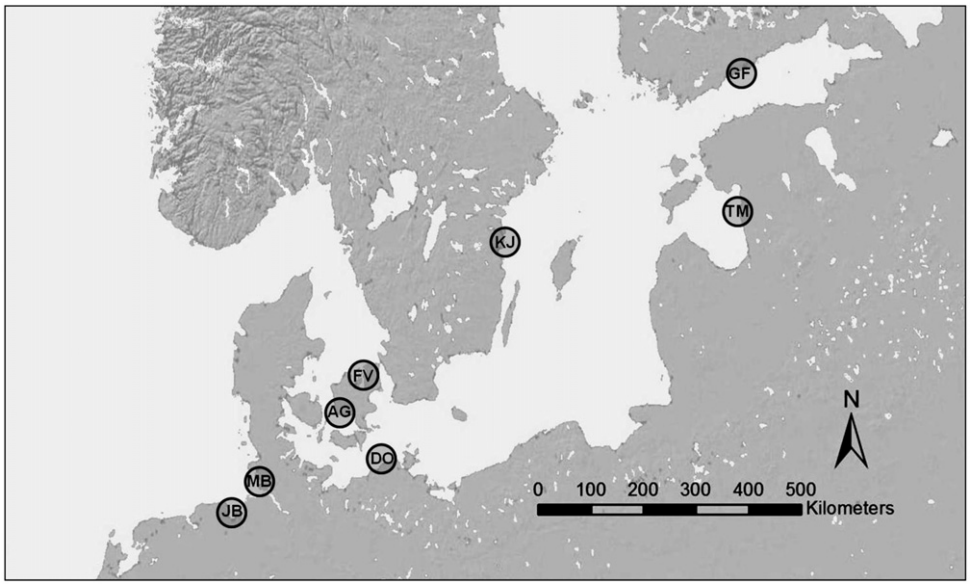

Fig. 1. Locations of the sampling sites: Jadebusen (JB), Meldorf Bay (MB), Frederiksværk (FV), Agersø (AG), Darßer Ort (DO), Kvädöfjärden (KJ), Treimani (TM), and Gulf of Finland (GF).

Table 1
Summarized statistics across all loci for samples from each location: n, number of individuals; N_A (obs.), mean number of observed alleles; N_A (eff.), mean number of effective alleles; AR, allelic richness (based on the min sample size of $n = 21$); H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient.

Region	Location	n	N_A (obs.)	N_A (eff.)	AR	H_O	H_E	F_{IS}
North Sea	JB	30	11.4	5.93	10.02	0.737	0.730	0.008
North Sea	MB	30	12.6	6.11	10.95	0.723	0.730	0.026
W Baltic Sea	FV	28	11.5	5.58	10.30	0.768	0.740	−0.019
W Baltic Sea	AG	29	11.7	6.46	10.42	0.800	0.749	−0.050
W Baltic Sea	DO	30	10.3	5.70	9.24	0.740	0.723	−0.006
C Baltic Sea	KJ	30	10.4	5.13	9.12	0.720	0.725	0.024
C Baltic Sea	TM	22	9.3	5.33	9.15	0.709	0.703	0.014
C Baltic Sea	GF	21	7.7	4.66	7.70	0.724	0.705	−0.003

manufacturer's protocol. Ten recently developed microsatellite loci for *Z. viviparus* (Molecular Ecology Resources Primer Development Consortium et al., 2012) were scored in all samples. All ten loci were co-amplified in 5 μl multiplex polymerase chain reactions (PCR) with an amount of approximately 20 ng template DNA per sample. Microsatellite primers were end-labeled with fluorescent dyes. Multiplex reactions were composed as follows: [1] *D10_Zvi1* (TAMRA), *F03_Zvi2* (FAM) and *A01_Zvi1* (HEX), [2] *B10_Zvi1* (FAM), *B08_Zvi2* (HEX) and *C01_Zvi1* (FAM) and [3] *B12_Zvi1* (HEX), *E10_Zvi1* (FAM), *H03_Zvi1* (TAMRA) and *D01_Zvi1* (HEX). Reactions were performed using the Type-it Microsatellite PCR Kit (QIAGEN) according to the manufacturer's protocol with an initial denaturation of 5min at 95 °C followed by 30 cycles of denaturation (30s at 95 °C), annealing (90s at 56 °C) and elongation (30s at 72 °C), with a final elongation step of 30min at 60 °C. All PCR's were run in a Biometra T Gradient thermocycler. A GE Healthcare Life Sciences MegaBACE 1000 sequencer was used for genotyping. Allele frequencies were scored using Fragment Profiler 1.2 (Amersham Biosciences).

2.3. Statistical analysis

Raw data were checked for potential scoring errors, such as stutter bands, null alleles and large allele dropout in Micro-Checker 2.2.3 (van Oosterhout et al., 2004). FSTAT 2.9.3.2 (updated from Goudet, 1995) was used to perform a test on linkage disequilibrium using a log-likelihood ratio G-statistic with Bonferroni corrections. FSTAT was also used to calculate inbreeding coefficients and allelic richness (El Mousadik and Petit, 1996), a measure that is independent of sample size, based on the minimum sample size ($n = 21$). The mean numbers of observed and effective alleles as well as the observed and expected heterozygosities were calculated in GenAlEx 6.41 (updated version from Peakall and Smouse, 2006). A Chi^2-test for the assessment of departures from Hardy–Weinberg-Equilibrium (HWE) was also performed in GenAlEx.

A nested analysis of molecular variance (AMOVA) with sampling sites as populations and three regions ('North Sea': JB, MB; 'Western Baltic Sea': FV, AG, DO, 'Central Baltic Sea': KJ, TM, GF) was calculated in GenAlEx with 9999 permutations, based upon F_{ST} and additionally on R_{ST} (Slatkin, 1995). R_{ST}-values are based on a stepwise mutation model (SMM) and are therefore thought to be more suited for microsatellite markers at evolutionary time scales (Balloux and Lugon-Moulin, 2002), while F_{ST}-values are based on an infinite allele model and more appropriate for studying recent patterns with a lower influence of mutations. We used both measures as the geographic scale of our study was rather high and the degree of differentiation unknown prior to this study. A third measure of genetic differentiation, D_{est} (Jost, 2008), based on the number of effective alleles, was calculated using the package DEMEtics in R 2.14 (Gerlach et al., 2010). This measure has been shown to be a more reliable measure of population differentiation than F_{ST}, particularly if genetic diversity is high (Ryman and Leimar, 2009; Heller and Siegismund, 2009; Jost, 2009; Whitlock, 2011). It has been recommended to be used in combination with F_{ST} (Meirmans and Hedrick, 2011). Since all three measures correlated significantly with each other ($F_{ST} - D_{est}$: $r^2 = 0.937$, $P < 0.001$; $R_{ST} - F_{ST}$: $r^2 = 0.386$, $P < 0.001$; $R_{ST} - D_{est}$: $r^2 = 0.314$, $P < 0.01$), F_{ST} was used for further analyses, since it is still the most commonly used measure.

To infer the optimal number of genetic clusters (K), the program STRUCTURE 2.3.3 (updated from Pritchard et al., 2000) was used. In this program, individuals are iteratively clustered based on a user-defined number of populations (K). The settings were as follows: correlated frequencies model, burn-in period of 100,000 simulations and 1,000,000 Markov Chain Monte Carlo simulations afterwards. The number of populations was tested from $K = 1$ to $K = 8$ with ten replications for each K. The admixture model was chosen as fully discrete populations could not be assumed based upon the results by Bergek et al. (2012). Additionally, the package Geneland 4.0 in R 2.11 was used to infer K (Guillot et al., 2005), which is based on a similar algorithm as STRUCTURE but handles geo-referenced individual multilocus genotypes.

IBD was tested with a Mantel test for matrix correlation between pairwise genetic and pairwise geographical distances with 10,000 randomizations and a reduced major axis regression (RMA) to calculate the slope in the program IBDWS (Jensen et al., 2005). This method is currently believed to be the most powerful statistical way to test for isolation by distance, as RMA is less sensitive to errors (Hellberg, 1994; Jensen et al., 2005). Genetic distances were calculated as described above and geographical distances between all sample locations were calculated as the shortest possible distances up to a water depth of 40 m, since this is the maximum depth reported for this species (Kristofferson and Oikari, 1975; Lajus et al., 2003). We calculated the distances with the Geographical Distance Matrix Generator 1.2.3 (Ersts, 2012), which uses spherical calculation.

3. Results

3.1. Genetic diversity

No scoring errors were detected and no linkage disequilibrium was suggested for any pair of loci. All measures of genetic diversity showed a decrease from the North Sea to north-eastern Baltic Sea (Table 1). Allelic richness decreased from 10.95 in the southern North Sea (MB) to 7.70 in the Gulf of Finland (GF). Observed and effective number of alleles dropped from 12.6 to 7.7 and 6.11 to 4.66, respectively. Observed and expected heterozygosities were rather evenly distributed across the geographic range, ranging between 0.71 and 0.80 with the highest values found in the Kattegat (FV, AG). The inbreeding coefficients (F_{IS}) were low with values ranging between −0.050 (TM) and 0.026 (MB) (Table 1). Significant departures from Hardy–Weinberg-Equilibrium were detected in six populations at one up to three loci. However, none of these departures was locus-specific.

Table 2
Pairwise F_{ST} values and pairwise geographical distances: pairwise F_{ST} values below the diagonal (*P < 0.05, **P < 0.01) and pairwise geographical distances (in km) above the diagonal (sea distances up to 40 m water depth).

Location	JB	MB	FV	AG	DO	KJ	TM	GF
JB	–	86	779	844	959	1354	1920	1863
MB	0.005	–	730	795	910	1305	1871	1814
FV	0.020**	0.018**	–	158	283	688	1255	1198
AG	0.012**	0.007*	0.005	–	116	621	1187	1130
DO	0.003	0.011**	0.016**	0.006*	–	586	1058	1095
KJ	0.021**	0.020**	0.012**	0.007*	0.011**	–	1115	509
TM	0.027**	0.031**	0.023**	0.017**	0.017**	0.007	–	605
GF	0.032**	0.036**	0.032**	0.023**	0.026**	0.015**	0.011*	–

Table 3
Pairwise R_{ST} and pairwise D_{est} values: Pairwise D_{est} values below the diagonal and pairwise R_{ST} values above the diagonal (*P < 0.05, **P < 0.01).

Population	JB	MB	FV	AG	DO	KJ	TM	GF
JB	–	0.005	0.008	0.014	0.066**	0.027*	0.173**	0.126**
MB	0.033**	–	−0.003	−0.009	0.074**	0.057**	0.216**	0.165**
FV	0.099**	0.089**	–	−0.005	0.061**	0.059**	0.208**	0.164**
AG	0.041**	0.043*	0.018	–	0.078**	0.065**	0.235**	0.181**
DO	0.024*	0.070**	0.082**	0.022**	–	0.012	0.081**	0.074**
KJ	0.078**	0.098**	0.062**	0.039**	0.053**	–	0.056*	0.033*
TM	0.115**	0.139**	0.114**	0.054**	0.081**	0.041*	–	0.006
GF	0.152**	0.175**	0.146**	0.107**	0.143**	0.072**	0.037	–

3.2. Genetic differentiation

Genetic differentiation was low, with only 0.97% of the genetic variability explained by regions and 0.87% explained by populations. F_{ST} values were low, ranging from 0.003 to 0.036. However, nearly all pairwise F_{ST} values were significant, except for the two Danish locations, the two North Sea locations, JB and DO and the two mid Baltic sea locations (Tables 2 and 3). Population assignment analyses revealed no genetic structure (i.e. $K = 1$) in both STRUCTURE and Geneland. However, the correlation of pairwise genetic and pairwise geographical distance showed a clear IBD pattern ($r^2 = 0.62$, $P < 0.001$; Fig. 2). This pattern also remained significant when D_{est} or R_{ST} were used instead of F_{ST} (D_{est}: $r^2 = 0.56$, $P < 0.001$; R_{ST}: $r^2 = 0.45$, $P < 0.01$; Fig. 3a and b).

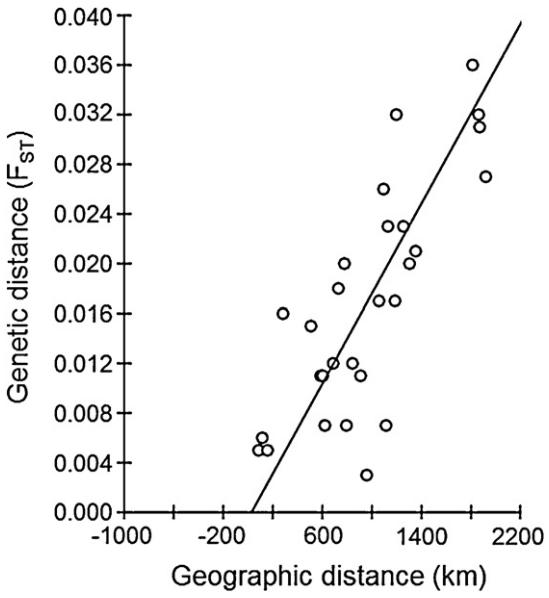

Fig. 2. Plot of isolation-by-distance, i.e. the correlation between pairwise genetic and pairwise geographical (sea) distances (up to 40 m depth) based upon a Mantel test for matrix correlations ($r^2 = 0.62$, $P < 0.001$, $Z = 554.0$, $r = 0.79$, intercept = −0.00052 ± 0.0023, slope = 1.81e−05 ± 2.19e−06).

4. Discussion

Our results falsify the hypothesis of LDD, as both IBD and a decrease in genetic diversity toward the northern range margin are unlikely to be found if long distance migration occurs regularly. Complete philopatry can also be falsified as no genetic structuring was found, supporting the idea that gene flow and migration of *Z. viviparus* have been underestimated in the past (Bergek et al., 2012). The unusually strong IBD supports a SSM. Hence, it has to be assumed that gene flow occurs regularly between adjacent populations of the eelpout, but not among distant ones (Kimura and Weiss, 1964). The lack of any population structure suggests that no environmental barriers among populations exist (Exeler et al., 2008, 2010). It is virtually impossible to assess the real number of migrants per generation between populations based on genetic data alone (Whitlock and McCauley, 1999). In contrast, it has recently been shown that it is possible to use effective population densities to infer migration rates (Pinsky et al., 2010), but these are difficult to obtain for a benthic fish species, such as the eelpout. Thus, the level of migration and the real migration distances remain unknown and should be the focus of future studies. However, it is also remarkable that both STRUCTURE and Geneland failed to detect clusters of Hardy–Weinberg populations even though both programs are known to detect genetic population structure even at low levels of genetic differentiation (Latch et al., 2006) and even tend to overestimate genetic structure under IBD (Frantz et al., 2009).

Due to their strong variability, microsatellite markers have been shown to be a powerful genetic marker system for detecting population structure in other highly mobile fish species, such as the Atlantic herring (*Clupea harengus*; André et al., 2011). It is thus unlikely that the low genetic population structure is a result of an insufficient resolution of the analyzed microsatellite loci. In contrast, it is more likely that the strong differentiation found in allozyme studies is a result of natural selection, which is supported by strong salinity gradients in allele frequencies (Christiansen and Frydenberg, 1974). Microsatellite studies in other mainly benthic fish species in the North Sea and the Baltic Sea revealed the existence of significant population structure. For example, Nielsen et al. (2004) found a sharp cline of genetic differentiation between Baltic Sea and North Sea populations in the turbot (*Scophthalmus maximus*), while no population structure (and even no IBD) was

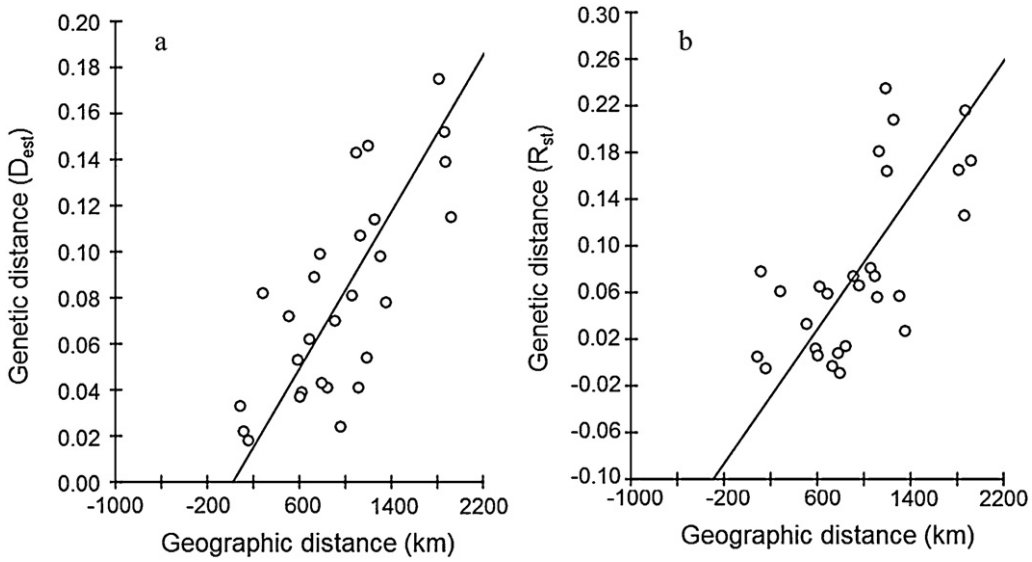

Fig. 3. Mantel test for matrix correlation between pairwise genetic and pairwise geographical (sea) distances (up to 40 m depth) based upon (a) D_{est} ($r^2 = 0.56$, $P < 0.001$, $Z = 2592.0$, $r = 0.75$, intercept = -0.002 ± 0.012, slope = $8.5e{-}05 \pm 1.1e{-}05$) and (b) R_{ST} ($r^2 = 0.45$, $P < 0.01$, $Z = 2825.1$, $r = 0.67$, intercept = -0.058 ± 0.02, slope = $0.00014 \pm 2.1e{-}05$).

found within the Baltic Sea (Florin and Höglund, 2007). In flounder (*Platichthys flesus*) another fish species with a similar geographic range, Hemmer-Hansen et al. (2007) and Florin and Höglund (2008) found significant population structures and detected differences among demersal and pelagic spawners. However, since the eelpout is viviparous and exclusively benthic, such a pattern is unlikely to occur.

Our results are in line with those of a previous analysis (Bergek et al., 2012), which showed that eelpout populations in the Baltic Proper show near-panmictic patterns. As the environment in the Baltic Proper is rather homogeneous, it has been suggested that the strong population differentiation found in allozyme studies in Danish fjords (e.g. Frydenberg et al., 1973; Christiansen et al., 1988; Simonsen and Strand, 2010) might be due to the stronger geographic structure (and existence of potential migration barriers) in these areas (Bergek et al., 2012). However, we found no steep genetic breaks across our sample locations, although these covered a broad range of environmental conditions, including salinity gradients of about 30–5 psu and temperature gradients ranging from 14 °C to 5 °C (bottom temperature in June) from JB to GF (Janssen et al., 1999, Feistel et al., 2010). Therefore, the genetic similarity of samples from the Baltic Proper are unlikely to be a result of environmental homogeneity, but rather a result of the small spatial scale of this study. This also supports the idea that the results of allozyme studies have been influenced by selection (see above). This is particularly underlined by the low pairwise F_{ST} between the Danish sample locations, which were also part of previous allozyme studies, where they showed significant differentiation (e.g. Simonsen and Strand, 2010).

Although Bergek et al. (2012) found only a weak trend for an IBD ($r^2 = 0.105$; $P = 0.056$), they suggested that IBD might become significant at a larger geographical scale, which is corroborated by our results. IBD in the eelpout is accompanied by a loss of genetic diversity from Southwest to Northeast, which is probably a consequence of range expansion during the Littorina stage of the Baltic Sea (<7500 y BP) and has been observed in several species in the Baltic Sea as well (Johannesson and André, 2006). If LDD would occur frequently, one would expect that genetic diversity remains more or less constant across the entire range (Bialozyt et al., 2006). Furthermore, thermal tolerance and morphometrics of *Z. viviparus* varies across its distributional range (Schladot et al., 1997; Zakhartsev et al., 2003), a pattern which is unlikely to evolve under complete admixture. Interestingly, F_{ST} was not significant between two non-adjacent locations (JB and DO). This might be explained by gene flow through the Kiel Canal, which connects the North Sea and Baltic Sea from the estuary of the Elbe to Kiel Bay, reducing the migration distance between these two locations by >600 km. Including the Kiel Canal as a potential migration corridor in the IBD analysis increased the effect size of the geographic distance, supporting this hypothesis. In fact, the Kiel Canal has been described as a dispersal route for several non-native species (e.g. Herborg et al., 2003; Gollasch and Nehring, 2006) and local anglers report that eelpout is regularly caught in the canal.

The results of this study have strong implications for marine environmental monitoring studies, in which the eelpout is used as bioindicator. Bergek et al. (2012) propose that the eelpout population is panmictic and questioned the reliability of studies, in which the distance of reference sites to the study sites was <90km (e.g. Napierska and Podolska, 2006). On the other hand, Bergek et al. (2012) correctly state that already low levels of migration can lead to near-panmictic genetic patterns, while they are less problematic for environmental monitoring. Our results indicate that gene flow is low enough to produce a clear IBD pattern, suggesting that LDD is very rare (or even absent) and the influence of adjacent sites depends on the geographic scale. From a demographic point of view a low level of gene flow may not severely compromise data on the accumulation of pollutants in eelpout populations. This is supported by analytic results of the German Environmental Specimen Bank, which showed strongly divergent concentrations of environmental pollutants (e.g. beta-hexachlorocyclohexane) over 18 years at JB and MB, which are 86 km apart (Umweltprobenbank, 2012). Only at very small distances (<10 km) immigration rates might be strong enough to challenge the use of reference stations. In fact, it should also be noted that immigration can even be an advantage for monitoring programs, as it allows integration of environmental pollutants over larger areas. Nevertheless, there is a need for clarification of the dispersal rates of *Z. viviparus* in order to be able to evaluate the potential influence of migrants. Monitoring programs may contribute to this, e.g. if it was possible to match chemical concentrations or stable isotopes reliably to a geographic area (Hobson, 1999). Future developments in technology might also improve the possibilities to use telemetry for studying dispersal behavior in the

eelpout. The Kiel Canal seems to be particularly suited for field studies on dispersal due to its linearity and limited spatial expansion, which increases recapture probabilities.

5. Conclusions

In conclusion, our study suggests that populations of *Z. viviparus* are interconnected by step-wise migration among adjacent populations resulting in a strong isolation-by-distance pattern. As long-distance migration can be ruled out, our analyses indicate that immigration does not substantially influence the usefulness of *Z. viviparus* as a bioindicator for chemical pollutants. Nevertheless, reference stations should be placed in an appropriate distance to avoid misleading results from migrating individuals to minimize effects of possible immigrants.

Acknowledgements

Parts of this study (samples from the German coast) were analyzed in the frame of the project "Klimabedingte Allelveränderungen in ausgewählten UPB-Probenarten" of the German Environmental Specimen Bank, financed by the German Federal Environment Agency (UBA). S. Bergek and J. Strand were kindly funded by the BONUS+ project BALCOFISH.

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