



Recolonization origin and reproductive locations, but not isolation from the sea, lead to genetic structure in migratory lagoonal fishes

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ABSTRACT

The assessment of connectivity in marine ecosystems is a requirement to adequate fisheries management. In this study we have selected two commercially exploited migratory species, European perch (*Perca fluviatilis*) and European smelt (*Osmerus eperlanus*), to evaluate the connectivity between the Curonian Lagoon and the coastal Baltic Sea. Our results indicate that isolation between the coastal lagoon and the adjacent sea area does not lead to the formation of genetic structure in migratory fish species. However, both species do register subpopulations coexisting in the area without interbreeding. This indicates that the fisheries management for migratory fishes in coastal lagoons affects a wider area than just the coastal lagoon. European perch, being a postglacial recolonizer from various refugees, has four different subpopulations, while the mechanism that maintains this division remains unexplored. The feeding migrations of European perch to the coastal zone suggest that the reproduction might occur elsewhere and that the factors for genetic structure suggested at the Baltic Sea scale might operate during these migrations. For European smelt, we discuss the existence of two different ecotypes, one lagoonal and one diadromous, and the different registered spawning locations as explicative causes for the maintenance of two genetically divergent clusters. The lagoonal ecotype reproduces and spawns inside the Curonian Lagoon while the diadromous one lives in the open Baltic Sea, performing spawning migrations to the lagoon and the mouth of Nemunas river, thus, maintaining the genetic divergence among them. However, our results indicate that there are no differences in size between both clusters, while the lagoonal population is expected to be smaller, forbidding the determination of two genetically different ecotypes. We conclude that there are no geographically and genetically separated populations of these two species in the lagoon-sea-terrestrial inlets continuum, and unified stock management for the coastal Baltic Sea and the Curonian lagoon is required.

1. Introduction

The maintenance of genetic flows in the marine and lagoon ecosystems depends on the connectivity between locations and the exchange of individuals between them, either as larvae, juveniles, or adults, by passive transport or trophic or reproductive active migrations. This connectivity may be disrupted or diminished when the distance increases (Johannesson and André, 2006), due to oceanographic dynamics (Cowen et al., 2006), by the presence of physical or chemical barriers such as those produced by salinity and temperature, the depth between islands (Johnson and Black, 2006; Spies, 2012) or the bathymetry in

general (Olsson et al., 2011), or the larval dispersal ability mainly in species with short larvae pelagic phase and little mobility (Cowen et al., 2006; Vergara-Chen et al., 2013).

Coastal lagoons are transitional ecosystems, between the sea and the mainland, isolated or semi-isolated by sand bars or spits sometimes with connections named channels or inlets (Pérez-Ruzafa et al., 2019a). The shape of the inlets and the tidal range determine the water exchange and, therefore, the main restrictions to the connectivity between the lagoon and the adjacent open sea area (Umgiesser et al., 2014). Depending on their restriction in water exchange, coastal lagoons can be classified as choked, restricted or leaky lagoons (Kjerfve, 1994). In

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addition, connectivity restrictions influence the colonization probabilities of a given species, introducing a random component that is reflected in the heterogeneity of these ecosystems (Pérez-Ruzafa et al., 2019a, b). This heterogeneity of the lagoons is particularly reflected in their fauna, where it has been observed that up to 40% of the species composition of ichthyoplankton (Quispe, 2014) and benthic macrofauna (Sigovini, 2011) change from one year to another. Thus, it is expected that these restricted connectivity and lagoon heterogeneity are also reflected in restrictions in gene flow and in structural complexity (Pérez-Ruzafa et al., 2019a).

Previous studies in genetic connectivity between coastal lagoons and the adjacent open sea areas reported an asymmetrical effect varying in accordance with the life cycle and strategies of the studied species (Pérez-Ruzafa et al., 2019b). In these environments, species with low larvae and adult dispersal capability show a high genetic structure increased by distance (Vergara-Chen et al., 2013) or between lagoons and the adjacent coastal areas (Liu and Hsu, 2021). The species with low adult dispersal but pelagic larvae can reach a sufficient exchange of individuals to show exclusive haplotypes in the sampling locations, but no genetic structure (Vergara-Chen et al., 2010a, 2010b), or even maintain a homogenous population between separated coastal lagoons (Peyran et al., 2021). Finally, native marine migrant species show no relationship between genetic distance and connectivity or geographical distance (Pérez-Ruzafa et al., 2019b). Despite the restrictions on gene flow, which can experience alternation between soft and strong restriction regimes in accordance with physiological and physical barriers (Seixas et al., 2018), there exists a higher probability of exporting particles out of the lagoon than that of receiving (Pérez-Ruzafa et al., 2019b). Thus, coastal lagoons could play a role in the genetic organization of the adjacent areas, amplifying alleles through bottleneck processes, and exporting those rare alleles and haplotypes (Pérez-Ruzafa et al., 2019b).

With this study, we analyze the population genetic structure of two marine migratory fish species, European perch *Perca fluviatilis* (Linnaeus, 1758), and European smelt *Osmerus eperlanus* (Linnaeus, 1758) in the Curonian lagoon and adjacent Baltic Sea, and relate it with the life cycle characteristics of both species and their migratory behavior. Both species spawn in the Curonian lagoon or adjacent rivers, while other parts of the life cycle are related to marine environment. The European perch is an species that lives in the edge of its salinity tolerance (Christensen et al., 2021), being a completely marine species in the north Baltic where the salinity is near 0. However, the salinity varies from 0 to 30 in the Baltic Sea. In the south-eastern Baltic, the European perch could be considered a freshwater straggler species (Razinkovas-Baziukas et al., 2017), according to the classification of Elliott et al. (2007), while the European smelt is a typical diadromous fish species. The brackish water environment in the Baltic Sea influences genetic lineages of migratory species, while knowledge of the genetic structure of fish populations is needed for their sustainable management and conservation, especially where anthropogenic activity is intense and lead to habitat modifications and stocks' overexploitation (Wennerström et al., 2017).

2. Material and methods

2.1. Study area, species, and sample collection

2.1.1. Study area

This study was carried out in the Lithuanian part of the Curonian lagoon and adjacent coastal areas in the south-eastern Baltic Sea. The Curonian is a coastal lagoon with an area of 1584 km², an average depth of 3.8 m approximately and a maximum of 5.8 m (Žaromskis, 1996; Gasiunaite et al., 2008), whose circulation is determined mainly by river discharge in the northern part and by the wind in the southern area (Umgiesser et al., 2014). In the lagoon, salinity ranges from 0 to 7 psu due to irregular marine water intrusions of ~7 psu (in the south-eastern Baltic

along the Lithuanian coast; Fig. 1) (Umgiesser et al., 2014). A narrow (0.4–1.1 km) strait (Klaipeda port area) in the northern part of the lagoon connects it to the southeastern Baltic Sea (Gasiunaite et al., 2008).

2.1.2. Fish species

Perca fluviatilis is a common freshwater species in coastal areas of the Baltic Sea. It is one of the few key indicator species used to assess the structure and development of the coastal fish community throughout the Baltic Sea (HELCOM, 2006). This includes the monitoring of the catch per unit of effort (CPUE) (HELCOM, 2006), or the study of its parasites and mercury content to assess pollution of the area (Valtonen et al., 2003; Luczyńska et al., 2020). The size that adult specimens could reach is up to 55 cm, but in general they are not expected to travel long distances (Kipling and Le Cren, 1984; Imbrock et al., 1996). It prefers warm-temperate waters environment and migrate seasonally between different locations. Its pelagic larval stage, when larvae can be passively transported, lasts a few weeks (Craig, 1987). The European perch is a recolonizing species from the different European areas in which it took refuge after the last glacial period (Nesbø et al., 1999; Toomey et al., 2020). It shows a detectable genetic structure within some lakes (Gerlach et al., 2001), between connected lakes (Ben Khader et al., 2019) and between a lake and a close not-connected coastal location (Pukk et al., 2016). In the Baltic Sea, it inhabits brackish waters (Karås and Hudd, 1993), and migratory (freshwater spawning) and resident forms are found in coastal waters (Berzins, 1949; Lutz, 1972; Karås, 1996; Overton et al., 2008). In Lithuanian coastal waters, the European perch spawns in the Curonian Lagoon and, after that, a part of its population makes feeding migrations to the Baltic coastal zone, where it spends the summer and early autumn before migrating back to the lagoon and the rivers or terrestrial inlets (Ložys, 2004; Razinkovas-Baziukas et al., 2017). As it is a stenohaline freshwater species that lacks the osmoregulatory capability that a saline environment would require (Collette et al., 1977; Brown et al., 2001), only the largest individuals can cope with slight changes in salinity and perform movements from freshwaters to the more saline waters of the coastal zone (Ložys, 2004; Tibblin et al., 2012).

Osmerus eperlanus, distributed from the White and Barents seas in Russia and Norway to the Garonne estuary in France, is a diadromous fish inhabiting brackish waters (Shpilev et al., 2005; Hagenlund et al., 2015; Sendek & Bogdanov, 2019). Its spawning areas include rivers, bights, and low salinity inlets (Shpilev et al., 2005). Its pelagic phase, including the egg and larvae stages, is estimated to last less than 30 days (Sepulveda, 1994). In the south-eastern Baltic Sea, adult European smelt inhabits marine waters, but during spawning migrations it is abundant in coastal waters and the Curonian Lagoon where the main spawning grounds occur. Within the Curonian Lagoon, the species is considered a lagoon inhabitant with two recognized ecotypes (Shpilev et al., 2005), although there are no previous genetic studies on them. One ecotype is a permanent resident of the lagoon, and the other, characterized by its anadromous behavior, inhabits the open Baltic Sea but enters the lagoon during spawning migrations to river mouths. The ecotypes are not differentiated by morphological characteristics and the variations in the size of the adults have been related to the feeding conditions in the marine or lagoon environments (Virbickas, 1986; Gaigalas, 2001).

Both species are commercial and economically important in the Curonian Lagoon and in the Lithuanian Baltic Sea coastal area, while overexploitation is the main reason for their decline within the Baltic Sea (Gasiunaite et al., 2008; HELCOM, 2006).

2.1.3. Sampling details

A total of 158 individuals of *P. fluviatilis* and 240 individuals of *O. eperlanus* were collected from fishermen's catches. Perch samples were collected during summer feeding time in July 2015 in four sites along the Lithuanian coast and in September 2015 when it migrates from the sea to the Curonian Lagoon. European smelt samples were collected from August 2015 to January 2016 in different locations along the Baltic Sea coast and, from January to March 2016, in the Nemunas river's delta

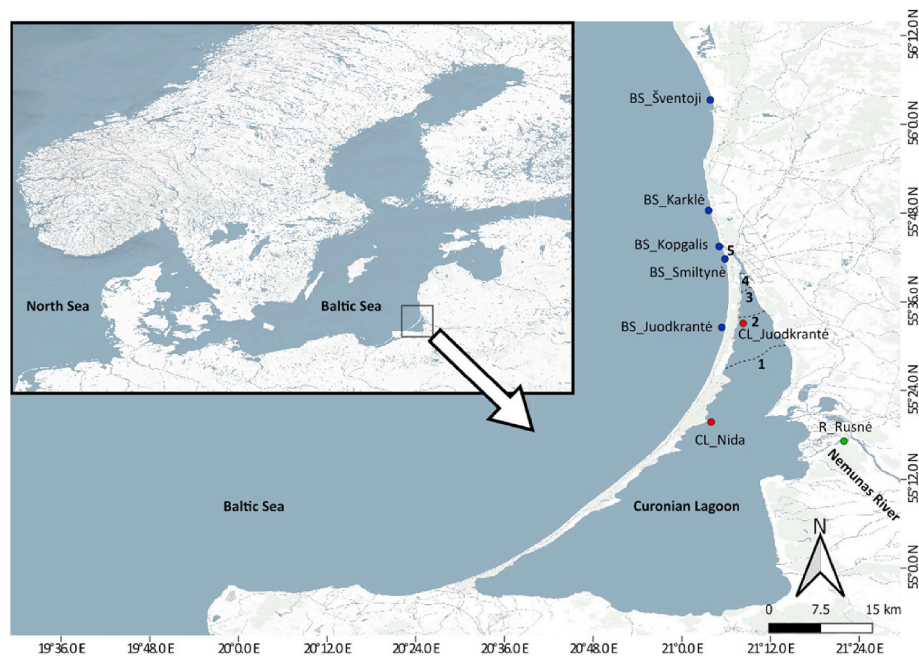


Fig. 1. Study area: Curonian Lagoon (CL), Baltic Sea (BS), and Nemunas River (R) sampling areas. Dot colors indicate sea (blue), lagoon (red), and river (green) locations. Dashed lines indicate the mean annual salinity isolines (1–5 psu) (Morkūnė et al., 2022).

and the lagoon (Fig. 1, Table 1). Fish lengths were adjusted to the nearest mm, and data on their sizes are provided as supplementary material (Table S1).

2.2. DNA extraction and microsatellite genotyping

DNA was extracted from 20 mg of muscle tissue (preserved in absolute ethanol) by ammonium acetate-isopropanol precipitation (Sambrook et al., 1989). DNA concentration was measured using a NanoDrop2000 Spectrophotometer (Thermo Scientific, Willmington, DE, USA) standardizing to 10 ng/μL with sterile distilled water (Gibco).

Two tetra-, three tri-, and six dinucleotides microsatellites loci for *P. fluviatilis* (Pukk et al., 2016), and four tetra- and eleven dinucleotides microsatellites loci for *O. eperlanus* (Hagenlund et al., 2015) were amplified through multiplex polymerase chain reactions (PCR) in a BioRad thermocycler (Table S2).

P. fluviatilis 10 μl PCR mixture consisted of 1μl template DNA, 1X reaction Buffer (Biotools®), 2 mM MgCl₂, 0.3 mM dNTP, 1 mg/ml Bovine Serum Albumin, forward and reserve primers (final concentrations shown in Table S2) and 0.75 U Taq polymerase (Biotools®). PCR profile was adapted from Pukk et al. (2016) with an initial heat activation at 95 °C for 5', followed by 28 cycles of denaturation at 95 °C for 30", annealing at 56 °C for 90" and extension at 72 °C for 30". The

reaction was terminated with a final extension at 60 °C for 30'.

O. eperlanus 10 μl PCR mixture consisted of 20 ng template DNA, 1X Multiplex PCR Master Mix (Qiagen), 1X Primers Mix (final concentrations of forward and reserve primers on PCR shown in Table S2) (Hagenlund et al., 2015). PCR profile was adapted from Hagenlund et al. (2015) with an initial heat activation at 95 °C for 15', followed by 25 cycles of denaturation at 94 °C for 30", annealing at 59 °C for 3' and extension at 72 °C for 1'. The reaction was terminated with a final extension at 60 °C for 30".

The PCR products were diluted in sterile distilled water (Gibco), according to the size and intensity of its band in the agarose gel and separated by capillary electrophoresis using an ABI Prism 310 automated genetic analyzer (Applied Biosystems) in the molecular biology section of the Research Support Service of the University of Murcia (Spain).

2.3. Data analysis

Allele sizes were edited by GENEMAPPER v3.7 software (Chatterji and Pachter, 2006) and manually inspected. In *P. fluviatilis*, two loci (Pflu10174 and Pflu7585) were excluded due to excess of missing data from doubtful amplification, while in *O. eperlanus* three loci (Oep6.10, Oep1.35, and Oep5.59) were excluded due to the excess of missing data

Table 1
Sampling specifications: number of individuals, dates and locations of sampling.

Species	Number of Individuals	Habitat	Locality	Date of Capture	Geographical Coordinates
<i>Perca fluviatilis</i> N = 158	29	Curonian Lagoon	Nida	08/09/2015	55° 19' 47.69", 21° 3' 58.51"
	30	Curonian Lagoon	Juodkrantė	10/09/2015	55° 33' 10.28", 21° 8' 21.19"
	31	Baltic Sea	Kopgalis	02/09/2015	55° 43' 30.13", 21° 5' 2.55"
	15	Baltic Sea	Juodkrantė	23/09/2015	55° 32' 37.0", 21° 5' 26.00"
	30	Baltic Sea	Karklė	03/07/2015	55° 48' 22.33", 21° 3' 36.65"
	23	Baltic Sea	Šventoji	15/09/2015	56° 3' 17.05", 21° 3' 51.15"
<i>Osmerus eperlanus</i> N = 240	60	River	Rusnė	25/03/2016	55° 17' 12", 21° 21' 58.28"
	30	Curonian Lagoon	Nida	15/01/2016	55° 17' 6.57", 21° 7' 37.89"
	30	Curonian Lagoon	Juodkrantė	28/01/2016	55° 31' 58.08", 21° 7' 23.73"
	30	Baltic Sea	Kopgalis	15/10/2015	55° 43' 30.13", 21° 5' 2.55"
	30	Baltic Sea	Smiltynė	04/09/2015	55° 41' 50.44", 21° 5' 50.59"
	30	Baltic Sea	Karklė	12/01/2016	55° 48' 9.47", 21° 3' 51.36"
	30	Baltic Sea	Šventoji	31/08/2015	55° 58' 44.57", 21° 4' 9.99"

derived from doubtful amplifications, and other three loci (Oep5.67, Oep7.11, and M-Omo4) were excluded because of their monomorphic allelic results. MICROCHECKER v2.2.3 (Van Oosterhout et al., 2004) was used to test the presence of null alleles in the data and they were detected in Pflu29376 across four out of six localities, so this locus was also excluded. Thereby, the final selection included 8 microsatellites for each species with 135 samples in *P. fluviatilis* and 222 in *O. eperlanus*.

Genotypes were analyzed by GENALEX v6.503 (Peakall and Smouse, 2012) to obtain different parameters of genetic diversity: sample size (N), the average number of alleles (A), average number of effective alleles (EA), number of private alleles (PA), observed (H_o) and expected (H_e) heterozygosity. Inbreeding index (F_{IS} , F_{IT} , F_{SC} , and F_{CT}) was tested in ARLEQUIN v3.5.2.2 (Excoffier and Lischer, 2010), with 10000 permutations, and deviations from Hardy-Weinberg equilibrium (H-W) were tested using GENEPOP v4.7.5 (Raymond and Rousset, 1995).

The genetic differences among sampling locations were estimated by analysis of molecular variance (AMOVA) in ARLEQUIN v3.5.2.2 (Excoffier and Lischer, 2010) with 10000 permutations. AMOVA showed no genetic structure between locations, and this was confirmed by the absence of structure in STRUCTURE v2.3.4 (Pritchard et al., 2000; Fig. S1). Thus, Discriminant Analysis of Principal Components (DAPC) without *a priori* knowledge of the sample origin was used to identify the population genetic clusters within populations using the 'Adegenet' package (Jombart, 2008) of the R v3.5.2 software (R Development Core Team, 2011). Unlike Principal Component Analysis (PCA) or Principal Coordinates Analysis (PCoA), DAPC seeks synthetic variables (discriminant functions) which show differences between groups as best as possible while minimizing variation within groups (Jombart et al., 2010). The number of clusters for each species was determined by applying the function find.cluster from the 'Adegenet' package with 'diffNgroup' as criterion and manual inspection of the BIC values. After the identification of clusters, individuals were assigned to their most probable group according to DAPC criteria, and genetic differences for the resulting matrix were estimated by AMOVA in ARLEQUIN (Excoffier and Lischer, 2010) and GENALEX (Peakall and Smouse, 2012). To know the genetic flows between clusters pairwise F_{ST} were estimated in ARLEQUIN v3.5.2.2 (Excoffier and Lischer, 2010) with 10000 permutations.

3. Results

3.1. Genetic diversity

For both species, almost all loci were polymorphic at all locations, with the exceptions of Pflu12189 that only presented one allele in BS_Juodkrantė and Oep3.80 that only presented one allele in Šventoji in smelt.

In the case of perch, the total number of alleles per locus ranged from 2 (Pflu5951) to 19 (Pflu29742). The average number of alleles (A) varied from 4.250 (BS_Juodkrantė) to 6.250 (BS_Karklė) and the average number of effective alleles per locus (EA) ranged from 1.962 (CL_Nida) to 2.462 (BS_Karklė). The number of private alleles (PA) varied from 0 (BS_Šventoji) to 5 (BS_Kopgalis). Observed heterozygosity (H_o) among localities varied between 0.380 (CL_Nida) and 0.474 (BS_Juodkrantė), and the average genetic diversity expressed as expected heterozygosity (H_e) varied between 0.414 (CL_Nida) and 0.487 (BS_Šventoji). Inbreeding index (F_{IS}) ranged from -0.0378 (CL_Juodkrantė) to 0.1191 (BS_Kopgalis). Hardy-Weinberg equilibrium test (H-W) indicated that no locality deviated significantly from H-W equilibrium proportions.

For smelt, the total number of alleles per locus ranged from 3 (M-Omo6) to 17 (Oep7.50). A ranged from 5.375 (CL_Juodkrantė) to 6.125 (R_Rusnė) and EA ranged from 2.738 (R_Rusnė) to 2.956 (CL_Juodkrantė). PA varied from 1 (BS_Kopgalis and BS_Smiltynė) to 4 (BS_Karklė and BS_Šventoji). H_o among localities varied between 0.448 (BS_Kopgalis) and 0.509 (BS_Smiltynė), and H_e varied between 0.495

(R_Rusnė) and 0.514 (BS_Smiltynė). Hardy-Weinberg equilibrium test indicated that CL_Nida deviated significantly from H-W equilibrium proportions (Table 2).

3.2. Genetic differentiation between lagoon and sea

In the case of perch, the analysis of molecular variance (AMOVA) showed no variation between the individuals from the Curonian Lagoon and Baltic Sea habitats or among localities. The 97.83% of genetic variation was within individuals although this was not statistically significant ($P > 0.05$) (Table 3). Analysis of smelt samples, on the contrary, showed small but significant ($F_{CT} = 0.00535$; $P = 0.03079$) differences between lagoon, seacoast, and river mouth habitats. Most of the genetic variability was registered among smelt individuals within habitats (5.23%) and within individuals (94.75%) showing, in both cases, small, but significant, genetic structure (Table 3).

Pairwise F_{ST} comparisons (Table 4) indicated a high genetic homogeneity among the six localities for both species since the genetic distances between them are null or very low without being statistically significant. Although the absence of significant differences among localities was to be expected in *P. fluviatilis*, it is surprising in the case of *O. eperlanus* after recording significant differences among habitats (Table 3). All the registered, not-significant, differences for *O. eperlanus* were detected between the lagoon populations and the ones situated in the Nemunas river or the Baltic Sea.

3.3. Population genetic structure

In accordance with the absence of significant differences in the pairwise F_{ST} , the STRUCTURE software did not show any structure between locations (Fig. S1). However, DAPC without *a priori* knowledge of the origin of the samples, both by manual inspection of the BIC values and by the diffNgroup criterion (Fig. S2), revealed four clusters in *P. fluviatilis* and two in *O. eperlanus* (Fig. 2). The probability of an individual belonging to the assigned cluster was approximately 100% for all the individuals.

Out of the 135 individuals of *P. fluviatilis*, 27 belong to cluster 1, 40 to cluster 2, 42 to cluster 3, and 26 to cluster 4. In the case of *O. eperlanus* 101 belong to cluster 1 and 121 to cluster 2. The individuals from all genetic clusters are present in all the sampling locations for both species in accordance with the absence of significant differences between locations (Fig. 3). The individuals of *P. fluviatilis* from the different clusters show, however, different proportions in the different locations. Clusters 1 and 2 show antagonistic proportions, being cluster 1 more abundant in the northern part and almost disappearing in the southernmost sampling stations, while cluster 2 proportion decreases from the most south location, northward. In the case of *O. eperlanus*, cluster 2 is more abundant in the lagoon and river mouth habitats.

3.4. Genetic diversity among clusters

In the case of perch, the mean number of alleles per locus varied from 5.125 (Cluster 4) to 6.625 (Cluster 2), and the total number of private alleles per locus ranged from 1 (Cluster 1) to 6 (Cluster 3). None of the subpopulations significantly differ from the H-W equilibrium, but all of them show an excess of heterozygotes. For smelt, the mean number of alleles and effective alleles per locus, and total private alleles are slightly superior in Cluster 2. Also, this cluster significantly differs from the H-W equilibrium, being the observed heterozygosity higher than expected (Table 5).

3.5. Genetic differentiation among clusters

Both species showed a significant genetic structure between the identified clusters. For *P. fluviatilis* the fixation index (F_{ST}) was 0.21341 ($p = 0$), explicative for 21.34% of the variation. In the case of

Table 2

Variability of the genetic pattern of *P. fluviatilis* and *O. eperlanus* across the different locations. CL - Curonian Lagoon; BS - Baltic Sea; R - river; N - sample size; A - mean number of alleles per locus; EA - mean number of effective alleles per locus; PA - total number of private alleles; H_o - observed heterozygosity; H_e - expected heterozygosity; F_{IS} - inbreeding index; P_{H-W} - P-value of Hardy-Weinberg probability test. Bolded numbers indicate significance ($p < 0.05$).

<i>Perca fluviatilis</i>								
Habitat_Locality	N	A	EA	PA	H_o	H_e	F_{IS}	P_{H-W}
CL_Nida	23	4.875	1.962	1	0.380	0.414	0.04379	0.5686
CL_Juodkrantė	27	6.000	2.178	4	0.474	0.457	-0.05208	0.9956
BS_Kopgalis	25	5.625	2.354	5	0.397	0.451	0.10000	0.3386
BS_Juodkrantė	11	4.250	2.148	1	0.422	0.415	-0.02921	0.3329
BS_Karklė	29	6.250	2.462	3	0.444	0.455	0.02535	0.3211
BS_Sventoji	20	4.625	2.262	0	0.469	0.487	0.03814	0.3122
<i>Osmerus eperlanus</i>								
Habitat_Locality	N	A	EA	PA	H_o	H_e	F_{IS}	P_{H-W}
R_Rusnė	56	6.125	2.738	3	0.480	0.495	0.0301	0.2571
CL_Nida	28	6.000	2.751	2	0.491	0.513	0.0439	0.0397
CL_Juodkrantė	27	5.375	2.956	2	0.449	0.507	0.1166	0.2141
BS_Kopgalis	27	5.625	2.820	1	0.448	0.499	0.0950	0.2975
BS_Smiltynė	28	5.750	2.803	1	0.509	0.514	0.0097	0.6255
BS_Karklė	28	5.875	2.797	4	0.473	0.507	0.0681	0.3703
BS_Sventoji	28	5.750	2.841	4	0.496	0.511	0.0309	0.3436

Table 3

Analysis of molecular variance test (AMOVA) of 8 microsatellites markers of *P. fluviatilis* and *O. eperlanus*. d.f - degrees of freedom; SS - sum of the squares.

<i>Perca fluviatilis</i>						
Source of variation	d.f.	SS	Variance components	% Variation	Fixation index	P-value
Among habitats	1	1.219	-0.00464 Va	-0.27	(F_{CT}) 0.00266	0.9397
Among localities within habitats	4	7.208	0.00023 Vb	0.01	(F_{SC}) 0.00013	0.4895
Among individuals within localities	129	231.177	0.04233 Vc	2.43	(F_{IS}) 0.02419	0.1401
Within individuals	135	230.500	1.70741 Vd	97.83	(F_{IT}) 0.02173	0.1501
Total	269	470.104	1.74533			
<i>Osmerus eperlanus</i>						
Source of variation	d.f.	SS	Variance components	% Variation	Fixation index	P-value
Among habitats	2	5.629	0.01079 Va	0.53	(F_{CT}) 0.00535	0.03079
Among localities within habitats	5	6.159	-0.01054 Vb	-0.52	(F_{SC}) -0.00525	0.97653
Among individuals within localities	215	456.534	0.10563 Vc	5.23	(F_{IS}) 0.05235	0.00089
Within individuals	222	424.500	1.91216 Vd	94.75	(F_{IT}) 0.05246	0.00109
Total	443	892.822	2.01774			

Table 4

Pairwise F_{ST} values (below the diagonal) and P-values (above the diagonal) of 8 microsatellites markers of *P. fluviatilis* and *O. eperlanus* among the 6 localities. The negative F_{ST} values were replaced by zero.

Perca fluviatilis							
Habitat_Locality	CL_Nida	CL_ Juodkrantė	BS_Kopgalis	BS_ Juodkrantė	BS_Karklė	BS_Šventoji	
CL_Nida	-	0.10484	0.58331	0.67884	0.30730	0.36650	
CL_ Juodkrantė	0.00919	-	0.59865	0.28651	0.45213	0.87288	
BS_Kopgalis	0.00000	0.00000	-	0.88783	0.53005	0.82170	
BS_ Juodkrantė	0.00000	0.00320	0.00000	-	0.48084	0.45758	
BS_Karklė	0.00335	0.00000	0.00000	0.00000	-	0.59964	
BS_Šventoji	0.00273	0.00000	0.00000	0.00048	0.00000	-	
Osmerus eperlanus							
	R_Rusnė	CL_Nida	CL_ Juodkrantė	BS_Kopgalis	BS_Smiltynė	BS_Karklė	BS_Šventoji
R_Rusnė	-	0.13761	0.45461	0.55559	0.91931	0.93179	0.77161
CL_Nida	0.00548	-	0.79289	0.19335	0.22958	0.15187	0.45669
CL_ Juodkrantė	0.00070	0.00000	-	0.25829	0.25225	0.25374	0.42521
BS_Kopgalis	0.00000	0.00639	0.00560	-	0.71547	0.88457	0.88862
BS_Smiltynė	0.00000	0.00432	0.00478	0.00000	-	0.62509	0.82249
BS_Karklė	0.00000	0.00686	0.00542	0.00000	-	-	0.93139
BS_Šventoji	0.00000	0.00067	0.00160	0.00000	0.00000	0.00000	-

O. eperlanus, F_{ST} was 0.08848 ($p = 0$) and explicative for 8.85% of the variation. As it occurred at the location level, most of the variation was recorded within individuals (89.91% in the perch and 90.65% in the smelt). Moreover, F_{IT} showed a significant genetic structure in the smelt ($F_{IT} = 0.09345$, $p = 0.0002$) (Table 6).

4. Discussion

4.1. Genetic structure between Curonian Lagoon and Baltic Sea

The relative isolation of the Curonian Lagoon, connected to the Baltic Sea by an 11 km long strait providing a mean residence time of 130 days

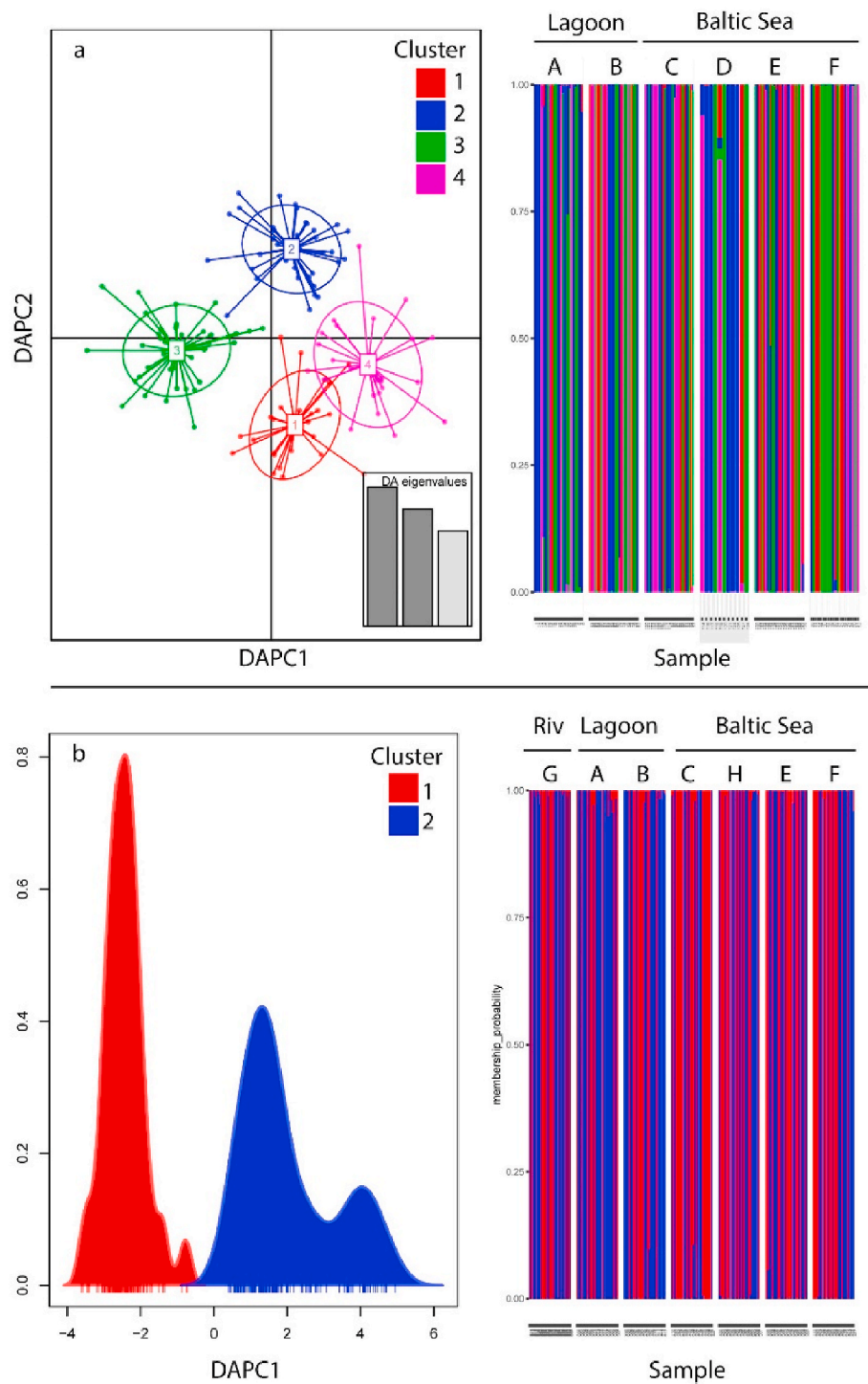


Fig. 2. Discriminant analysis of principal components (DAPC) and barplot of *P. fluviatilis* (a) and *O. eperlanus* (b) individuals without *a priori* information on the origin samples. Each color and number correspond with one cluster. A: CL_Nida; B: CL_Juodkrantė; C: BS_Kopgalis; D: BS_Juodkrantė; E: BS_Karklė; F: BS_Šventoji; G: R_Rusnė; H: BS_Smiltynė.

(Idzelytė et al., 2020), does not lead to the formation of any genetic structure at the level of localities or habitats (lagoon vs. Baltic and Nemunas River Delta) in *P. fluviatilis* (Tables 3 and 4). *O. eperlanus* showed a small but significant genetic structure between habitats (Table 3), not reflected at a smaller scale (Table 4). Those results support the idea that both species, despite some degree of isolation in the coastal lagoon, are capable of maintaining the genetic fluxes with the adjacent areas (Pérez-Ruzafa et al., 2019b).

However, our results also indicate that different populations of the

same species coexist in the same area performing non-random mating (Fig. 2; Table 6) as it has previously been observed for the European eel, *Anguilla anguilla* (Linnaeus, 1758) in the Sargasso Sea (Wirth and Bernatchez, 2001), the European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the Mediterranean Sea and the East Atlantic Ocean (Montes et al., 2016; Catanese et al., 2017, 2020), or for *Diplodus sargus* (Linnaeus, 1758) in other coastal lagoons (Hernández-García et al., 2015). The existence of four well-differentiated clusters of *P. fluviatilis* and two of *O. eperlanus* in every location (Fig. 3) with a strong and significant

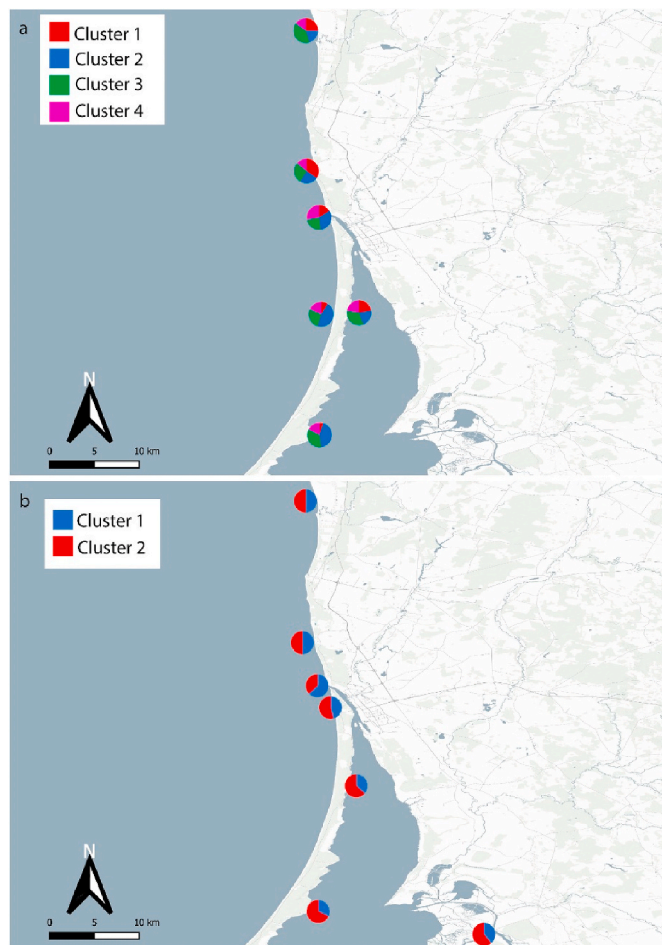


Fig. 3. Relative abundance of the different genetic clusters of *P. fluviatilis* (a) and *O. eperlanus* (b) in each sampling location.

divergence between them (Table 6) can only indicate that individuals belong to different reproductive populations. However, the formation of genetic structure at locality or ecosystem scale is prevented by a random distribution of migrants among localities. Here we discuss the different factors that might be operating in the maintenance of different clusters.

4.1.1. Genetic structure based on recolonization origin in perch

The genetic structure of *P. fluviatilis* has been observed to differ from one European ecosystem to another. Within individual lakes, perch genetic structure varies from an absence of the same (Ben Khadler et al., 2015) to the coexistence of 2–9 populations (Gerlach et al., 2001; Ben Khadler et al., 2019). In non-connected locations, a small but significant genetic structure has been found between lakes and coastal areas and

between non-connected lakes (Pukk et al., 2016; Ben Khadler et al., 2019). Toomey et al. (2020) suggested that the asymmetrical genetic structure found over Europe was due to postglacial colonization from different refugia after the last ice age instead of the physical separation of drainage basins. Following this suggestion, the Baltic Sea, being one of the last reservoirs covered by the ice sheet, would have been colonized by *P. fluviatilis* specimens from, at least, four different refugia (Nesbø et al., 1999; Toomey et al., 2020). Our results support the hypothesis of colonization from a variety of origins to explain the coexistence of different subpopulations with a reproductive separation (Fig. 2a; Table 5), but more research on the migratory patterns or lifecycle of the species is necessary to explain how these genetic differences are maintained at present. It should be noted that *P. fluviatilis* is a species whose subpopulations show strong natal homing and live on the edge of its salinity tolerance (Christensen et al., 2021). In another estuarine species, *E. encrasicolus*, two different ecotypes, coastal and off-shore, with different salinity tolerances and spatial dispersion, have been identified (Montes et al., 2016; Catanese et al., 2017, 2020). This suggests that similar mechanisms could be operating in the case of the European perch.

Even though, to elucidate the mechanisms maintaining these genetic differences it is also important to consider the variability between Baltic Sea basins and the management of the areas. First, the existing differences in salinity, temperature, and nutrient availability between different regions of the Baltic Sea have been reported to affect fish community structure as well as other habitat conforming factors such as seabed types and algal or seagrass conditions (HELCOM, 2006). This broad spectrum of ecosystems has already been suggested as the reason behind the different haplotypes of European perch (Wawrzyniak et al., 2020). Moreover, it has been proved that not all Baltic Sea basins show the same pattern of genetic structuring in *P. fluviatilis* (Olsson et al., 2011). A barrier to gene flow associated with depth has been described in the Gulf of Bothnia and the Central Baltic. To the north of this barrier exists strong isolation by distance in *P. fluviatilis*, but this effect is not found southwards (Olsson et al., 2011). Not only the genetic structure of perch differs between the regions of the Baltic Sea, but also the status of the stocks, with increases in the CPUE in the southern Gulf of Bothnia and north Baltic proper and declines in the West-Estonian archipelago (HELCOM, 2006). Within the Curonian Lagoon, perch is considered a freshwater straggler (Razinkovas-Baziukas et al., 2017) which performs seasonal migrations and feeding movements. Feeding movements from freshwater or estuarine environments to marine coastal areas have been attested by stable isotope analysis in tissues of perch and other freshwater stragglers, showing a wide range of isotopic signatures of freshwater fishes that indicates possible mixing of populations from coastal areas (Morkūnė, 2017; Lesutienė et al., 2018).

We find that none of the clusters detected deviate from the H-W equilibrium, indicating that the size of the different populations is large enough to maintain the heterozygote proportion. Additional mark-recapture studies, and the increase in the sampled area northward and southward, will certainly serve to clarify whether the factors operating

Table 5

Variability of the genetic pattern of *P. fluviatilis* and *O. eperlanus* in the different clusters; N - sample size; A - mean number of alleles per locus; EA - mean number of effective alleles per locus; PA - total number of private alleles; H_o - observed heterozygosity; H_e - expected heterozygosity; F_{IS} - inbreeding index; P_{H-W} - P-value of Hardy-Weinberg probability test. Bolded numbers indicate significance (p < 0.05).

<i>Perca fluviatilis</i>								
Cluster	N	A	EA	PA	H _o	H _e	F _{IS}	P _{H-W}
Cluster 1	27	5.750	2.374	1	0.421	0.386	−0.09870	1.000
Cluster 2	40	6.625	2.198	6	0.421	0.405	−0.03325	0.818
Cluster 3	42	6.500	2.084	5	0.487	0.446	−0.28125	0.240
Cluster 4	26	5.125	1.887	3	0.378	0.369	−0.11491	0.731
<i>Osmerus eperlanus</i>								
Cluster	N	A	EA	PA	H _o	H _e	F _{IS}	P _{H-W}
Cluster 1	101	7.750	2.796	10	0.434	0.453	0.04197	0.637
Cluster 2	121	8.125	2.802	13	0.516	0.506	−0.02200	0.000

Table 6

Analysis of molecular variance test (AMOVA) of 8 microsatellites markers of *P. fluviatilis* and *O. eperlanus*. d.f. - degrees of freedom; SS - sum of the squares. ARLEQUIN v3.5.2.2.

<i>Perca fluviatilis</i>						
Source of variation	d.f.	SS	Variance components	% Variation	Fixation index	P-value
Among clusters	3	21903.338	104.8887 Va	21.34	(F _{ST}) 0.21341	0.00000
Among individuals within clusters	131	43403.114	−55.2893 Vb	−11.25	(F _{IS}) −0.14301	0.99822
Within individuals	135	59656.500	441.9000 Vc	89.91	(F _{IT}) 0.10091	0.17030
Total	269	124962.952	491.4994			
<i>Osmerus eperlanus</i>						
Source of variation	d.f.	SS	Variance components	% Variation	Fixation index	P-value
Among clusters	1	43.027	0.18662 Va	8.85	(F _{ST}) 0.08848	0.00000
Among individuals within clusters	220	425.295	0.01050 Vb	0.50	(F _{IS}) 0.00546	0.34990
Within individuals	222	424.500	1.91216 Vc	90.65	(F _{IT}) 0.09345	0.00020
Total	443	892.822	2.10928			

in the Baltic Sea, i.e. temperature, salinity, habitat, depth, and management (HELCOM, 2006; Olsson et al., 2011; Wawrzyniak et al., 2020), could also be operating in the Curonian lagoon-Baltic Sea continuum and in what way in maintaining genetic differences between clusters.

4.1.2. Genetic structure in smelt

Within the Curonian Lagoon and the adjacent Baltic Sea area it has been suggested the existence of two different ecotypes of *O. eperlanus*, one anadromous population that spawns in the lagoon and migrates to open waters afterward, and a lagoon non-migrant population. Also, between the two ecotypes, it has been observed differences in the sexual maturation rate reaching maturity in two to three years in the case of the migrant population and only in one year in the case of the lagoon population (Shpilev et al., 2005). In accordance with these authors (Shpilev et al., 2005), we did not attempt to make a phenotypic distinction between both ecotypes given the absence of morphological differences, and we assumed that fishermen's catches in the Nemunas river and Curonian Lagoon include a greater representation of the lagoon population, but being in any case our samples representative of the existing diversity (Švagždys, 2009).

In our study, we have confirmed the existence of two different groups that inhabit both the Curonian lagoon and the adjacent Baltic Sea area (Fig. 2b) without interbreeding (Table 6). So, our results indicate that there is a small, but significant, genetic structure between habitats that support the idea of the existence of mentioned two different ecotypes of smelt. However, this small difference is not detected in smaller scale comparisons (Table 4). The individuals from both groups have a random distribution within the localities (Fig. 3b) and, thus, don't allow the determination of which cluster corresponds to which population. However, it is worth noting that the mean size from both subpopulations is almost the same (Table S1) and challenges the existence of two genetically different subspecies (Shpilev et al., 2005; Švagždys, 2009). Another plausible explanation for the maintenance of these two subpopulations of smelt is the existence of different reproductive and spawning locations. This mechanism has been proposed for *D. sargus* in the Mar Menor coastal lagoon (Spain), where age 0+ recruits from different locations enter the lagoon and randomly distribute inside (Hernández-García et al., 2015). For the Curonian Lagoon, spawning migrations of *O. eperlanus* have been recorded to the Nemunas river and to a smaller river, born as a diversion from the Nemunas, known as Matrosovka river (Shibaev et al., 2012), being it possible that the subpopulations belong to different watercourses. Further studies to elucidate whether the existence of different reproductive or spawning spots or the ecotype are responsible for the maintenance of the two subpopulations are required.

The genetic diversity studies on *Osmerus* spp. are extremely limited (Wennerstörn et al., 2017), being the information consistent with our findings. The data for *O. eperlanus* in the northern part of the Baltic Sea showed an absence of intraspecific divergence (Kovpak et al., 2011)

through an isolation gradient, which is in agreement with the absence of genetic structure between the Curonian Lagoon and the Baltic Sea (Table 3). Even though, in Norway, the introduction of a species from a single location into a separated lake has been determined through microsatellites analysis (Hagenlund et al., 2015) indicating that different reproductive stocks can coexist within a given area and that the separation events can facilitate the formation of subpopulations, supporting the idea of two different clusters coexisting in the Curonian Lagoon.

4.2. Management implications

Our study, even if different reproductive spots could not be assured and identified, detected that marine migrant species subpopulations coexist within the same coastal lagoon without interpopulation reproduction. In these ecosystems, knowledge of this behavior was, as far as we know, limited to *D. sargus* in the Mar Menor coastal lagoon (Hernández-García et al., 2015), but it can be hypothesized that it is more widespread than previously thought for migratory fish species. This implies that the management of coastal lagoons got influence over a wide area beyond the lagoon limits (Pérez-Ruzafa et al., 2019a). The migratory behavior of the main target species in lagoon fisheries makes that the management of lagoon fisheries must consider both lagoon and open coastal population dynamics where both fishing and natural mortality and recruitment-stock relationships probably differ and are regulated by different processes (Pérez-Ruzafa and Marcos, 2012). To the effect that migrant fish fisheries management has over adjacent areas of the coastal lagoons, it should be added that these migrant populations are shaped by the conditions of the coastal lagoons (Pérez-Ruzafa et al., 2019a). Management plans for coastal lagoons should be constructed considering that there are no geographically and genetically separated migrant fish populations in the lagoon-sea continuum. In the area of study, the management of the coastal Baltic Sea and the Curonian Lagoon needs to be unified.

The coexistence of subpopulations in the same coastal lagoon is particularly interesting for species suffering from stock overexploitation due to poor management (HELCOM, 2006) given that this coexistence makes the area a suitable candidate for the selection of reproductive individuals for aquaculture destined to human consumption or the replenishment of overexploited areas. Moreover, as perch is one of the key indicator species in assessing coastal fish community structure and development in the entire Baltic Sea, knowledge on the genetic structure of its populations might help to ensure its sustainable fishery and effective management. Also, there exists a huge gap in the knowledge of the genetic diversity of *Osmerus* spp. in the Baltic Sea (Wennerstörn et al., 2017), being this the first study in which two genetically different subpopulations of *O. eperlanus* are determined to coexist in a given area.

CRediT author statement

Alfredo Fernández-Álías: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review and editing; **Artūras Razinkovas-Baziukas:** Conceptualization, Validation; **Rasa Morkūnė:** Investigation, Writing – review and editing, Validation; **Helena Ibáñez-Martínez:** Data curation, Investigation; **Egidijus Bacevičius:** Investigation, Writing – review and editing; **Irene Muñoz:** Investigation; **Concepción Marcos:** Conceptualization, Funding acquisition, Project administration, Writing – review and editing; Validation; **Ángel Pérez-Ruzafa:** Conceptualization, designed the research, Funding acquisition, Supervision, Writing – review and editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2022.105732>.

References

- Ben Khadher, S., Agnès, J.F., Milla, S., Teletchea, F., Fontaine, P., 2015. Patterns of genetic structure of Eurasian perch (*Perca fluviatilis* L.) in Lake Geneva at the end of the spawning season. *J. Great Lake Res.* 41 (3), 846–852. <https://doi.org/10.1016/j.jglr.2015.04.006>.
- Ben Khadher, S., Teletchea, F., Fontaine, P., 2019. Strong genetic difference of Eurasian perch *Perca fluviatilis* from two Alpine lakes used as founder populations for farming. *Cybum: Rev. Int. Ichtyol.* <https://doi.org/10.26028/cybum/2019-433-002>.
- Berzins, B., 1949. On the biology of the Latvian perch (*Perca fluviatilis* L.). *Hydrobiologia* 2 (1), 64–71.
- Brown, J.A., Moore, W.M., Quabius, E.S., 2001. Physiological effects of saline waters on zander. *J. Fish. Biol.* 59 (6), 1544–1555. <https://doi.org/10.1046/j.1095-8649.1996.tb00001.x>.
- Catanese, G., Watteaux, R., Montes, I., Barra, M., Rumolo, P., Borme, D., Buongiorno Nardelli, B., Botte, V., Mazzocchi, M.G., Genovese, S., Di Capua, I., Iridondo, M., Estonba, A., Ruggeri, P., Tirelli, V., Caputo-Barucchi, V., Basilone, G., Bonanno, A., Iudicone, D., Procaccini, G., 2017. Insights on the drivers of genetic divergence in the European anchovy. *Sci. Rep.* 7 (1), 1–15. <https://doi.org/10.1038/s41598-017-03926-z>.
- Catanese, G., Di Capua, I., Iridondo, M., Bonanno, A., Estonba, A., Procaccini, G., 2020. Application of high-throughput single nucleotide polymorphism genotyping for assessing the origin of *Engraulis encrasicolus* eggs. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 30 (7), 1313–1324.
- Chatterji, S., Pachter, L., 2006. Reference based annotation with GeneMapper. *Genome Biol.* 7 (4), 1–10. <https://doi.org/10.1186/gb-2006-7-4-r29>.
- Christensen, E.A.F., Svendsen, M.B.S., Steffensen, J.F., 2021. Population ecology, growth, and physico-chemical habitat of anadromous European perch *Perca fluviatilis*. *Estuar. Coast Shelf Sci.* 249, 107091. <https://doi.org/10.1016/j.ecss.2020.107091>.
- Collette, B.B., Ali, M.A., Hokanson, K.E.F., Nagić, M., Smirnov, S.A., Thorpe, J.E., Weatherley, A.H., Willemsen, J., 1977. Biology of the percids. *J. Fish. Board Can.* 34 (10), 1890–1899. <https://doi.org/10.1139/j77-255>.
- Cowen, R.K., Paris, C.B., Srinivasan, A., 2006. Scaling of connectivity in marine populations. *Science* 311 (5760), 522–527. <https://doi.org/10.2307/3843412>.
- Craig, J.F., 1987. *The Biology of Perch and Related Fishes*. Croom-Helm. Timber Press, London and Sydney. Portland, Oregon.
- Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J., Cyrus, D.P., Nordlie, F.G., Harrison, T.D., 2007. The guild approach to categorizing estuarine fish assemblages: a global review. *Fish. Fish.* 8 (3), 241–268. <https://doi.org/10.1111/j.1467-2679.2007.00253.x>.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567.
- Gaigalas, K., 2001. Fish and fisheries in the Curonian Lagoon (Kursių marių baseino žuvis ir žvejyba. Klaipėda, Eglė. 369.
- Gasiūnaite, Z.R., Daunys, D., Olenin, S., Razinkovas, A., 2008. The curonian lagoon. *Ecology of baltic coastal Waters book. Ecol. Stud.* 197. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>.
- Gerlach, G., Schardt, U., Eckmann, R., Meyer, A., 2001. Kin-structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). *Heredity* 86, 213–221. <https://doi.org/10.1046/j.1365-2540.2001.00825.x>.
- Hagenlund, M., Østbye, K., Langdal, K., Hassve, M., Pettersen, R.A., Anderson, E., Gregersen, F., Præbel, K., 2015. Fauna crime: elucidating the potential source and introduction history of European smelt (*Osmerus eperlanus* L.) into Lake Storsjøen, Norway. *Conserv. Genet.* 16 (5), 1085–1098. <https://doi.org/10.1007/s10592-015-0724-2>.
- HELCOM (Helsinki Commission-Baltic Marine Life Protection Commission), 2006. Assessment of coastal fish in the Baltic Sea. In: *Baltic Sea Environment Proceedings*, vol. 103.
- Hernández-García, R., Muñoz, I., López-Capel, A., Marcos, C., Pérez-Ruzafa, A., 2015. The influence of environmental variability of a coastal lagoon ecosystem on genetic diversity and structure of white seabream (*Diplodus sargus* (Linnaeus, 1758)) populations. *Mar. Ecol.* 36, 1144–1154. <https://doi.org/10.1111/maec.12210>.
- Idzelytė, R., Mėzinė, J., Zemlys, P., Umgiesser, G., 2020. Study of ice cover impact on hydrodynamic processes in the Curonian Lagoon through numerical modeling. *Oceanologia* 62 (4), 428–442.
- Imbrock, F., Appenzeller, A., Eckmann, R., 1996. Diet and seasonal distribution of perch in Lake Constance: a hydroacoustic study and in situ observations. *J. Fish. Biol.* 49, 1–13. <https://doi.org/10.1111/j.1095-8649.1996.tb00001.x>.
- Johannesson, K., André, C., 2006. Life on the margin: genetic isolation and loss of variation in a peripheral marine ecosystem. *Mol. Ecol.* 15. <https://doi.org/10.1111/j.1365-294x.2006.02919.x>, 2013–2030.
- Johnson, M., Black, R., 2006. Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. *Evolution* 60 (12), 2498–2506. <https://doi.org/10.1111/j.0014-3820.2006.tb01885.x>.
- Jombart, T., 2008. Adegnet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24, 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>.
- Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* 11, 94. <https://doi.org/10.1186/1471-2156-11-94>.
- Karås, P., 1996. Recruitment of perch (*Perca fluviatilis* L.) from Baltic coastal waters. *Arch. Hydrobiol.* 99–121. <https://doi.org/10.1127/archiv-hydrobiol/138/1996/99>.
- Karås, P., Hudd, R., 1993. Reproduction areas of fresh-water fish in the northern quark (Gulf of Bothnia). *Aqua Fenn.* 23 (1), 39–49.
- Kipling, C., Le Cren, E., 1984. Mark-recapture experiments on fish in windermere. *J. Fish. Biol.* 24, 395–414. <https://doi.org/10.1111/j.1095-8649.1984.tb04811.x>.
- Kjerfve, B., 1994. Coastal Lagoons. In: Kjerfve, B. (Ed.), *Coastal Lagoon Processes*. Elsevier Oceanographic Series 60. Elsevier, Amsterdam, pp. 1–8.
- Kovpak, N.E., Skurikhina, L.A., Kuchlevsky, A.D., Oleinik, A.G., Sendek, D.S., 2011. Genetic divergence and relationships among smelts of the genus *Osmerus* from the Russian waters. *Russ. J. Genet.* 47 (8), 958–972.
- Lesutienė, J., Ložys, L., Dainys, J., Karosienė, J., Pilkaitytė, R., Pūtyš, Ž., Bukaveckas, P. A., Gasiūnaite, Z.R., 2018. Migratory patterns and cyanotoxin concentrations of pikeperch (*Sander lucioperca*) in the coastal waters of the Baltic Sea. *Boreal Environ. Res.* 23, 315–327.
- Liu, S.Y.V., Hsu, C.H., 2021. Genetic analyses reveal fine-scale genetic structure between lagoon and open water spoon seagrass (*Halophila ovalis*) populations around Dongsha Island. *Aquat. Bot.* 174, 103421. <https://doi.org/10.1016/j.aquabot.2021.103421>.
- Ložys, L., 2004. The growth of pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) under different water temperature and salinity conditions in the Curonian Lagoon and Lithuanian coastal waters of the Baltic Sea. *Hydrobiologia* 514 (1), 105–113. <https://doi.org/10.1023/b:hydr.0000018211.26378.b9>.
- Lutz, P.L., 1972. Ionic and body compartment responses to increasing salinity in the perch *Perca fluviatilis*. *Comp. Biochem. Physiol. Physiol.* 42 (3), 711–717. [https://doi.org/10.1016/0300-9629\(72\)90449-5](https://doi.org/10.1016/0300-9629(72)90449-5).
- Luczyńska, J., Paszczyk, B., Luczyński, M.J., Kowalska-Górska, M., Nowosad, J., Kucharczyk, D., 2020. Using *Rutilus rutilus* (L.) and *Perca fluviatilis* (L.) as bioindicators of the environmental condition and human health: lake Łańskie, Poland. *Int. J. Environ. Res. Publ. Health* 17 (20), 7595. <https://doi.org/10.3390/ijerph17207595>.
- Montes, I., Zarraonandia, I., Iridondo, M., Grant, W.S., Manzano, C., Cotano, U., Conklin, D., Irigoien, X., Estonba, A., 2016. Transcriptome analysis deciphers evolutionary mechanisms underlying genetic differentiation between coastal and

- offshore anchovy populations in the Bay of Biscay. *Mar. Biol.* 163 (10), 1–13. <https://doi.org/10.1007/s00227-016-2979-7>.
- Morkūnė, R., 2017. Food Web of the Lithuanian Baltic Sea Coastal Zone: Structure and Organic Matter Flows. Doctoral dissertation, Klaipėda university.
- Morkūnė, R., Bučas, M., Katarzytė, M., Politi, T., Vaičiūtė, D., Vizzini, S., Martin, G., 2022. Food sources for benthic grazers in trophic networks of macrophyte habitats in a transitional baltic ecosystem. *Water* 14 (10), 1565.
- Nesbø, C.L., Fosshem, T., Vøllestad, L.A., Jakobsen, K.S., 1999. Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonisation. *Mol. Ecol.* 8, 1387–1404. <https://doi.org/10.1046/j.1365-294x.1999.00699.x>.
- Olsson, J., Mo, K., Florin, A.B., Aho, T., Ryman, N., 2011. Genetic population structure of perch *Perca fluviatilis* along the Swedish coast of the Baltic Sea. *J. Fish. Biol.* 79 (1), 122–137. <https://doi.org/10.1111/j.1095-8649.2011.02998.x>.
- Overton, J.L., Bayley, M., Paulsen, H., Wang, T., 2008. Salinity tolerance of cultured Eurasian perch, *Perca fluviatilis* L.: effects on growth and on survival as a function of temperature. *Aquaculture* 277 (3–4), 282–286. <https://doi.org/10.1016/j.aquaculture.2008.02.029>.
- Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28, 2537–2539. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>.
- Pérez-Ruzafa, A., Marcos, C., 2012. Fisheries in coastal lagoons: an assumed but poorly researched aspect of the ecology and functioning of coastal lagoons. *Estuar. Coast Shelf Sci.* 110, 15–31. <https://doi.org/10.1016/j.ecss.2012.05.025>.
- Pérez-Ruzafa, A., Pérez-Ruzafa, I.M., Newton, A., Marcos, C., 2019a. Coastal lagoons: environmental variability, ecosystem complexity, and goods and services uniformity. In: *Coasts and Estuaries*. Elsevier, pp. 253–276.
- Pérez-Ruzafa, A., De Pascalis, F., Ghezzi, M., Quispe-Becerra, J.I., Hernández-García, R., Muñoz, I., Vergara, C., Pérez-Ruzafa, I.M., Umgieser, G., Marcos, C., 2019b. Connectivity between coastal lagoons and sea: asymmetrical effects on assemblages' and populations' structure. *Estuar. Coast Shelf Sci.* 216, 171–186. <https://doi.org/10.1016/j.ecss.2018.02.031>.
- Peyran, C., Boissin, E., Morage, T., Nebot-Colomer, E., Iwankow, G., Planes, S., 2021. Genetic homogeneity of the critically endangered fan mussel, *Pinna nobilis*, throughout lagoons of the Gulf of Lion (North-Western Mediterranean Sea). *Sci. Rep.* 11 (1), 1–12. <https://doi.org/10.1038/s41598-021-87493-4>.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155 (2), 945–959. <https://doi.org/10.1093/genetics/155.2.945>.
- Pukk, L., Gross, R., Vetemaa, M., Vasemägi, A., 2016. Genetic discrimination of brackish and freshwater populations of Eurasian perch (*Perca fluviatilis* L.) in the Baltic Sea drainage: implications for fish forensics. *Fish. Res.* 183, 155–164. <https://doi.org/10.1016/j.fishres.2016.05.027>.
- Quispe, J.I., 2014. Dinámica espacio-temporal del ictioplancton del Mar Menor (SE España) y factores ambientales asociados. Tesis Doctoral, Universidad de Murcia.
- R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-90005107-0.
- Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249.
- Razinkovas-Baziukas, A., Morkūnė, R., Bacevičius, E., Gasiūnaitė, Z.R., 2017. Trophic network model of exposed sandy coast: linking continental and marine water ecosystems. *Estuar. Coast Shelf Sci.* 195, 110–123. <https://doi.org/10.1016/j.ecss.2017.04.030>.
- Sambrook, E., Fritsch, F., Maniatis, T., 1989. *Molecular Cloning: a Laboratory Manual*. Cold spring Harbour, New York.
- Seixas, V.C., Paiva, P.C., Russo, C.A.D.M., 2018. Comparative population genetics and demographic history of two polychaete species suggest that coastal lagoon populations evolve under alternate regimes of gene flow. *Mar. Biol.* 165 (11), 1–16. <https://doi.org/10.1007/s00227-018-3437-5>.
- Sendek, D.S., Bogdanov, D.V., 2019. European smelt *Osmerus eperlanus* in the eastern Gulf of Finland, Baltic Sea: stock status and fishery. *J. Fish. Biol.* 94 (6), 1001–1010. <https://doi.org/10.1111/jfb.14009>.
- Sepulveda, A., 1994. Daily growth increments in the otoliths of European smelt *Osmerus eperlanus* larvae. *Mar. Ecol. Prog. Ser.* 108 (1), 33–42.
- Shibaev, S., Sokolov, A., Tylik, K., Svirina, L., Smirnova, M., Shkitsky, V., 2012. The spawning migration of smelt (*Osmerus eperlanus* L.) in the rivers of the Curonian lagoon basin. *Vestnik IKBFU* (7).
- Shpilev, H., Ojaveer, E., Lankov, A., 2005. Smelt (*Osmerus eperlanus* L.) in the Baltic Sea. *Proc. Est. Acad. Sci. Biol. Ecol.* 54 (3), 230–241.
- Sigovini, M., 2011. Multiscale dynamics of zoobenthic communities and relationships with environmental factors in the Lagoon of Venice. Tesi di Dottorato. Università Ca' Foscari di Venezia.
- Spies, I., 2012. Landscape genetics reveals population subdivision in Bering Sea and Aleutian Islands Pacific cod. *Trans. Am. Fish. Soc.* 141 (6), 1557–1573.
- Svagzdys, A., 2009. Impact of environmental conditions on smelt catch fluctuations in the Nemunas River and the Curonian Lagoon. *Ekologija* 55 (3–4), 204–214.
- Tibblin, P., Koch-Schmidt, P., Larsson, P., Stenroth, P., 2012. Effects of salinity on growth and mortality of migratory and resident forms of Eurasian perch in the Baltic Sea. *Ecol. Freshw. Fish* 21 (2), 200–206. <https://doi.org/10.1111/j.1600-0633.2011.00537.x>.
- Toomey, L., Dellicour, S., Vanina, T., Pegg, J., Kaczowski, Z., Kouril, J., Teletchea, F., Bláha, M., Fontaine, P., Lecocq, T., 2020. Getting off on the right foot: integration of spatial distribution of genetic variability for aquaculture development and regulations, the European perch case. *Aquaculture* 521, 734981. <https://doi.org/10.1016/j.aquaculture.2020.734981>.
- Umgieser, G., Ferrarin, C., Cucco, A., De Pascalis, F., Bellafiore, D., Ghezzi, M., Bajo, M., 2014. Comparative hydrodynamics of 10 Mediterranean lagoons by means of numerical modeling. *J. Geophys. Res.: Oceans* 119 (4), 2212–2226. <https://doi.org/10.1002/2013JC009512>.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4, 535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>.
- Valtonen, E.T., Holmes, J.C., Aronen, J., Rautalahti, I., 2003. Parasite communities as indicators of recovery from pollution: parasites of roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in Central Finland. *Parasitology* 126 (7), S43–S52. <https://doi.org/10.1017/S0031182003003494>.
- Vergara-Chen, C., González-Wangüemert, M., Marcos, C., Pérez-Ruzafa, A., 2010a. High gene flow promotes the genetic homogeneity of the fish goby *Pomatoschistus marmoratus* (Risso, 1810) from Mar Menor coastal lagoon and adjacent marine waters (Spain). *Mar. Ecol.* 31 (2), 270–275. <https://doi.org/10.1111/j.1439-0485.2009.00342.x>.
- Vergara-Chen, C., González-Wangüemert, M., Marcos, C., Pérez-Ruzafa, A., 2010b. Genetic diversity and connectivity remain high in *Holothuria polii* (Delle Chiaie 1823) across a coastal lagoon-open sea environmental gradient. *Genetica* 138 (8), 895–906. <https://doi.org/10.1007/s10709-010-9472-x>.
- Vergara-Chen, C., González-Wangüemert, M., Marcos, C., Perez-Ruzafa, A., 2013. Small-scale genetic structure of *Cerastoderma glaucum* in a lagoonal environment: potential significance of habitat discontinuity and unstable population dynamics. *J. Molluscan Stud.* 79 (3), 230–240. <https://doi.org/10.1093/mollus/eyt015>.
- Virbickas, J., 1986. *Fish of Lithuania*. Mokslas, Vilnius [in Lithuanian].
- Wawrzyniak, W., Kiepińska, J., Czerniejewski, P., Bugaj, A., 2020. Genetic characterization of selected populations of European perch, *Perca fluviatilis* (Actinopterygii: perciformes: Percidae), in the waters of north-western Poland with recommendations for fishing and stocking policies. *Acta Ichthyol. Piscatoria* 50 (2), 161–170. <https://doi.org/10.3750/AIEP/02494>.
- Wennerström, L., Jansson, E., Laikre, L., 2017. Baltic Sea genetic biodiversity: current knowledge relating to conservation management. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 27 (6), 1069–1090. <https://doi.org/10.1002/aqc.2771>.
- Wirth, T., Bernatchez, L., 2001. Genetic evidence against panmixia in the European eel. *Nature* 409 (6823), 1037–1040. <https://doi.org/10.1038/35059079>.
- Žaromskis, R., 1996. *Oceans, Seas, Estuaries*. Debesija, Vilnius (in Lithuanian).