Arch. Hydrobiol. 159 4 531–554 Stuttgart, April 2004

Genetic and morphometric variations in the pikeperch (*Sander lucioperca* L.) of a fragmented delta

Nicolas Poulet[1], Patrick Berrebi[2], Alain J. Crivelli[3], Sovan Lek[4] and Christine Argillier[1]

With 5 figures and 8 tables

Abstract: Agricultural development modified the connectivity of the Rhône River delta waterbodies (also called the Camargue) which is now isolated from the Rhône River by dikes. Furthermore, the hydrographic network of the Camargue is constituted of irrigation and drainage canals, which are not directly connected. Pikeperch (*Sander lucioperca* L.), an allochthonous freshwater species, colonised the Rhône and the Camargue more than 50 years ago. We used morphometric and meristic features, otolith shape descriptors and protein electrophoresis in order to assess whether the Camargue houses one or several pikeperch populations. All characters except the meristic counts highlighted the existence of two subpopulations: one in the drainage network and one in the irrigation network. Electrophoresis showed that the irrigation network population is closer to the Rhône population and that the drainage network population displayed a high inbreeding rate. The causes of such isolation and the implications for the pikeperch population dynamics are discussed.

Key words: allozyme, delta, fragmentation, morphometrics, otolith, population genetics, *Sander lucioperca*.

Introduction

River fragmentation is nowadays widespread worldwide and causes an important loss of biodiversity (Dynesius & Nilsson 1994). Furthermore a river can

[1] **Authors' addresses:** Cemagref, Unité de Recherche RIPE, 361, rue JF Breton BP 5095 34033 Montpellier Cedex, France;
E-Mail: christine.argillier@montpellier.cemagref.fr

[2] UMR 5119 "Ecosystèmes Lagunaires", Université Montpellier II, CC 093, Place E. Bataillon, 34095 Montpellier Cedex 5, France.

[3] Station Biologique de la Tour du Valat, 13200 Arles, France.

[4] LADYBIO (CNRS-UPS) Laboratoire Dynamique de la Biodiversité, Université Paul Sabatier, 118 route de Narbonne 31062 Toulouse cedex 4, France.

DOI: 10.1127/0003-9136/2004/0159-0531 0003-9136/04/0159-0531 $ 6.00

be submitted to two types of connectivity disruption between its different parts: i) longitudinal disruption preventing upstream/downstream passage and ii) lateral disruption preventing the passage between the main channel of a river and its annexes (side arms) or its floodplain. The longitudinal disruption is mainly induced by hydroelectric dams or weirs and their effects on the riverine ecosystem are well known (WARD & STANFORD 1983). Lateral disruption is often due to dikes erected to prevent floods and/or to establish agricultural plans. Lateral waterbodies play a major role in the dynamics of river ecosystems (WARD & STANFORD 1995) including fish population dynamics. Indeed, many species use these annexes as reproduction grounds, nurseries, resting places, etc. (SCHIEMER & SPINDLER 1989). Although connectivity can be sometimes established by an exceptional flood or by irrigation pumps, isolated floodplain fish populations remain exposed to falls in numbers and even risk local extinction (SCHMUTZ & JUNGWIRTH 1999). It is crucial to determine the degree of isolation in order to estimate whether the number of migrants from the river population is sufficient to sustain the floodplain population. Although this problem is commonly investigated in anthropogenic longitudinal disruption (LAROCHE et al. 1999, NERAAS & SPRUELL 2001), it is not so in the case in artificial lateral disruptions.

A typical case of such engineering is the Camargue development completed in the middle of the nineteenth century on the Rhône delta by the elevation of two dikes along the Rhône and a third one between the Mediterranean Sea and the lagoons. These constructions were erected to prevent flooding by freshwater. Since these management structures were built, the Rhône delta has been isolated from the river and fragmented into many canal networks.

The pikeperch (*Sander lucioperca* L.) is a percid fish allochthonous to the French hydrographic network. Its life history traits (nest guarder, high fecundity) and its tolerance for the quality of the ecosystem make it a good coloniser (DEELDER & WILLEMSEN 1964, KIENER 1968, MARSHALL 1977). Thus, pikeperch should be able to colonise the different parts of the delta. The aim of this paper is to determine the degree of isolation of pikeperch populations between the different spatially and temporarily connected compartments of the delta.

Many tools can be used to identify and discriminate fish stock for estimating the degree of connectivity between artificially isolated aquatic ecosystems. We used morphometric and meristic characters (MENG & STOCKER 1984, HURLBUT & CLAY 1998, CADRIN 2000), otolith shape (BIRD et al. 1986, CAMPANA & CASSELMAN 1993, FRIEDLAND & REDDIN 1994, BEGG & BROWN 2000) and protein electrophoresis (ALLENDORF & PHELPS 1981, BERREBI et al. 2000). The use of several methods to discriminate stocks is better than only one (BEGG & WALDMAN 1999).

Study site

The Camargue is situated in southern France (43° 34′ N, 4° 34′ E) in the Rhône delta (Fig. 1). Since 1869, the Camargue has been completely diked. Therefore, it is hydraulically isolated from the Rhône River (CHAUVELON 1998). Our study site was the Fumemorte catchment (68 km^2) located in the eastern part of the Camargue and composed of three parts: the irrigation system, the drainage system and the Vaccarès Lagoon (Fig. 2). The main characteristics of these three compartments are given in Table 1. The irrigation and the drainage systems include an extensive network of canals. Our sampling sites were located in the Bouic Canal, in the Fumemorte Canal and in the Vaccarès Lagoon.

The Fumemorte basin is equipped with 15 pumping stations on the Rhône River. From April to October, these stations provide freshwater for irrigations canals supplying hunting marshes and agriculture (rice fields). For the rest of the year, most of these canals remain almost dry. The Bouic Canal, with $24.5.10^6$ of the $70.1.10^6$ m^3 supplied yearly in the Fumemorte basin, is the major irrigation canal (CHAUVELON et al. 1996).

The Fumemorte Canal collects drainage water from a complex 400 km-long network (CHAUVELON 1998). The water collected from the marshes and rice fields is

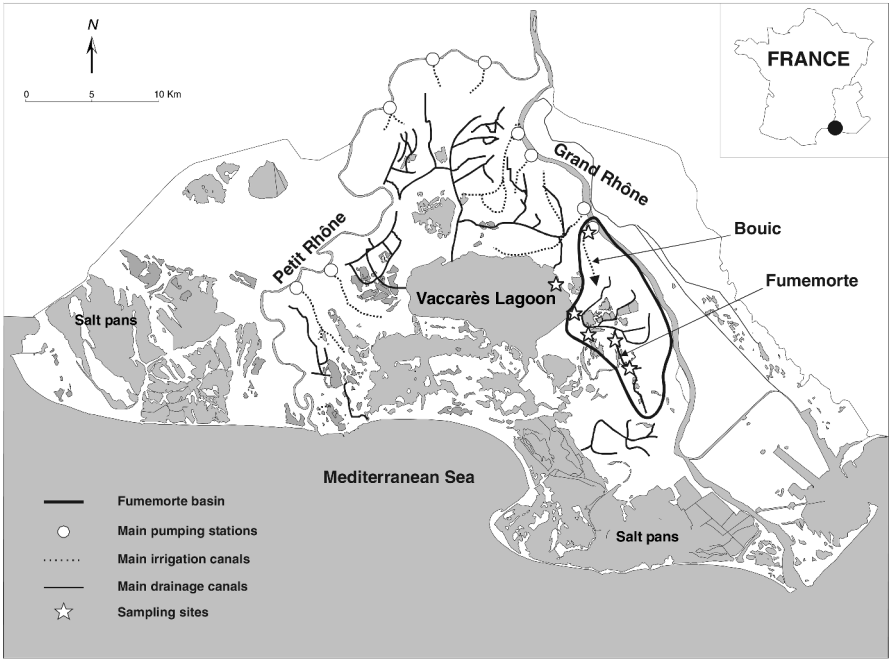

Fig. 1. The Camargue (Rhône Delta) and the irrigation/drainage networks. The canal networks have been simplified.

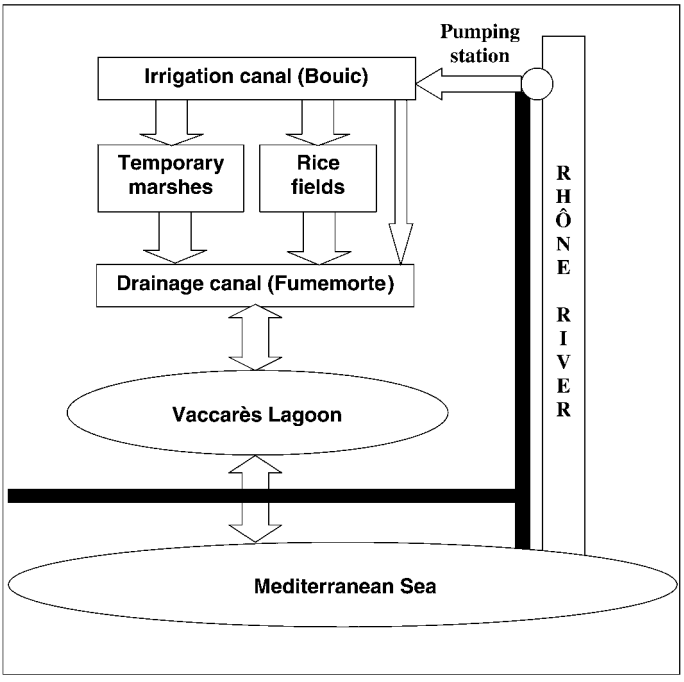

Fig. 2. Hydraulic functioning of the Fumemorte basin (modified from CHAUVELON et al. 1996). Arrows indicate the direction of flow and thick black lines represent the dikes.

Table 1. Characteristics of each Fumemorte basin compartment. Mean temperature and salinity were calculated from the monthly data between 1993 and 2000.

	Bouic Canal (irrigation)	Fumemorte Canal (drainage)	Vaccarès Lagoon
Length (km)	10	14.6	6600 Ha
Width (m)	2.5	15	
Depth (m)	<1	1	1.5
Mean temperature (°C)	14.5 ± 6.7	14.5 ± 6.9	14.6 ± 6.5
Mean salinity (g/L)	<0.1	0.97 ± 0.7	8.25 ± 3.8

brackish but salinity varies according to the cultivation period and to the force and direction of the wind: it can reach 5 g/L near the mouth.

The Vaccarès Lagoon is permanently flooded. Because of drainage water and precipitation, salinity of the Vaccarès Lagoon varied between 5 g/L and 38 g/L during the last 50 years (HEURTEAUX 1992). The Vaccarès Lagoon is supplied both by the sea and by the drainage system. However, the capacity of all the Camargue lagoons is insufficient to drain the 400 million m^3 of water pumped every year from the Rhône into the irrigation system without flooding. Most of the drainage canals are blocked by sluices

at their outlets and the water collected is pumped back into the Rhône (CHAUVELON 1998). At its outlet, the Fumemorte Canal just has a raisable barrier (erected in 1987) in order to prevent the entrance of salt; the water flows under gravity to the Vaccarès Lagoon and most of the aquatic organisms can freely pass from the canal to the lagoon and vice versa when the barrier is opened (ROSECCHI & CRIVELLI 1995). The Fumemorte Canal remains the largest drainage canal with no major obstacles. The connections between irrigation and drainage compartments are indirect, via rice fields and seasonally-flooded marshes. In the rice fields, environmental conditions are too harsh for most fish species: the temperature is high (25 °C, see PONT 1977), the depth shallow (10 cm) and the concentration of pesticides can be high (ROCHE et al. 2002). Most fish would not survive in such conditions. The marshes are the more likely way for fish to cross between the drainage and the irrigation networks. If a marsh is irrigated and then drained, the fish can pass from the irrigation canal to the drainage system. Occasional direct overflow from the irrigation to the drainage system does occur and fish could use it to pass down into the drainage system. The probability of a fish going from the drainage to the irrigation network, however, is extremely low. The only possibility for this to occur would be that a flooded marsh, already colonised by fish, be connected to the irrigation canal so fish can swim upstream from the marsh into the irrigation system (POIZAT et al. 1999).

Materials and methods

Data collection

Sampling

Pikeperch were caught between March and December 2001 in Fumemorte Canal, Bouic Canal and Vaccarès Lagoon with fyke nets (mesh size 6 mm), gill nets (mesh

Table 2. Pikeperch mean fork length and sample size (± standard error) for the four methods.

Method	Site	Fork length (mm)	Numbers
Genetics	Rhône	62 (± 15)	24
	Bouic	214 (± 18)	36
	Fumemorte	306 (± 27)	30
	Vaccarès	219 (± 5)	5
Meristics	Bouic	258 (± 23)	41
	Fumemorte	301 (± 22)	31
	Vaccarès	213 (± 5)	4
Landmarks	Bouic	218 (± 14)	41
	Fumemorte	305 (± 23)	38
	Vaccarès	215 (± 15)	5
Otolith shape	Bouic	210 (± 4)	33
	Fumemorte	311 (± 20)	43
	Vaccarès	231 (± 9)	7

size 40, 55, and 80 mm in Fumemorte only) and electrofishing (in Bouic only) (Table 2). Fish were anaesthetised in a bath containing 5 mL/L of 2-phenoxyethanol, measured (fork length to the nearest millimetre), weighed (to the nearest gram), photographed for morphometric purposes (see below) and frozen at −20 °C for allozyme analysis.

Note that the number of pikeperch captured in the Vaccarès Lagoon was very low, probably due to the salinity which was over the reported pikeperch tolerance (12 g/L, Craciun et al. 1982) but see Brown et al. (2001).

Furthermore, 20 additional juveniles from the Rhône were captured in June in the pump outflow (Poizat et al. 1999). Because of their small size (< 50 mm), these fish were not retained for morphometric analyses.

Electrophoresis

Eyes, muscle and liver were individually homogenised in an equal volume of pH 6.8 Tris-NADP-EDTA buffer, and centrifuged (5500 g, 30 min, 4 °C). The supernatant was

Table 3. Loci and protein investigated; organs and buffers used. L: Liver, M: Muscle and E: Eye. Resolution: Good: clear spot; Fair: light spot; Poor: too light or missing spot; N.D.: Not determined.

Protein	E. C. number	Locus	Tissue	TC8	TCB
Alcohol dehydrogenase	1.1.1.1	*ADH**	L	N.D.	Good
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	L	Fair	N.D.
Sorbitol dehydrogenase	1.1.1.14	*SDH**	L, M, E	N.D.	Poor
Lactate dehydrogenase	1.1.1.27	*LDH-C1**	E	Good	N.D.
Malate dehydrogenase	1.1.1.37	*MDH-1**	L, E	Good	N.D.
		*MDH-2**	L, E	Good	N.D.
Malic enzyme	1.1.1.40	*ME**	M	Good	N.D.
Isocitrate dehydrogenase	1.1.1.42	*IDHP-1**	L	Good	N.D.
		*IDHP-2**	E, M	Good	N.D.
6-phosphogluconate dehydrogenase	1.1.1.44	*6PGDH-2**	L, M, E	Poor	N.D.
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PD**	L, M, E	Poor	N.D.
Superoxide dismutase	1.15.1.1	*SOD**	L, M, E	Poor	N.D.
Asparate aminotransferase	2.6.1.1	*AAT-1**	M	N.D.	Fair
Creatine kinase	2.7.3.2	*CK-1**	E	Good	N.D.
Adenylate kinase	2.7.4.3	*AK-1**	L, M, E	Poor	N.D.
Phosphoglucomutase	2.7.5.1	*PGM-1**	L, M, E	Good	N.D.
Esterase	3.1.1.1	*EST-1**	L	N.D.	Good
Aconitase	4.2.1.3	*ACO**	L, M, E	N.D.	Poor
Mannose phosphate isomerase	5.3.1.8	*MPI-2**	L, E	Good	N.D.
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1**	L, M, E	Good	N.D.
		*GPI-B2**	M, E	Good	N.D.
Muscle protein	–	*PROT-1**	M	Good	N.D.
		*PROT-2**	M	Good	N.D.
		*PROT-3**	M	Good	N.D.

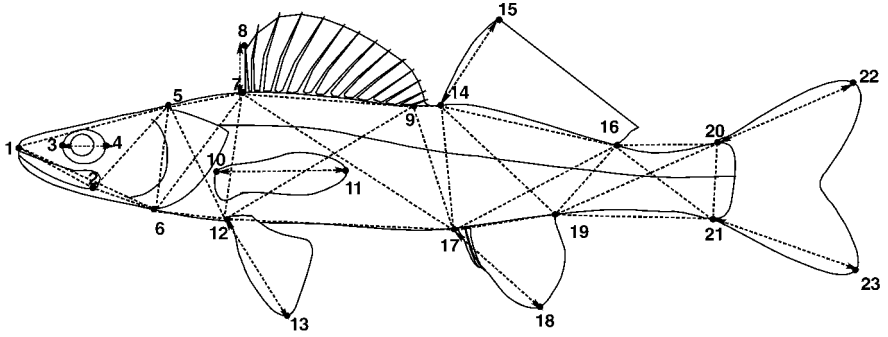

Fig. 3. Landmarks used and distances taken from the fish: (1) Tip of snout; (2) Tip of maxillary; (3) Pre orbital; (4) Post orbital; (5) Forehead; (6) Branchiosteges (base of gill opening); (7) Insertion of first dorsal-fin; (8) Tip of first spiny ray; (9) Insertion of last spiny ray; (10) Insertion of pectoral-fin; (11) Tip of pectoral-fin; (12) Insertion of pelvic-fin; (13) Tip of pelvic-fin; (14) Insertion of second dorsal-fin; (15) Tip of first spiny ray; (16) Insertion of last soft ray; (17) Insertion of anal-fin; (18) Tip of first spiny ray; (19) Insertion of last soft ray; (20) Insertion of first caudal fin ray; (21) Insertion of last caudal fin ray; (22) Upper tip of caudal fin; (23) Lower tip of caudal fin.

frozen at – 20 °C. Electrophoresis was performed on 12 % horizontal starch gels according to the method reported by PASTEUR et al. (1987). The buffers employed were TC8 (continuous Tris-citrate) and TCB (discontinuous Tris-citrate) (BILLINGTON et al. 1990).

First, 19 enzymes were tested on all three tissues (eye, liver and muscle) of 7 individuals. For each enzyme, the relative intensity of the different spots was noted (Table 3). Then, the enzymes providing the best results were selected for the study. Electrophoresis of the 0+ fish enzymes was conducted with eye and muscle only due to the small size of the fish. A total of 95 pikeperch were genetically analysed.

Morphometric and meristic analysis

A picture of the left side of 83 pikeperch was taken with a digital camera (Nikon Coolpix® 950) fixed on an L-shaped bracket. A metric ruler was placed alongside the fish to provide a baseline scale. The fins were held spread with fine needles. TpsDIG32 software (free download at: http://life.bio.sunysb.edu/morph/) was used to take the Cartesian co-ordinates of 23 landmarks. Twenty eight distances were measured according to the box truss method (STRAUSS & BOOKSTEIN 1982) and eight classic distances (HOLCIK et al. 1989) were added (Fig. 3).

Meristic counts were performed directly on 79 fish (Table 4).

Otolith shape analysis

Two types of shape descriptors commonly employed in biology were used: shape ratio descriptors and elliptic Fourier coefficients. Both are independent of the size, translation and/or rotation of the otolith on the picture (DE PONTUAL & PROUZET 1988).

Table 4. Meristic variables used.

No.	Meristic count
1	Spiny rays of the first dorsal fin
2	Spiny rays of the second dorsal fin
3	Soft rays of the second dorsal fin
4	Spiny rays of the left pelvic fin
5	Soft rays of the left pelvic fin
6	Spiny rays of the right pelvic fin
7	Soft rays of the right pelvic fin
8	Rays of the anal fin
9	Rays of the left pectoral fin
10	Rays of the right pectoral fin

Sagittal otolith pairs were removed from 83 pikeperch. The right one was kept for ageing analysis. The left otolith was placed on a glass slide and photographed with a digital camera fixed on a microscope. In order to have a clear outline, the otolith was lighted from below.

Then, for each otolith, the perimeter, area, length, width and two shape ratio descriptors (circularity and rectangularity) were calculated using OPTIMAS (1996). Circularity was defined as the perimeter of the otolith squared and divided by its area, and rectangularity was as the otolith area divided by the area of its enclosing rectangle, oriented along the length of the otolith.

The elliptic Fourier coefficients were computed from the digital pictures of each otolith (MANH 2001). Here, the empiric contour of a plane shape is decomposed into a series of ellipses. An ellipse is described by an equation made up of many components called harmonics whose coefficients may be used as shape descriptors (see BIRD et al. 1986 for details). These coefficients are: A_n, the semi-major axis of an ellipse; B_n, the semi-minor axis; Φ_n, the orientation of the major axis with respect to the major axis A_1 of the first ellipse, θ_n, the dephasing angle, and n the number of harmonics.

The minimum number of harmonics to accurately describe the shape of the otolith was determined by computing the Fourier coefficients of 7 otoliths with 4, 8, 16, 24 and 32 harmonics. The curve fitted between the residuals (i.e. the differences between the model and the actual otolith) and the number of harmonics (results not shown), showed by interpolation that with 13 harmonics, the shape of the otolith was described very accurately.

Statistical methods

Genetic data

Observed heterozygosity (H_0), average unbiased heterozygosity (H_{nb}) (NEI 1978) based on the Hardy-Weinberg expectation and percentage of polymorphic loci ($P > 95\,\%$) were computed for Rhône, Bouic, Fumemorte and Vaccarès samples. WEIR & COCKERHAM (1984) method was used to estimate Wright's F-statistics (WRIGHT 1951). Hardy-Weinberg equilibrium conformance was tested using F_{is} which is a measure of devia-

tion from panmixia due to a heterozygotes deficiency: at Hardy-Weinberg equilibrium, $F_{is} = 0$. F_{st} is a measure of population divergence: when $F_{st} = 0$, populations are not genetically differentiated. Tests of significance of F_{is} and F_{st} were carried out by permutation. In the case of F_{is}, 1000 random permutations between loci within a population were performed from the original matrix in order to simulate panmixia. In the case of F_{st}, 1000 random permutations between populations were performed from the original matrix. In both cases, the relative frequencies of the estimation which were equal to or greater than the true estimation of F gave the relevant one-tailed p-value. All treatments were performed using the computer package GENETIX 4.02 (BELKHIR 1997) available at http://www.univ-montp2.fr/~genetix/genetix.htm.

The number of migrant individuals arriving in the population per generation can be estimated using the formula given by WRIGHT (1969): $Nm = (1\text{-}F_{st})/(4.F_{st})$ where N is the effective population size and m is the rate of gene flow per generation. This formula can be employed if $m << 1$ and if the subpopulations are at equilibrium with respect to the effect of genetic drift and migration (i. e. Hardy-Weinberg equilibrium).

Morphometric, meristic and otolith shape data

Discriminant analysis was performed (using the Mahalanobis metric) to assess if there were phenotypic (morphometric, meristic and otolith shape) differences between pikeperch from the Fumemorte Canal and those from the Bouic Canal. As the Vaccarès sample size was too small to be used in the analysis, individuals from this origin were used as supplementary rows to investigate their membership to one of the two sites. The discriminant analysis using the Mahalanobis metric tends to maximise the variance between the classes by taking into account the ratio of between-class variance to within-class variance. As the discriminant analysis requires a reduced set of characters, a stepwise procedure was used to reduce the number of variables.

Significance was tested by a permutation test: 1000 random permutations between all the individuals from all the populations were performed and the discriminant analysis inertia was calculated for each case. The p value is given by the frequency of simulated inertia greater than or equal to the observed inertia.

A cross-validation test was performed to assess the ability of the phenotypes to discriminate between the populations. In cross-validation, one individual is removed from the original matrix and discriminant analysis is performed from the remaining observations and is then used to classify the omitted individual.

Since the otolith shape may vary between age classes, e. g. CASTONGUAY et al. (1991), and/or between sexes (CAMPANA & CASSELMAN 1993), we also tested whether the differences between the samples were due to sexual dimorphism or to an age effect by performing discriminant analyses using the variables retained by the first stepwise discriminant analysis on the population criteria. The same tests were also performed for the meristic counts and for the morphometric distances.

Relationships between morphometric distances were linearised by a logarithm transformation according to the general formula for allometry (HUXLEY 1932). Then, in order to remove the body size differences between samples, the residuals from the regression of the log transformed morphometric variables against the log transformed

centroid (i.e. the sum of the squared morphometric distances) were calculated (Ehlinger 1991, Robinson et al. 1993, Robinson & Wilson 1996, Brinsmead & Fox 2002). The centroid was highly correlated ($r^2 = 0.998$, $p < 0.001$) to the first principal component of the PCA which is also considered as a size axis (Jolicoeur 1963). The residuals were used as new size-free morphometric variables since none of them was correlated to the centroid ($p > 0.05$).

As the meristic characters are fixed early during ontogeny and remain stable throughout life (Barlow 1961), no size correction was necessary.

The Fourier coefficients are size dependent, so A_n and B_n were divided by the amplitude A1 (De Pontual & Prouzet 1988).

The features used to describe otolith shape were:

$$A_2/A_1, \ldots, A_n/A_1; B_1/A_1, \ldots, B_n/A_1; \Phi_2, \ldots, \Phi_n; \theta_2 \ldots, \theta_n.$$

It is difficult to relate the shape quantification to some fundamental properties of the analysed otoliths since the physical meaning of Fourier coefficients remains unclear (De Pontual & Prouzet 1988). Nevertheless, if the aim is to find descriptors whose variations are sufficient to distinguish between given groups, this limitation is not a real problem (Rohlf & Ferson, 1983).

All statistical tests were performed with Ade-4.0 software for PC (Thioulouse et al. 1997) (free download at: http://pbil.univ-lyon1.fr/ADE-4/ADE-4.html) and Spss 11.0 (SPSS Inc 1999).

Results

Genetic variation and population structure

Among the 24 loci tested 18 had a sufficient resolution to be used (Table 3). Among them, *ADH*, G3PDH*, MDH-1*, MDH-2*, ME*, IDHP-1*, IDHP-2*, AAT-1*, CK-1*, EST-1*, GPI-B1*, GPI-B2*, PROT-1*, PROT-2* and PROT-3** were monomorphic. Only *LDH-C1*, PGM-1*,* and *MPI-2** revealed a polymorphism.

Allelic frequencies were computed for each sample but results for Rhône and Vaccarès samples ($n = 4$ and $n = 5$, respectively) have to be interpreted with care because of the small number of individuals (Table 5). *LDH* exhibited two bands which could be interpreted as one or two loci. If we consider that two loci occur, because some individuals displayed a strong spot on one of the bands whereas the other spot was light or even absent we should also consider that both loci share the same two alleles. This hypothesis is difficult to maintain. It is therefore clear that we cannot reach the exact genotypes and a phenetic interpretation was used noting: A = one spot above; B = a spot above and a lighter one below; C = two equal spots; D = one spot below and one lighter above; E = one spot below (Fig. 4). Only one case of pattern A and one case of E were recorded. Therefore, they were attributed to their nearest pattern for the

Table 5. Allele frequencies in the polymorphic loci. P_{95} is the proportion of polymorphic loci by the 0.95 criterion (a locus is considered polymorphic if the most frequent allele does not exceed 95 %). H_o: observed heterozygosity, H_{nb}: unbiased heterozygosity (Nei, 1978) (± standard error), F_{is}: see text for details, NS $p > 0.05$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. As *LDH-C1** was interpreted using patterns (see text and Fig. 4), it was not taken into account to calculate H_o, H_{nb} and F_{is} (see text for details).

Locus	Rhône	Bouic	Fumemorte	Vaccarès
*LDH-C1**	24	31	30	5
A	0.00	0.00	0.03	0.00
B	0.17	0.16	0.40	0.00
C	0.42	0.26	0.37	0.00
D	0.42	0.55	0.20	1.00
E	0.00	0.03	0.00	0.00
*PGM-1**	4	36	30	5
100	1.00	0.75	0.68	0.9
120	0.00	0.25	0.32	0.1
*MPI-2**	2	34	28	5
100	0.25	0.87	0.68	0.8
120	0.75	0.13	0.32	0.2
P_{95}	0.12	0.17	0.17	0.19
H_o	0.031 (± 0.12)	0.035 (± 0.12)	0.042 (± 0.12)	0.013 (± 0.05)
H_{nb}	0.035 (± 0.14)	0.036 (± 0.10)	0.052 (± 0.15)	0.037 (± 0.10)
F_{is}	0.143	0.042	0.189*	0.670***

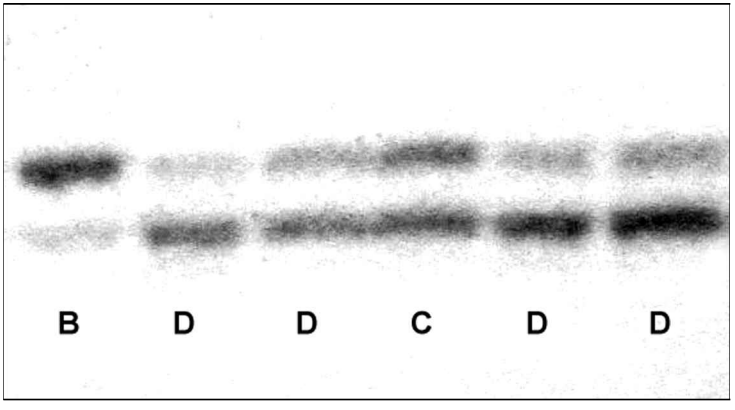

Fig. 4. Examples of *LDH-C1** patterns.

statistical analysis (A became B and E became D). Consequently, the genetic structure was interpretable for Bouic and Fumemorte samples with *PGM-1**, *PI-2** and *LDH-C1** and for the Rhone population with *LDH-C1** only. Whatever the locus considered, the Vaccarès sample displayed too few individuals to make reliable interpretations.

Table 6. Pairwise comparisons of each Fumemorte basin compartment for *PGM-1** and *MPI-2** using permutation tests on F_{st} (see text for details) and for *LDH-C1** patterns using Chi^2 tests. F_{st} values are noted above the diagonal and Chi^2 values below.

	Rhône	Bouic	Fumemorte	Vaccarès
Rhône	–	0.20*	0.06 (NS)	0.00 (NS)
Bouic	1.76 (NS)	–	0.04*	−0.03 (NS)
Fumemorte	5.21 (NS)	10.01**	–	0.00 (NS)
Vaccarès	5.64 (NS)	3.28 (NS)	12.7**	–

NS: non significant, $p > 0.05$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Observed heterozygosity using the same loci for the four sites ranged between 0.013 ± 0.05 (Vaccarès) and 0.042 ± 0.12 (Fumemorte). The range of the unbiased heterozygosity was smaller: from 0.035 ± 0.14 (Rhône) to 0.052 ± 0.15 (Fumemorte). For the Fumemorte and the Vaccarès, observed heterozygosity was lower than expected, tending to indicate a departure from Hardy-Weinberg expectations. This was confirmed by significant F_{is} which indicated a departure from panmixia (Fumemorte: $F_{is} = 0.189$, permutation test 1000 permutations, $p < 0.05$ and Vaccarès: $F_{is} = 0.670$, permutation test, 1000 permutations, $p < 0.001$). The same was observed when F_{is} was calculated for the four samples pooled ($F_{is} = 0.15$, permutation test, 1000 permutations, $p < 0.05$). Consequently, the number of migrants (Nm) between the Fumemorte and the Bouic Canal cannot be estimated using Wright's formula.

The only significant F_{st} differences were observed between the Bouic and the Rhône ($F_{st} = 0.20$, permutation test, 1000 permutations, $p < 0.05$) and the Fumemorte and the Bouic ($F_{st} = 0.04$, permutation test, 1000 permutations, $p < 0.05$). Concerning the *LDH-C1** phenotype, in order to assess whether the difference in distribution was due to ontogeny, we tested the pattern distribution between populations with the length of fish (log-transformed) as a covariable. For this purpose, we used a multinomial regression which showed that both the length ($Chi^2 = 13.3$, df = 2, $p < 0.01$) and the population ($Chi^2 = 21.3$, df = 6, $p < 0.01$) significantly explained the *LDH-C1** pattern. Even though ontogeny influenced the *LDH-C1** pattern, the populations displayed significant differences. On that basis, using Chi^2 test, we tested the differences between samples (Table 6): Fumemorte and Bouic samples displayed significant differences in *LDH-C1** frequencies and so did Fumemorte and Vaccarès. The Rhône sample did not show any significant difference with the Bouic sample or with the Fumemorte sample.

Nevertheless, concerning the results about the Rhône and Vaccarès populations, the samples were too small to make reliable interpretations (Table 5).

Phenotypic variations

Meristics

The stepwise procedure did not retain any variables. The permutation test performed with all the variables showed no significant differences between sites, sexes or ages.

Landmark method

The stepwise analysis retained 6 morphometric variables (Table 7; Fig. 5) that significantly discriminated the Bouic sample from the Fumemorte sample (permutations test, $p < 0.001$). Pikeperch from the Fumemorte canal displayed a higher body and head depth with a higher tail peduncle while pikeperch from the Bouic canal exhibit a rather elongated head and longer pelvic fins. Using

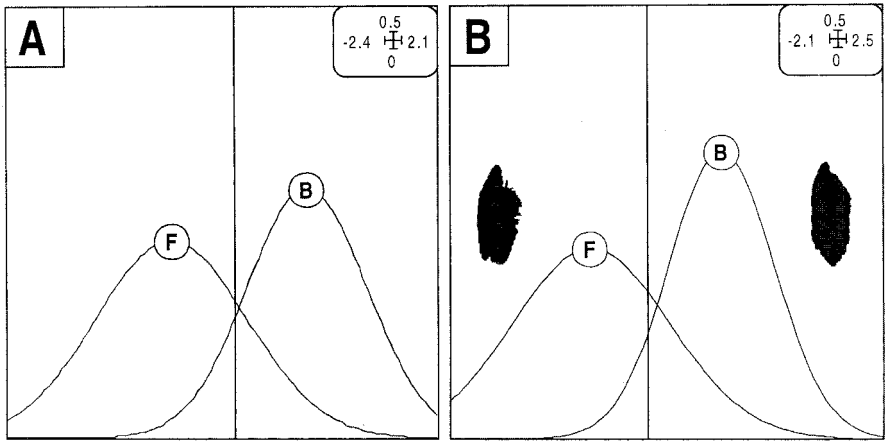

Fig. 5. Canonical scores of individuals from discriminant analysis of the landmark method (**A**) and the otolith shape method (**B**) fitted according to Gauss curves with the silhouette of the otholiths. F: Fumemorte individuals and B: Bouic individuals.

Table 7. Standardised canonical scores of the discriminant analysis on the landmark method. The morphological variables are given according to their landmarks (see Fig. 3).

Morphometric variable	Canonical scores
2– 5	0.769
5– 6	–1.316
5–12	1.319
14–17	–0.422
16–21	–0.334
12–13	0.760

Table 8. Discriminant analysis of the landmark method and the otolith shape method. For both methods, the table presents the number of individuals (and the %) classified in each group (irrigation and drainage canals) from the original matrix and from the cross validation procedure (see text for details).

Method	Matrix	Observed group	Predicted group membership		Total
			Bouic	Fumemorte	
Landmarks	Original	Bouic	33 (80.5 %)	8 (19.5 %)	41
		Fumemorte	8 (21.1 %)	30 (78.9 %)	38
	Cross validation	Bouic	33 (80.5 %)	8 (19.5 %)	41
		Fumemorte	11 (28.9 %)	27 (71.1 %)	38
Otolith shape	Original	Bouic	29 (90.6 %)	3 (9.4 %)	32
		Fumemorte	9 (20.5 %)	35 (79.5 %)	44
	Cross validation	Bouic	29 (90.6 %)	3 (9.4 %)	32
		Fumemorte	9 (20.5 %)	35 (79.5 %)	44

these variables, 75.9 % fish were classified into their correct sample (cross-validation method). Misclassification was higher for the Fumemorte sample (28.9 %) than for the Bouic sample (19.5 %) (Table 8). These variables did not allow discrimination between sex (permutation test, $p = 0.37$) or age classes (permutation test, $p = 0.48$). Using this model, 4 out of the 5 supplementary individuals from the Vaccarès Lagoon were assigned to the Fumemorte population.

Otolith shape

The stepwise analysis retained seven variables (Circularity; A5; A12; B12; Φ7; Φ9; Φ13) that significantly discriminated the Bouic from the Fumemorte samples (permutation test, $p = 0.018$) (Fig. 5). Discriminant analysis allowed 84.2 % of the individuals to be correctly classified. Misclassification was higher for the Fumemorte sample (20.0 %) than for the Bouic sample (9.4 %) (Table 8). No difference in the otolith shape was noted between sexes using the seven variables (permutation test, $p = 0.87$) nor between age classes (permutation test, $p = 0.11$). Among the seven supplementary individuals from the Vaccarès Lagoon, 5 were assigned to the Fumemorte population.

Discussion

Sample size

The formerly abundant pikeperch population in the Fumemorte Canal is nowadays reduced: in 2001, only 28 adult pikeperch were captured during four days

of sampling per month with fyke nets and two days with gill nets. Using the same protocol (except gill nets), 12 adults were caught in the Bouic Canal in 2001. In the Vaccarès Lagoon, no adults were captured in 2001, probably due to too high salinity (10 to 14 g/L).

Thus the capture of a large number of individuals was quite difficult, first because it would have required a very great sampling effort and second because it would threaten the pikeperch populations.

Genetic variation in the populations

Few genetic studies of pikeperch have been reported and most of them concerned small samples for phylogenetic purposes (BILLINGTON et al. 1990, FABER & STEPIEN 1997, BILLINGTON 1998, FABER & STEPIEN 1998, NESBO et al. 1998). Thus, the study analysing the genetic structure of a pikeperch population in the Baltic Sea and in a lagoon (PAULAUSKA & LOZYS 2001) is the only one that could be taken as an informative reference.

Allozyme polymorphism in our samples was low, unlike the finding of PAULAUSKA & LOZYS (2001) (4 polymorphic loci out of 7). BILLINGTON et al. (1990) also observed a low polymorphism for pikeperch but their sample was small (2 individuals). As we have screened a fair number of loci (i.e. 17 over 24 tested), the low number of polymorphic loci can be due to a characteristic of the species when allozymes are used, as observed for the other percid species like the saugere (*Sander canadensis*) (BILLINGTON et al. 1990), the yellow perch (*Perca flavescens*) (TODD & HATCHER 1993) and the European perch (*Perca fluviatilis*) (GYLLENSTEN et al. 1985, HELDSTAB & KATOH 1995). It could be also due to a founding effect: the low diversity and/or the small number of fish from which the Camargue population issued.

In our study the mean heterozygosity for the pooled samples was H_{nb} = 0.043 which is in the same range as that of other freshwater fish (H_e = 0.046, WARD et al. 1994). But it remains much lower than the heterozygosity found by PAULAUSKA & LOZYS (2001) in the Curonian Lagoon and in the Baltic Sea (H_{nb} = 0.342 and 0.136, respectively, recalculated from the allelic frequencies). However, the polymorphic loci found by PAULAUSKA & LOZYS (2001) were esterases which are quite difficult to interpret (BERREBI et al. 1990). So any comparison with our results should be done with caution since the number of loci they tested was lower and the electrophoresis method (polyacrylamide gel) was not the same. We cannot conclude as to the genetic variability of the pikeperch population in the Camargue since there have been too few population genetic studies of this species.

The genetic distance between the Bouic and the Fumemorte samples was small but significant, suggesting a recent segregation of the populations. This is congruent with the results of the *LDH-C1** pattern comparison that emphasi-

ses a significant difference between the irrigation and the drainage pikeperch populations. The *LDH-C1** pattern showed that the Rhône sample was significantly different neither from the Bouic nor from the Fumemorte sample. But, in the case of the Fumemorte sample, the p value was near the significance threshold ($p = 0.07$) whereas in the Bouic sample it was quite far ($p = 0.41$). So we can presume that the Rhône population is genetically closer to the irrigation population than to the drainage one.

The Fumemorte population showed a significant deficiency in heterozygotes which suggests a high inbreeding level, recent immigration, or a selection against some heterozygote genotypes, possibly due to an ecological change. This was not the case for the Bouic sample and considering the *LDH-C1** pattern, it suggests that this population has a continuous input from the Rhône population, which prevents inbreeding.

Phenotypic variation in the populations

The body morphology and the otolith shape significantly discriminated between the Bouic and Fumemorte samples, which is congruent with the genetic results. This difference is not due to age classes nor to a sexual dimorphism, which is consistent with a previous study (Goubier 1975). In addition, meristic characters are less efficient discriminators than morphometric ones (Meng & Stocker 1984, Waldman et al. 1997, Hurlbut & Clay 1998).

In his biometric study of different French pikeperch populations (including that of the Vaccarès Lagoon), Goubier (1975) concluded as to the steadiness of the pikeperch morphometric features. On the other hand, Krpo Cetkovic & Stamenkovic (1996) discriminated among four pikeperch populations in the Danube using morphometric features. The absence of significant results in Goubier (1975) may be due to the statistical method employed: he compared the correlation coefficient of the linear relationship between each non log-transformed feature and the total length (or the head length) without performing any statistical tests between populations.

Using otolith shape or morphometric features, most of the pikeperch from the Vaccarès Lagoon were assigned to the Fumemorte population. This supports the hypothesis that pikeperch from the Vaccarès Lagoon come from the Fumemorte Canal and that there is a real phenotypic difference between the irrigation compartment (Bouic) and the drainage compartment (Fumemorte and Vaccarès). However, the Vaccarès sample is too small to definitely prove this. Unfortunately, we failed to catch a sufficient number of adults from the Rhône River to have a phenotypic proof for the Rhône origin of the Bouic population as the biochemical marker *LDH-C1** tends to indicate.

Causes of the genetic and phenotypic variations

The genetic and phenotypic results tend to show an isolation of the pikeperch population inhabiting the Fumemorte Canal.

This could be explained by the recent history of the pikeperch population. This species appeared in France in 1912 in the canal between the Rhône and the Rhine (GOUBIER 1972). However, it remains unclear whether pikeperch came naturally from lake Constance or were introduced by man, both hypotheses being probably true (GOUBIER 1972). In the 1930 s the pikeperch went down the Rhône River and then, in 1948–1949 it naturally colonised the brackish water (< 8 g/L) of the Vaccarès Lagoon in the Camargue where it found suitable conditions to reproduce. Furthermore, after a sharp decrease of the numbers in 1960, the lagoon was restocked with fry in 1962, 1963 and 1964 but without success (KIENER 1968). In the autumn of 1981 after a severe drought, the salinity rose to 15 g/L in the Vaccarès Lagoon, forcing the pikeperch population to take refuge in the Fumemorte Canal. The salinity reached 35 g/L in 1984. At the beginning of the 1990 s the population in the canal had almost collapsed due to the pressure of the numerous anglers and to lack of recruitment (unpublished data). During the winters of 1990 and 1991, a total of 1049 young of the year from a hatchery were marked (oxytetracycline and clipping) and stocked in the Fumemorte Canal (GAAMOUR 1993). Only 43 were recaptured and since then no more stocking occurred. From 1993 to 2001, pikeperch numbers grew and then slightly decreased in 2002.

Thus for more than 50 years a pikeperch population has been present in the Vaccarès Lagoon and in the Fumemorte Canal where environmental conditions and population dynamics were probably very different from those found in the Rhône River. Indeed the fluctuating environmental conditions and the size of the population in the Vaccarès-Fumemorte system made the population dynamics more chaotic than in the Rhône River. So the Vaccarès-Fumemorte population could have genetically diverged from the Rhône population through both demographic processes (genetic drift) and selective processes (natural selection). This divergence has probably been enforced by the stocking events. Even though no reliable conclusions may be drawn concerning the Rhône and the Vaccarès data due to the small size of these samples, this interpretation is confirmed by i) the significant F_{is} (0.15) found for the Camargue population (i.e. the four samples pooled) that tends to indicate a structuring of the Rhône delta population, ii) the significant F_{st} (0.04) between the Fumemorte and Bouic populations and iii) the significant differences in *LDH-C1** patterns between the Fumemorte and Bouic populations but not between the Rhône and Bouic populations. So no or very little migration occurs between the Bouic and the Fumemorte Canals but as no significant differences were observed in *LDH-C1** patterns, migration may exist between the Rhône River and the Bouic Canal through the pumping stations.

The causes of morphological differences between populations are often quite difficult to explain (CADRIN 2000). Phenotype is under the double control of environmental conditions and genotype, e. g. HARD et al. (1999), but morphological changes can be rapid when new different environmental conditions occur (KLEPAKER 1993). The Bouic Canal undergoes large water level fluctuations and great parts of it remain dry during most of the agriculture growing season. Furthermore, prey density is much lower than in the Fumemorte Canal (unpublished data) that is inhabited by a large sandsmelt (*Atherina boyeri* R.) population (ROSECCHI & CRIVELLI 1995). Thus, Fumemorte is more suitable for pikeperch growth than the Bouic Canal, which is congruent with the results of FORGEOIS (2002). The growth rate is also often involved in the variation of otolith shape (CAMPANA & CASSELMAN 1993). Nevertheless, whatever the determinism of the phenotypic variation, the biometric results confirm the genetic results supporting the isolation of the Fumemorte population.

Isolation of the Camargue and consequence on the population dynamics

The isolation of the pikeperch population inhabiting the Fumemorte Canal would mean that the number of migrants from the Bouic Canal is quite low or null. Nevertheless, passage between the two canals remains possible since CRIVELLI & POIZAT (2001) describe the downstream migration of 0+ shad (*Alosa fallax rhodanensis* R.) from the Rhône to the Vaccarès Lagoon via the pumping stations, the Bouic and the Fumemorte Canals. The shad may use the marshes to pass from the irrigation to the drainage system although, as for pikeperch, no 0+ shad were captured in the marshes (POIZAT & CRIVELLI 1997). The presence of 0+ shad in the drainage canal can be explained by the migratory behaviour of the shad (an anadromous species). Furthermore, the number of 0+ pikeperch pumped from the Rhône is lower than the number of 0+ shad (POIZAT et al. 1999). Typical riverine species like nase (*Chondrostoma nasus* L.), chub (*Leuciscus cephalus* L.) or common barbel (*Barbus barbus* L.) are also pumped from the Rhône river in greater numbers than shad (POIZAT et al. 1999), whereas few are captured in the drainage canal in which they do not reproduce (unpublished data). So it seems that the degree of isolation of a population depends on species behaviour. This is congruent with the results of TIBBETS & DOWLING (1996) showing that the degree of isolation between populations is affected by the intrinsic characteristics such as dispersal capabilities and the reproductive behaviour of the species.

If fish may travel from the Rhône to the Vaccarès Lagoon, presumably they cannot go back. If a species is reproductively successful in both compartments (i. e. Rhône and Camargue), with a limited number of one-way migrants per

generation, this generates a particular case of metapopulation: both subpopulations are spatially separated but linked unidirectionally. This could be the case for the pikeperch if the salinity in the Vaccarès Lagoon decreased to 5 g/L. There, it would find optimal conditions to reproduce. If the subpopulation receiving migrants has a low or a null reproduction, the case is a source-sink model. This could hold for the pikeperch because recruitment in the drainage canal (and in the irrigation canal also) can fluctuate greatly (unpublished data). Thus, source-sink and metapopulation processes probably occur together or alternate.

Whatever the actual situation, pikeperch conservation in the Fumemorte Canal seems to mainly depend on the recruitment of this population rather than on the migrants from the Rhône River (via the Bouic Canal). The drainage canal is submitted to great human pressure that can be very damageable for several aquatic species. For instance every year, a large quantity of pesticide sprayed on rice fields enters the Fumemorte Canal. Residents said that in the past, accidents with algicides caused massive fish mortality. If the Vaccarès Lagoon remains unsafe for pikeperch, the limited number of migrants from the Rhône may not be sufficient to maintain the pikeperch population after a pesticide accident. It may need a long time for the Rhône population to re-colonise the Camargue.

We showed that the genetic variation observed in the Camargue population can just as well involve demographic as selective processes. It would be interesting to test whether microsatellites (Wirth et al. 1999) which are known to give a closer estimation of heterozygosity at large-scale levels than allozymic markers (Neff & Gross 2001) are able to give such a close estimation at a more restricted scale. Moreover, investigations with microsatellites which provide powerful inter-individual discrimination should allow to quantify the number of pikeperch (Nm) passing from the Rhône River into the Camargue, its diked delta, and to investigate more precisely the demographic versus selective hypotheses proposed in the present paper.

Acknowledgements

Thanks to Pascal Contournet and Gilles Segura (Ichtyosys Environnement) for their technical support, to Jean-Michel Roger for his help in statistical analysis, to Anne-Gaëlle Manh for her assistance with the image analysis, to Ghislaine Cattaneo-Berrebi for her help in protein electrophoresis, to Patricia Balaresque for her valuable comments, to two anonymous referees and to Prof. Eric Pattée for his patient help as co-editor.

References

Allendorf, F. W. & Phelps, S. R. (1981): Use of allelic frequencies to describe population structure. – Can. J. Fish. Aquat. Sci. **38:** 1507–1514.

Barlow, G. W. (1961): Causes and significance of morphological variation in fishes. – Syst. Zool. **10:** 105–117.

Begg, G. A. & Brown, R. W. (2000): Stock identification of haddock *Melanogrammus aeglefinus* on Georges bank based on otolith shape analysis. – Trans. Amer. Fish. Soc. **129:** 935–945.

Begg, G. A. & Waldman, J. R. (1999): An holistic approach to fish stock identification. – Fish. Res. **43:** 35–44.

Belkhir, K. (1997): GENETIX 4.0, Windows^{TM} software for population genetics. – CNRS UPR 9060, Université de Montpellier II, Laboratoire Génome et Population. Montpellier.

Berrebi, P., Landaud, P., Borsa, P. & Renno, J. F. (1990): The esterases of the flounder (*Platichthys flesus*, Pleuronectidae, Teleostean): development of an identification protocol using starch gel electrophoresis and characterization of loci. – Experientia **46:** 863–867.

Berrebi, P., Povz, M., Jesensek, D., Cattanéo-Berrebi, G. & Crivelli, A. J. (2000): The genetic diversity of native, stocked and hybrid populations of marble trout in the Soca river, Slovenia. – Heredity **85:** 277–287.

Billington, N. (1998): Genetic variation in percids determined by mitochondrial DNA analysis. – Ital. J. Zool. **65:** 35–40.

Billington, N., Hebert, P. D. N. & Ward, R. D. (1990): Allozyme and mitochondrial DNA variation among three species of *Stizostedion* (Percidae): phylogenetic and zoogeographical implications. – Can. J. Fish. Aquat. Sci. **47:** 1093–1102.

Bird, J. L., Eppler, D. T., Bird, J. L. & Checkley, D. M. (1986): Comparison of herring otoliths using Fourier series shape analysis. – Can. J. Fish. Aquat. Sci. **43:** 1228–1234.

Brinsmead, J. & Fox, M. G. (2002): Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. – J. Fish. Biol. **61:** 1619–1638.

Brown, J. A., Moore, W. M. & Quabius, E. S. (2001): Physiological effects of saline waters on zander. – J. Fish. Biol. **59:** 1544–1555.

Cadrin, S. X. (2000): Advances in morphometric identification of fishery stocks. – Rev. Fish. Biol. and Fish. **10:** 91–112.

Campana, S. E. & Casselman, J. M. (1993): Stock discrimination using otolith shape analysis. – Can. J. Fish. Aquat. Sci. **50:** 1062–1083.

Castonguay, M., Simard, P. & Gagnon, P. (1991): Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. – Can. J. Fish. Aquat. Sci. **48:** 296–302.

Chauvelon, P. (1998): A wetland managed for agriculture as an interface between the Rhone river and the Vaccares lagoon (Camargue, France): transfers of water and nutrients. – Hydrobiologia **374:** 181–191.

Chauvelon, P. A. S., Poizat, G. & Crivelli, A. J. (1996): Hydro-agricultural management in the Rhône river delta, France: consequences on dissolved and solid fluxes, potential impact on fish populations. – Second International Symposium on Habitat Hydraulics. Quebec. pp. 167–176.

Craciun, V., Craciun, M., Neacsu, I. & Trandafirescu, I. (1982): Activité de la pompe de Na+ – K+ chez le sandre au cours de son acclimatation aux conditions de salinité de la Mer Noire. – Cercetari Marine **15:** 227–233.

Crivelli, A. J. & Poizat, G. (2001): Timing of migration and exceptional growth of YOY *Alosa fallax rhodanensis* (Roule, 1924) in a lagoon in southern France. – Bull. Fr. Pêche et Piscic. 761–772.

Deelder, C. & Willemsen, J. (1964): Synopsis of biological data on pike-perch *Lucioperca lucioperca* (Linnaeus) 1758. – FAO Fisheries Synopsis. Rome. pp. 52.

De Pontual, H. & Prouzet, P. (1988): Numerical analysis of scale morphology to discriminate between Atlantic salmon stocks. – Aquat. Living Resour. **1:** 17–27.

Dynesius, M. & Nilsson, C. (1994): Fragmentation and flow regulation of river systems in the northern third of the world. – Science **266:** 753–762.

Ehlinger, T. J. (1991): Allometry and analysis of morphometric variation in the bluegill, *Lepomis macrochirus*. – Copeia **2:** 347–357.

Faber, J. E. & Stepien, C. A. (1997): The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae. – In: Kocher, T. D. & Stepien, C. A. (eds): Molecular Systematics of Fishes. – Academic Press, San Diego, pp. 129–143.

– – (1998): Tandemly repeated sequences in the mitochondrial DNA control region and phylogeography of the pike-perches *Stizostedion*. – Mol. Phyl. Evol. **10:** 310–322.

Forgeois, S. (2002): Traits de vie du sandre (*Stizostedion lucioperca*, L.) en Camargue. – Mémoire de Maîtrise, Université François Rabelais, Tours. 56 pp.

Friedland, K. D. & Reddin, D. G. (1994): Use of otolith morphology in stock discriminations of Atlantic salmon (*Salmo salar*). – Can. J. Fish. Aquat. Sci. **51:** 91–98.

Gaamour, A. (1993): Validation de l'estimation de l'âge du sandre (*Stizostedion lucioperca* L.) en Camargue. – Mémoire de DEA, Université de Bretagne Occidentale, 30 pp.

Goubier, J. (1972): Acclimatation du Sandre (*Lucioperca lucioperca* L.) dans les eaux françaises. – Verh. Internat. Verein. Limnol. **18:** 1147–1154.

– (1975): Biogéographie, biométrie et biologie du Sandre, *Stizostedion lucioperca* (L.), Osteichthyen, Percidé. – State thesis, University Lyon-I, 259 pp.

Gyllenstен, U., Ryman, N. & Gunnar, S. (1985): Monomorphism in allozymes in perch (*Perca fluviatilis* L.). – Hereditas **102:** 57–61.

Hard, J. J., Winans, G. A. & Richardson, J. C. (1999): Phenotypic and genetic architecture of juvenile morphometry in Chinook salmon. – J. Hered. **90:** 597–606.

Heldstab, H. & Katoh, M. (1995): Low genetic variation in perch (*Perca fluviatilis* L) from three major European drainage systems in Switzerland. – Aquat. Sci. **57:** 14–19.

Heurteaux, P. (1992): Modifications du régime hydrique et salin des étangs du système Vaccarès (Camargue, France) liées aux perturbations anthropiques des cinquantes dernières années. – Ann. Limnol. **28:** 157–174.

Holcik, J., Banarescu, P. & Diana, E. (1989): General introduction to fishes. – In: Holcik, J. (eds): The freshwater fishes of Europe – General introduction to fishes – Acipenseriformes. – AULA Verlag, Wiesbaden, pp. 19–59.

Hurlbut, T. & Clay, D. (1998): Morphometric and meristic differences between shallow- and deep-water populations of white hake (*Urophycis tenuis*) in the southern Gulf of St. Lawrence. – Can. J. Fish. Aquat. Sci. **55:** 2274–2282.

Huxley, J. S. (1932): Problems of relative growth. – Methuen & Co, London, 360 pp.

Jolicoeur, P. (1963): The multivariate generalization of the allometry equation. – Biometrics **19:** 497–499.

Kiener, A. (1968): L'évolution des populations de sandre dans deux étangs méditerranéens. – La Terre et la Vie **22:** 470–491.

Klepaker, T. (1993): Morphological changes in a marine population of threespine stickleback, *Gasterosteus aculeatus*, recently isolated in fresh water. – Can. J. Zool. **71:** 1251–1258.

Krpo Cetkovic, J. & Stamenkovic, S. (1996): Morphological differentiation of the pikeperch *Stizostedion lucioperca* (L.) populations from the Yugoslav part of the Danube. – Ann. Zool. Fenn. **33:** 711–723.

Laroche, J., Durand, J. D., Bouvet, Y., Guinand, B. & Brohon, B. (1999): Genetic structure and differentiation among populations of two cyprinids, *Leuciscus cephalus* and *Rutilus rutilus*, in a large European river. – Can. J. Fish. Aquat. Sci. **56:** 1659–1667.

Manh, A. G. (2001): Modèles déformables pour la reconnaissance d'adventices. – Thèse de doctorat, Université des Sciences et Techniques du Languedoc, Montpellier. 194 pp.

Marshall, T. R. (1977): Morphological, physiological, and ethological differences between walleye (*Stizostedion vitreum vitreum*) and pikeperch (*S. lucioperca*). – J. Fish. Res. Board Can. **34:** 1515–1523.

Meng, H. J. & Stocker, M. (1984): An evaluation of morphometrics and meristics for stock separation of Pacific herring (*Clupea harengus pallasi*). – Can. J. Fish. Aquat. Sci. **41:** 414–422.

Neff, B. D. & Gross, M. R. (2001): Microsatellite evolution in vertebrates: Inference from AC dinucleotide repeats. – Evolution 55: 1717–1733.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. – Genetics **89:** 583–590.

Neraas, L. P. & Spruell, P. (2001): Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. – Mol. Ecol. **10:** 1153–1164.

Nesbo, C. L., Arab, M. O. & Jakobsen, K. S. (1998): Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (*Perca fluviatilis, Acerina cernua, Stizostedion lucioperca*). – Genetics **148:** 1907–1919.

OPTIMAS (1996): OPTIMAS 6.0. User guide and technical reference, 8 th edition. – Media Cybernetics, Silver Spring, Maryland, USA, 630 pp.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. (1987): Manuel technique de génétique par électrophorèse des protéines. – Lavoisier, Paris, 232 pp.

Paulauska, A. & Lozys, L. (2001): Isoenzyme analysis of percids (*Stizostedion lucioperca, Perca fluviatilis, Gymnocephalus cernua*) in Lithuanian coastal zone of the Balic Sea, the Curonian Lagoon and Kaunas Water Reservoir. – Acta Zool. Lituanica **11:** 29–38.

Poizat, G., Chauvelon, P., Rosecchi, E. & Crivelli, A. J. (1999): Passage de poissons du Rhône par les pompes d'irrigation de Camargue: premiers résultats. – Bull. Fr. Pêche et Piscic. **352:** 31–43.

Poizat, G. & Crivelli, A. J. (1997): Use of seasonally flooded marshes by fish in a Mediterranean wetland: timing and demographic consequences. – J. Fish Biol. **51:** 106–119.

Pont, D. (1977): Structure et évolution saisonnière des populations de Copépodes, Cladocères et Ostracodes des rizières de Camargue. – Ann. Limnol. **13:** 15–28.

Robinson, B. W. & Wilson, D. S. (1996): Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). – Evol. Ecol. **10:** 631–652.

Robinson, B. W., Wilson, D. S., Margosian, A. S. & Lotito, P. T. (1993): Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. – Evol. Ecol. **7:** 451–464.

Roche, H. E., Buet, A. & Ramade, F. (2002): Relationships between persistent organic chemical residues and biochemical constituents in fish from a protected area: the French National Nature Reserve of Camargue. – Comp. Biochem. Physiol. B **133:** 393–410.

Rohlf, F. J. & Ferson, S. (1983): Image analysis. – In: Felstetein, J. (eds): Numerical taxonomy. – Springer Verlag, pp. 583–599.

Rosecchi, E. & Crivelli, A. J. (1995): Sand smelt (*Atherina boyeri*) migration within the water system of the Camargue, Southern France. – Hydrobiologia **301:** 289–298.

Schiemer, F. & Spindler, T. (1989): Endangered fish species of the Danube River in Austria. – Regul. Rivers **4:** 397–407.

Schmutz, S. & Jungwirth, M. (1999): Fish as indicators of large river connectivity: the Danube and its tributaries. – Arch. Hydrobiol. Suppl. **115/**Large Rivers **11:** 329–348.

SPSS Inc (1999): SPSS base 9.0 – User's guide. SPSSInc., Chicago, 740 pp.

Strauss, R. E. & Bookstein, F. L. (1982): The truss: body form reconstruction in morphometrics. – Syst. Zool. **31:** 113–135.

Thioulouse, J., Chessel, D., Dolédec, S. & Olivier, J. M. (1997): ADE-4: A multivariate analysis and graphical display software. – Stat. Comput. **7:** 75–83.

Tibbets, C. A. & Dowling, T. E. (1996): Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). – Evolution **50:** 1280–1292.

Todd, T. N. & Hatcher, C. O. (1993): Genetic variability and glacial origins of yellow perch (*Perca flavescens*) in North-America. – Can. J. Fish. Aquat. Sci. **50:** 1828–1834.

Waldman, J. R., Richards, R. A., Schill, W. B., Wirgin, I. & Fabrizio, M. C. (1997): An empirical comparison of stock identification techniques applied to striped bass. – Trans. Amer. Fish. Soc. **126:** 369–385.

Ward, J. V. & Stanford, J. A. (1983): The serial discontinuity concept of lotic ecosystems. – In: Fontaine, T. D. & Bartell, S. M. (eds): Dynamics of Lotic Ecosystems. – Ann Arbor Science, Ann Arbour, Michigan, USA, pp. 494.

– – (1995): The Serial Discontinuity Concept – extending the model to floodplain rivers. – Regul. Rivers **10:** 159–168.

Ward, R. D., Woodwark, M. & Skibinski, D. O. F. (1994): A comparison of genetic diversity levels in marine, fresh-water, and anadromous fishes. – J. Fish Biol. **44:** 213–232.

Weir, B. S. & Cockerham, C. (1984): Estimating *F*-statistics for the analysis of population structure. – Evolution **38:** 1358–1370.

WIRTH, T., SAINT-LAURENT, R. & BERNATCHEZ, L. (1999): Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross-species amplification within the family Percidae. – Mol. Ecol. **8:** 1960–1963.

WRIGHT, S. (1951): The genetical structure of populations. – Ann. Eugenics **15:** 323–354.

– (1969): Evolution and the genetics of populations. – University of Chicago Press, Chicago, USA, 512 pp.

Submitted: 1 August 2003; accepted: 17 December 2003.