Aquat. Sci. 67 (2005) 122–127
1015-1621/05/010122-06
DOI 10.1007/s00027-004-0772-▮

Aquatic Sciences

Research Article

Does fish scale morphology allow the identification of populations at a local scale? A case study for rostrum dace *Leuciscus leuciscus burdigalensis* in River Viaur (SW France)

Nicolas Poulet[1], Yorick Reyjol[2,3,*], Hélène Collier[2] and Sovan Lek[2]

[1] Cemagref, Unité de Recherche RIPE, 361 rue JF Breton, BP 5095, 34033 Montpellier cedex, France
[2] Laboratoire Dynamique de la Biodiversité (LADYBIO), CNRS, Université Paul Sabatier, 118 Route de Narbonne, F 31062 Toulouse cedex 4, France
[3] Present adress: Laboratoire Ecologie des Hydrosystèmes Fluviaux, U.M.R 5023, C.N.R.S, Université Claude Bernard, 43 Bd du 11 Novembre 1918, 69622 Villeubranne, France

Received: 21 July 2004; revised manuscript accepted: 11 October 2004

Abstract. The aim of the study was to investigate if scale morphology could be used to discriminate fish populations at a local scale. To this aim, 168 rostrum dace (*Leuciscus leuciscus burdigalensis*) were collected at 3 sites in the River Viaur (SW France), distributed along 82 Km of stream. Seventy-one measurements were taken from scales: 2 shape factors and 69 Fourier coefficients. A stepwise discriminant analysis revealed that fish from the 3 sites showed morphological differences ($p < 0.001$), with an average of 75.6% correct discrimination of the scales (65.5% after the cross-validation procedure). These results reveal that scale morphology can detect spatial structure in fish populations at a fine scale, which has implications for riverine fish conservation.

Key words. Fourier coefficients; river stretch; population.

Introduction

Identification of local populations and their connectivity between each other is a major point for the conservation and management of vulnerable species (Hanski and Simberloff, 1997). Geographical isolation can result in the development of different morphological features between fish populations because the interactive effects of environment, selection and genetics on individual ontogenies produce morphometric differences within a species (Cadrin, 2000). Thus, phenotypic features like body morphology, meristic counts, otolith or scale shape have been widely used in the identification and discrimination of fish populations (Begg and Brown, 2000; Casselman et al., 1981; Meng and Stocker, 1984; Poulet et al, 2004).

Scale characteristics such as overall shape and internal features have proven successful for population identification for many years, e.g., for walleye *Sander vitreus* (Jarvis et al., 1978), lake whitefish *Coregonus clupeaformis* (Casselman et al., 1981), Atlantic salmon (Pontual and Prouzet, 1987), dace *Leuciscus leuciscus* (Fraisse, 1990) and striped bass *Morone saxatilis* (Richards and Esteves, 1997). Scale morphology was shown to discrimate fish populations at relatively large spatial scales, e.g., within the great lakes (Jarvis et al., 1978; Casselman et al., 1981) or among rivers from different drainage basins (Pontual and Prouzet, 1987; Richards and Esteves, 1997). The smallest spatial scale at which populations were discriminated using phenotypic features was the river basin: Fraisse (1990) compared

---

* Corresponding author phone: +33 4 72 44 62 34;
fax: +33 4 72 43 28 92; e-mail: yorick.reyjol@univ-lyon1.fr

scale morphology of 2 rostrum dace samples originating from 2 French rivers of the same drainage basin; the River Rhône and its tributary, the River Ain.

The objective of this study was to assess the use of scales to discriminate rostrum dace *Leuciscus leuciscus burdigalensis* (Cuvier et Valencienne, 1844) at the spatial scale of the river stretch. Indeed, no study investigated whether scales could be used to individualize fish groups at this spatial scale, despite distinct local populations may exist (Carlsson et al., 1999). The rostrum dace is a subspecies of dace, endemic to South-western France (Spillmann, 1961; Chappaz et al., 1998). Since it was previously classified as 'vulnerable' by Lelek (1987), the use of scale morphology for this species is particularly appealing.

Study area

The River Viaur is located in the Adour-Garonne basin (SW France). This rain-fed stream has its source at an altitude of 1090 m in the piedmont zone of the Massif Central Mountains. Its confluence with the River Aveyron is situated 169 km downstream at an altitude of 150 m. Three sampling sites were selected along the river course. The distance between sites A and B was 30 km, while 52 km separated site B from site C. Moreover, numerous obstacles separated the 3 sampling sites, i.e. 7 weirs (height <2 m) between sites A and B and 9 weirs and 1 high dam (height = 30.7 m) between sites B and C (Fig. 1). The main available environmental features of the sampling sites are provided in Table 1.

Materials and methods

Fish were collected between March and late July 2000 using electrofishing. Scales were always removed from the same place, i.e. the left side of the fish, between the dorsal fin and the lateral line along a hypothetical line between the posterior edge of the dorsal fin and the dorsal edge of the anal fin. Each scale was cleaned with 5% KOH, stained with toluene blue, and mounted between microscope slides. For a clear outline, scales were lighted from below. The image analysis system was composed of a microscope assembled on a camera, itself being coupled to a computer. Magnification depended on the size of the scale: it was adjusted to have the largest picture and, as such, the best precision. A threshold procedure allowed to separate the object (i.e. scale) from the background for subsequent images analyses.

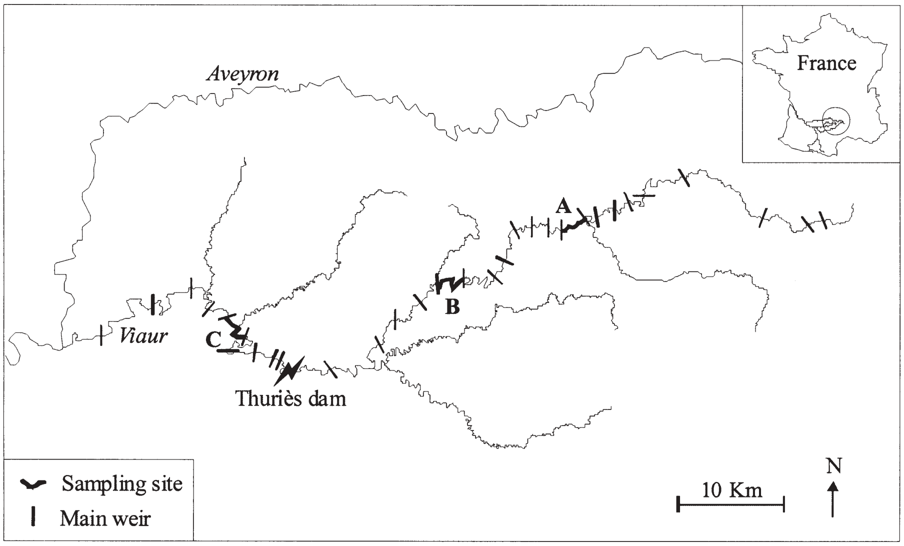

Figure 1. Hydrographical network of the River Viaur and tributaries, with location of the Thuriès dam, main weirs and 3 sampling sites.

Table 1. Characteristics of the main environmental variables of the 3 sampling sites.

Sites	Altitude (m)	Distance from the source (km)	Slope (%)	Width (m)	Mean annual temperature (°C)	Conductivity (µS/cm)
A	581 – 565	51 – 48	0.46	8 – 12	12	135
B	424 – 406	81 – 77	0.43	10 – 25	14	145
C	234 – 228	136 – 130	0.16	15 – 30	15	165

Two types of shape descriptor commonly employed were used: 2 ratio shape descriptors (circularity and rectangularity) and the elliptic Fourier coefficients (see Pontual and Prouzet, 1988; Fraisse, 1990). Circularity is defined as the perimeter of the scale squared and divided by its area, and rectangularity corresponds to the scale area divided by the area of its enclosing rectangle, oriented along the length of the scale. Circularity and rectangularity were computed using Optimas software. The ratio shape descriptors are independent of size and orientation (Pontual and Prouzet, 1988) and, therefore, required no transformation with regard to fish length. This independence was confirmed by a Pearson correlation test that showed no link existed between circularity or rectangularity and the size of the fish (circularity: $R = -0.03$, $p = 0.800$; rectangularity: $R = 0.03$, $p = 0.720$). In the case of elliptic Fourier coefficients, the empiric contour of a planar 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; Phi_n, the orientation of the major axis in respect to the major axis A_1 of the first ellipse, $Teta_n$, the dephasing angle, and n the number of harmonics.

Fourier coefficients being size and orientation (translation and rotation) dependent, the corrections recommended by Pontual and Prouzet (1988) were taken into account. Size effect was removed by dividing A_n and B_n by the amplitude A_1. Orientation effect was avoided by eliminating Phi_1 and $Teta_1$. Finally, (4n-3) normalized parameters were available to describe a contour approximated with n harmonics:

> $A_2/A_1, \ldots, A_n/A_1$; $B_1/A_1, \ldots, B_n/A_1$; $Phi_2, \ldots, Phi_n$; $Teta_2 \ldots, Teta_n$.

The parameters A_n/A_1 and B_n/A_1 will be called A_n and B_n respectively. Any regenerated, highly damaged or asymmetrical scales were discarded. Fourier coefficients were obtained using the software developed by Manh (2001) for Fourier coefficients. A picture of a typical rostrum dace scale, illustrations of the Fourier's adjustment with 1, 4 and 18 harmonics, and pictures of scales characterized by a high value of circularity and rectangularity are shown in Figure 2.

Statistical treatment

A stepwise discriminant analysis using the Mahalanobis metric was performed. This statistical analysis builds a predictive model of group membership based on observed characteristics of each sample (in our case the membership of each scale to sites A, B or C, based on its morphometric characteristics). The first step of this analysis is to select a subset of variables that best fit the observed data (i.e. the stepwise procedure), and then use it in order to compute the discriminant function. Wilk's lambda was used to test the significance of the discrimination.

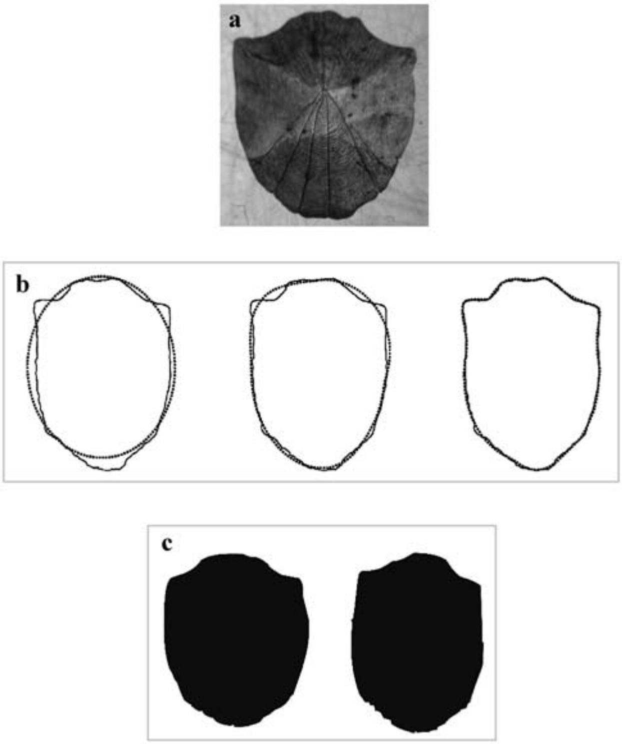

Figure 2. **a.** Picture of a typical rostrum dace scale. **b.** Illustrations of the Fourier's adjustment with an increasing number of harmonics (from left to right). **c.** Pictures of scales characterized by a high value of circularity C (left scale) and rectangularity R (right scale).

A cross-validation testing procedure was performed to assess the ability of the selected variables to predict fish from the 3 sites (i.e., to evaluate the model's quality). In cross-validation, one individual is removed from the original matrix. The discriminant analysis is then performed from the remaining observations and used to classify the omitted individual. The statistical treatment was carried out with SPSS 11.0 for Windows.

Results

A total of 168 rostrum daces was collected from the 3 sampling sites; i.e., 45 individuals from site A (mean size 192 ± 22 mm), 81 from site B (mean size 197 ± 21 mm) and 42 from site C (mean size 165 ± 28 mm).

Eighteen coefficients were found to be the appropriate number of harmonics correctly describing each scale's outline. Consequently, 69 Fourier coefficients and

Table 2. Subset of variables selected by the stepwise discriminant analysis, associated standardized canonical discriminant function coefficients, and within-groups correlations between variables and canonical scores.

	Std Can. Disc. Func. Coef.		Corr. Var.-Can. Scores	
	Function 1	Function 2	Function 1	Function 2
Circularity	0.397	0.143	–0.111	0.177
Rectangularity	–0.367	0.152	–0.046	0.075
A4	0.440	–0.227	–0.177	–0.056
A5	0.249	0.257	–0.078	0.030
A7	–0.455	–0.054	–0.204	–0.149
A8	–0.430	–0.358	–0.160	0.025
A12	0.420	–0.295	–0.159	0.155
A13	0.179	0.378	0.176	0.115
B1	–0.588	0.658	0.204	0.060
B3	–0.319	0.382	0.121	–0.098
Theta10	–0.266	–0.269	–0.200	0.048
Theta11	0.165	0.314	0.125	–0.046
Theta12	0.362	–0.114	–0.013	0.171
Theta14	0.121	0.416	–0.301	–0.018
Theta16	0.404	0.482	–0.006	0.028
Phi4	0.290	–0.112	0.000	0.016

2 shape factors were used for the discriminant analysis. Among these 71 morphometric variables, the best subset selected by the stepwise selection comprised 16 variables (Table 2). From the discriminant analysis of the 168 individuals, 3 populations of rostrum dace were distinguished, belonging to the 3 sampling sites (Wilk's lambda, $p < 0.001$). The first canonical discriminant function of the discriminant analysis explained 71% of the total variance while the second one accounted for 29% of the total variance. The first function opposed individuals from sites A and B from individuals from site C, while the second one discriminated site A individuals from site B individuals (Fig. 3).

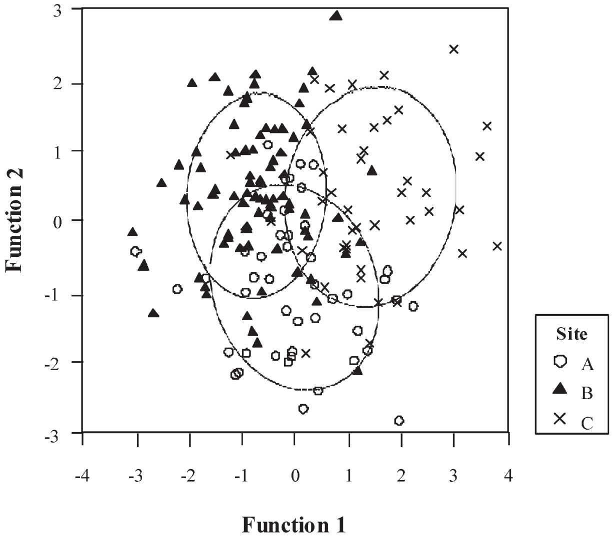

Figure 3. Scatter plot of the canonical scores from the discriminant analysis. An ellipse surrounds 95% of the individuals within a population.

The discriminant analysis correctly classified 127 of the 168 scales (75.6%), while the cross-validation testing procedure correctly classified 65.5% of the scales. For both analyses, site C members were the most correctly classified (83.3% from the original matrix and 76.2% from the cross-validation testing procedure), followed by site B members (75.3% and 67.9%, respectively) and then by site A members (62.2% and 51.1%, respectively). Considering site A, a higher proportion of misclassified individuals was allocated to site B than C (28.9% vs. 8.9% and 35.6% vs. 13.3%, respectively), while for site B a higher proportion was allocated to site A than C (19.8% vs. 4.9% and 22.2% vs. 9.9%, respectively), and for site C a higher proportion was allocated to site A than B (11.9% vs. 4.8% and 16.7% vs. 7.1%, respectively) (Table 3).

Table 3. Classification results for the discriminant analysis (Original) and the cross-validation testing procedure (Cross-validated).

		Site	Predicted group membership			Total
			A	B	C	
Original	Count	A	28	13	4	45
		B	16	61	4	81
		C	5	2	35	42
	%	A	62.2	28.9	8.9	100.0
		B	19.8	75.3	4.9	100.0
		C	11.9	4.8	83.3	100.0
Cross-validated	Count	A	23	16	6	45
		B	18	55	8	81
		C	7	3	32	42
	%	A	51.1	35.6	13.3	100.0
		B	22.2	67.9	9.9	100.0
		C	16.7	7.1	76.2	100.0

Discussion

In the present study, 3 groups of rostrum dace were identified in the stretch of the River Viaur based on the shape of their scales, corresponding to the 3 sampling sites. The level of classification we found (75.6% of scales correctly classified, 65.5% after the cross-validation procedure) was lower than previous studies using a similar methodology. Pontual and Prouzet (1988) found > 99% of the scales correctly classified when investigating group membership of 2 populations of Atlantic salmon from Norway and France. Similarly, Fraisse (1990) found 85% of correctly classified scales when comparing dace populations from 2 rivers of the same drainage basin. The difference in morphological variables used in these 2 studies compared to the present study probably partly explains the differences observed. Pontual and Prouzet (1988) used Fourier coefficients, moments invariant and seven shape factors for scale description, while Fraisse (1990) used the same variables excluding the moments invariant. However, the lower classification efficiency observed is probably inherent to the smaller spatial scale we considered, i.e. a river stretch compared to different rivers in Pontual and Prouzet (1988) and Fraisse (1990), even from the same drainage basin in Fraisse (1990).

The first canonical discriminant function of the discriminant analysis gathered sites A and B close together, indicating that fish from these sites have a more similar scale morphology compared to individuals from site C. This result was probably due to the smaller distance between sites A and B (30 km) compared to that between sites B and C (52 km). Indeed, because scale characteristics are strongly influenced by environmental conditions (Ihssen et al., 1981), the natural gradient in environmental conditions from upstream to downstream (e.g., with regard to water velocity, temperature, chemistry) probably explained most of the differences observed. On the other hand, damming has previously been reported to have important effects on fish genetics, by fragmenting formerly panmictic populations (e.g. Brito and Coelho, 1999; Guinand et al., 1996; Jager et al., 2001; Neraas and Spruell, 2001). Because the River Viaur is a highly-fragmented river (Sites A and B are separated by 7 weirs, while sites B and C are separated by 9 weirs and 1 dam), and rostrum dace needs to migrate for reproduction (Mann and Mills, 1986; Clough et al., 1998), it is possible that the differences observed in the present study originated from a balance between both environmental and genetic effects, or that fragmentation accentuated the differences observed.

Natural rivers are important corridors for the movement of animals through the natural landscape (Forman and Godron, 1986); however, dams act as barriers to the movement of fish and consequently the river's ability to act as a corridor is reduced (Malanson, 1993). Many riverine species have become fragmented due to this obstruction to organism dispersal (Dynesius and Nilsson, 1994). This phenomenon can be deleterious to the persistence of riverine fish populations and therefore biodiversity, especially for smaller populations which will be more easily eliminated than larger ones (Saccheri et al., 1998). Within this context, our study highlights that scale morphology can be successfully used to discriminate riverine fish populations at a fine spatial scale, i.e. a river stretch. The use of fish scale morphology is an easy-to-implement method, relatively rapid, inexpensive and does not require fish sacrifice. Since the identification of populations and their connectivity between each other is a major point for the conservation and management of vulnerable species (Hanski and Simberloff, 1997), the use of scale morphology to this purpose appears particularly promising.

References

Begg, G. A. and R. W. Brown, 2000. Stock identification of haddock *Melanogrammus aeglefinus* on Georges bank based on otolith shape analysis. Transactions of the American Fisheries Society **129:** 935–945.

Bird, J. L, D. T. Eppler and D. M. Checkley, 1986. Comparaison of herring otoliths using Fourier shape analysis. Canadian Journal of Fisheries and Aquatic Sciences **43:** 1228–1234.

Brito, R. M. and M. M. Coelho, 1999. Genetic structure of the Iberian chub, *Leuciscus pyrenaicus*, in the Tejo drainage. Hydrobiologia **392:** 169–177.

Cadrin, S. X. 2000. Advances in morphometric identification of fishery stocks. Reviews in Fish Biology and Fisheries **10:** 91–112.

Carlsson, J., K. H. Olsen, J. Nilsson, O. Overli and O. B. Stabell, 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. Journal of Fish Biology **55:** 1290–1303.

Casselman, J. M., J. J. Collins, E. J. Crossman, P. E. Ihssen and G. R. Spanger, 1981. Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. Canadian Journal of Fisheries and Aquatic Sciences **38:** 1772–1789.

Chappaz, R., A. Gilles, A. Miquelis, L. Cavalli and J. F. Martin, 1998. Genetic differentiation and hybridization in cyprinid Leuciscus leuciscus. C. R. Acad. Sci. Paris **321:** 933–940.

Clough, S., P. Garner, D. Deans and M. Ladle, 1998. Postspawning movements and habitat selection of dace in the River Frome, Dorset, southern England. Journal of Fish Biology **53**: 1060–1070.

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

Forman, R. T. T. and M. Godron, 1986. Landscape Ecology. John Wiley and Sons, New York, 619 pp.

Fraisse, L. 1990. Essai de discrimination de deux populations de vandoise (*Leuciscus leuciscus*, L., 1758) par étude de la forme de leurs écailles: aspects méthodologiques. Hydroécologie Appliquée **1/2:** 135–149.

Guinand, B., Y. Bouvet and B. Brohon, 1996. Spatial aspects of genetic differentiation of the European chub in the Rhone river basin. Journal of Fish Biology **49:** 714–726.

Hanski, I. and D. Simberloff, 1997. The metapopulation approach, its history, conceptual domain, and application to conservation. In: Hanski, I. and M.E. Gilpin, eds. Metapopulation biology, Ecology, Genetics, and Evolution. Academic press, San Diego, pp. 5–26.

Ihssen, P. E., H. E. Booke, J. M. Casselman, J. M. McGlade, N. R. Payne and F. M. Utter, 1981. Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences **38:** 1838–1855.

Jager, H. I., J. A. Chandler, K. B. Lepla and W. Van Winkle, 2001. A theoretical study of river fragmentation by dams and its effects on white sturgeon populations. Environmental Biology of Fishes **60:** 347–361.

Jarvis, R. S., H. F. Klodowski and S. P. Sheldon, 1978. New method of quantifying scale shape and an application to stock identification in Walleye *(Stizostedion vitreum vitreum*). Transactions of the American Fisheries Society **107:** 528–534.

Lelek, A. 1987. The freshwater fishes of Europe. Vol. 9. Threatened fishes of Europe. AULA-Verlag GmbH, Wiesbaden, 343 pp.

Malanson, G. P. 1993. Riparian Landscapes. Cambridge University Press, Cambridge, 296 pp.

Manh, A. G. 2001. Modèles déformables pour la reconnaissance d'adventices. Thèse de Doctorat, spécialité Systèmes automatiques et microélectroniques. Université des Sciences et Techniques du Languedoc, Montpellier, France.

Mann, R. H. K. and C. A. Mills, 1986. Biological and climatic influences on the dace *Leuciscus leuciscus* in a southern chalkstream. Annual Report of the Freshwater Biological Association **54:** 123–136.

Meng, H. J. and M. Stocker, 1984. An evaluation of morphometrics and meristics for stock separation of Pacific herring (*Clupea harengus pallasi*). Canadian Journal of Fisheries and Aquatic Sciences **41:** 414–422.

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

De Pontual, H. and P. Prouzet, 1987. Atlantic salmon, *Salmo salar* L., stock discrimination by scale shape analysis. Aquaculture and Fisheries Management **18:** 277–289.

De Pontual, H. and P. Prouzet, 1988. Numerical analysis of scale morphology to discriminate between atlantic salmon stocks. Aquatic Living Resources **1:** 17–27.

Poulet, N., P. Berrebi, A. J. Crivelli, S. Lek and C. Argillier, 2004. Genetic and morphometric variations in the pikeperch (*Sander lucioperca* L.) of a fragmented delta. Archiv für Hydrobiologie **159:** 531–554.

Richards, R. A. and C. Esteves, 1997. Stock-specific variation in scale morphology of atlantic striped Bass. Transactions of the American Fisheries Society **126:** 908–918.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius and I. Hanski, 1998. Inbreeding and extinction in a butterfly metapopulation. Nature **392:** 491–494.

Spillmann, J. 1961. Faune de France. Vol. 61. Paul Lechevalier, Paris, 303 pp.

To access this journal online:
http://www.birkhauser.ch