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Ecological traits of *Squalius lucumonis* (Actinopterygii, Cyprinidae) and main differences with those of *Squalius squalus* in the Tiber River Basin (Italy)

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ABSTRACT

Squalius lucumonis (Bianco, 1983) is an endemic species restricted to three river basins in central Italy and listed as endangered according to IUCN Red List. The aim of this research was to increase the information on ecological preferences of this species and to focus on its differences with *S. squalus* (Bonaparte, 1837). Data collected in 86 different watercourses throughout Tiber River basin were analysed in the research. For each of the 368 river sectors examined, the main environmental parameters and the fish community were considered. The information were analysed by means of the Canonical Correspondence Analysis (CCA) while the differences in ecological traits between *S. lucumonis* and *S. squalus* were compared by ANOVA. The results of the study showed significant differences in the ecological preferences of the two species: the *S. lucumonis* showed predilection for smaller watercourses characterised by a lower number of species and a higher degree of integrity of fish community than *S. squalus* This information allowed to increase the basic knowledge on population biology and ecology of *S. lucumonis* that could be very useful for the management and conservation of this Italian endemic species.

Key-words: *endemic species, Squalius lucumonis, Squalius squalus, longitudinal gradient, fish assemblage*

RÉSUMÉ

Caractéristiques écologiques de *Squalius lucumonis* (Actinopterygii, Cyprinidae) et leurs principales différences avec celles de *Squalius squalus* dans le bassin du Tibre (Italie)

Squalius lucumonis (Bianco, 1983) est une espèce endémique limitée à trois bassins fluviaux de l'Italie centrale. Elle est dans la liste des espèces menacées selon la Liste Rouge IUCN. L'objectif de cette recherche était d'améliorer l'information sur les préférences écologiques de cette espèce et de se concentrer sur ses différences avec *S. squalus* (Bonaparte, 1837). Les données recueillies dans 86 différents cours d'eau tout au long du bassin de la rivière Tibre ont été analysés dans cette étude. Pour chacun des 368 secteurs fluviaux examinés, les principaux paramètres environnementaux et ceux de la communauté de poissons ont été pris en compte. Les informations ont été analysées au moyen de l'Analyse Canonique des Correspondances (ACC), tandis que les différences dans les caractéristiques écologiques entre *S. lucumonis* et *S. squalus* ont été comparés par analyse de

Mots-clés : *espèces endémiques,* Squalius lucumonis*,* Squalius squalus*, gradien longitudinal,*

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communauté de poissons

variance. Les résultats de l'étude ont montré des différences significatives dans les préférences écologiques des deux espèces : *S. lucumonis* a montré une prédilection pour les petits cours d'eau caractérisés par un faible nombre d'espèces et un plus haut degré d'intégrité de la communauté de poissons que *S. squalus*. Ces informations ont permis d'accroître les connaissances de base sur la biologie des populations et l'écologie de *S. lucumonis* qui pourrait être très utile pour la gestion et la conservation de cette espèce endémique italienne.

INTRODUCTION

Squalius lucumonis (Bianco, 1983) is an Italian endemic species restricted to the Tuscany-Latium district in three drainages of central Italy: Tiber, Arno, Ombrone-Serchio, (Bianco and Ketmaier, 2003; Crivelli, 2006). It is one of the rheophilic cyprinid species inhabiting the secondary water courses (*i.e* brooks, creeks, small streams) within the "barbel zone" (Mearelli *et al*., 1995) which is characteristic of the intermediary sectors of the river basins in central Italy (Lorenzoni *et al*., 2006).

Many authors reported about a progressive decline in the original range of the species (Bianco and Taraborelli, 1984; Mearelli *et al*., 1996; Bianco and Ketmaier, 2001) and this reduction is mainly due to habitat modification and competition with non-native species (Bianco and Ketmaier, 2003). Thus, *S. lucumonis* has been assessed as endangered species according to IUCN Red List because of an estimated area of occupancy less than 500 km^2, and only by a small number of subpopulations (Crivelli, 2006). The species is also listed in the Appendix III of the Bern Convention and in the Annex II of the European Union Habitats Directive as a species requiring designation of Special Areas of Conservation.

Despite it conservational value, the information on ecology and biology of *S. lucumonis* are limited (Giannetto *et al*., 2012b), probably because this species was often assimilated with *S. squalus* (Bonaparte, 1837) a cyprinid endemic in Italian peninsula and Balkans (Kottelat and Freyhof, 2007) and one of the most widespread freshwater fish in Italy (Pompei *et al*., 2011). Indeed some authors considered *S. lucumonis* as a hybrid of *S. squalus* (Zerunian, 2002) with other cyprinids species (*i.e.*: *Rutilus rubilio* or *Telestes muticellus*) and refused their separation in two different species (Gandolfi *et al*., 1991). Currently, recent studies in morphological and molecular taxonomy confirmed the separation of *S. lucumonis* and *S. squalus* in two different species (Kottelat and Freyhof, 2007; Perea *et al*., 2010).

The aim of this study was to investigate the main ecological traits of *S. lucumonis* in Tiber River basin and to explore the different ecological preferences between *S. lucumonis* and *S. squalus*

MATERIALS AND METHODS

> *STUDY AREA*

The investigated area was the portion of the Tiber River basin (central Italy) located in Umbria region (9413 km^2) (Figure 1) The Tiber is the third-longest river in Italy (405 km) and its watershed is the second-largest; besides Umbria it extends also into the Italian Regions Lazio, Emilia Romagna, Tuscany, Marche, Molise and Abruzzo with a total extension of 17375 km^2 (Giannetto *et al.*, 2012a).

> *DATA COLLECTION*

Data from 162 sampling sites (Figure 1), collected from 1998 to 2010 in 86 different watercourses for the draft of the Fish Map of Umbria region were used for the analysis. All sampling

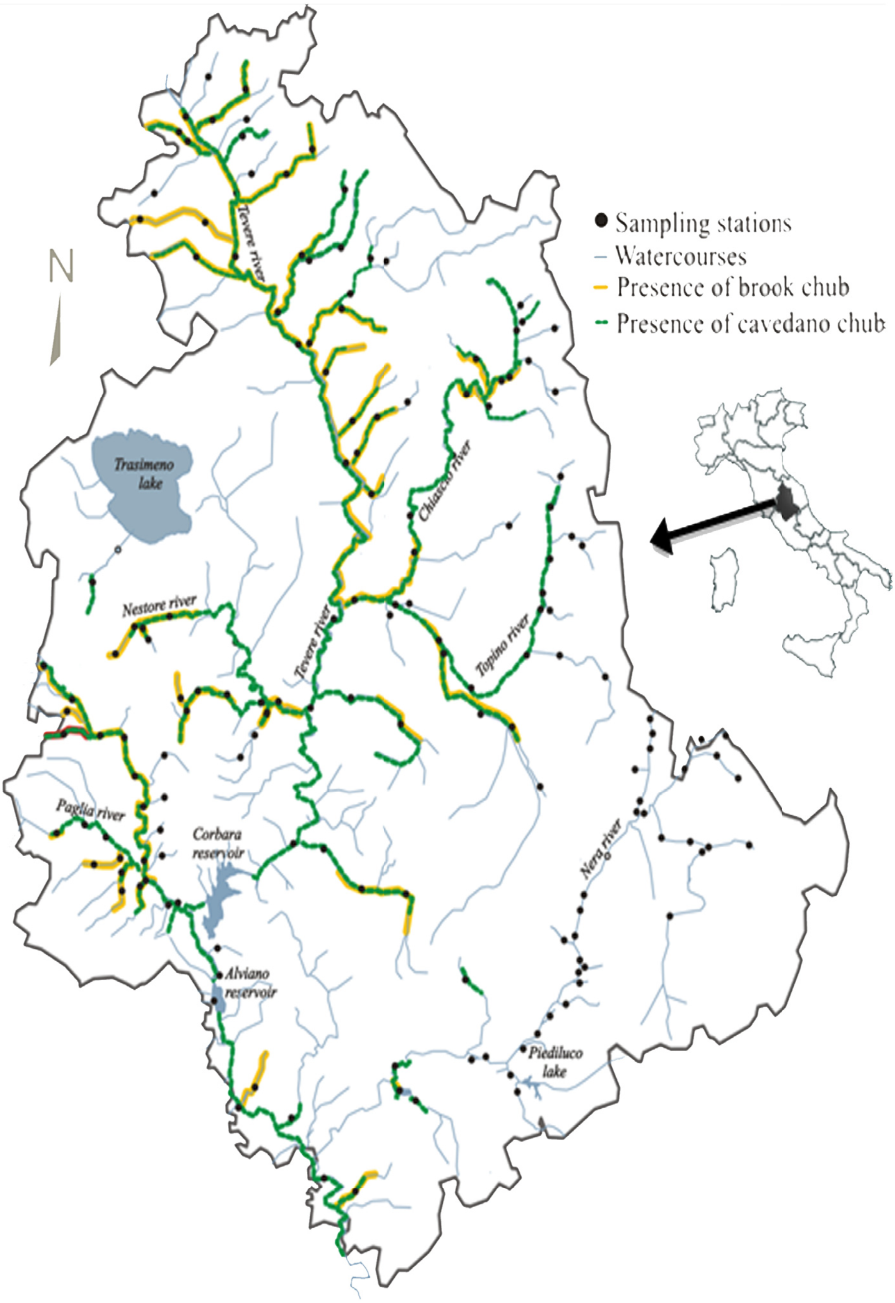

Figure 1
Study area and location of the sampling sites.

stations were sampled 2 times (1 in late spring and 1 in autumn) or 3 times (2 in late spring and 1 in autumn) for a total of 368 observations.

For each station a list of environmental parameters was used to characterise the river sectors (Table I) and a census of the fish fauna was carried out by electrofishing. In each location, a stretch with a lenght of 10 times of the wetted channel (min 50 m–max 400 m) was sampled, from downstream to upstream during daylight, using the removal method (Moran, 1951; Zippin, 1956). Within each sampling stretch, the environmental parameters were usually assessed on the same day or within several days of the fish collections. Hydrobiological variables were measured within each sampling stretch: (i) watershed area, distance from the source, average slope and altitude were determined from topographic maps; (ii) conductivity, pH, water temperature and dissolved oxygen were measured with electronic

Table I
Canonical and correlations coefficients of the environmental parameters with axis 1 and 2 of Canonical Correspondence Analysis (CCA) (in bold value of $p < 0.05$).

	Canonical coefficients		Correlations with axes			
Environmental variables	**AX1**	**AX2**	**AX1**	***p***	**AX2**	***p***
Distance from the source (km)	–0.059	–0.558	–0.112	0.067	0.266	**<0.05**
Watershed area (km²)	0.652	0.942	–0.044	0.472	0.321	**<0.05**
Altitude (m a.s.l)	–0.069	0.569	0.571	**<0.05**	0.149	**<0.05**
Average slope (%)	–0.022	0.164	–0.013	0.831	–0.200	**<0.05**
pH	0.109	–0.203	0.001	0.987	–0.088	0.151
Conductivity (μS/s^{-1} at 25 °C)	–0.627	–0.549	–0.733	**<0.05**	0.344	**<0.05**
Nitrate (mg·l^{-1} as N)	–0.131	–0.035	–0.358	**<0.05**	–0.081	0.187
Nitrite (mg·l^{-1} as N)	0.132	0.254	–0.253	**<0.05**	–0.019	0.750
Ammonia(mg·l^{-1} as N)	–0.026	–0.131	–0.234	**<0.05**	–0.037	0.542
Sulphates (mg·l^{-1} as S)	0.573	0.014	–0.499	**<0.05**	–0.140	**<0.05**
Chlorides (mg·l^{-1} as Cl)	0.002	–0.269	–0.727	**<0.05**	–0.396	**<0.05**
Water temperature (°C)	0.052	–0.195	–0.307	**<0.05**	–0.046	0.455
Dissolved oxigen (mg·l^{-1})	0.234	–0.383	0.238	**<0.05**	0.150	**<0.05**
Dissolved oxigen (%)	–0.081	0.309	0.228	**<0.05**	0.206	**<0.05**
Extended Biotic Index (index 0-7)	–0.139	0.042	0.390	**<0.05**	–0.010	0.874
Mean width (m)	0.420	–0.352	–0.108	0.076	0.185	**<0.05**
Mean depth (m)	0.009	0.149	0.013	0.836	0.140	**<0.05**
Current speed (m·s^{-1})	–0.003	0.298	0.448	**<0.05**	0.460	**<0.05**
Flow rate (m^3·s^{-1})	0.185	0.132	0.158	**<0.05**	0.341	**<0.05**
Wetted river section (m²)	–0.621	–0.497	–0.002	0.969	0.269	**<0.05**
Canopy cover (index 0-4)	–0.059	–0.096	0.175	**<0.05**	–0.091	0.137
Instream cover (index 0-4)	0.101	0.206	0.184	**<0.05**	0.045	0.460
Cover plant (index 0-4)	–0.050	0.382	0.054	0.377	0.138	**<0.05**
Substrate size (index 0-7)	–0.408	–0.407	0.101	0.096	–0.122	**<0.05**

meters; (iii) others chemical parameters of the water were determined according to APHA, AWWA and WPCF (1989) specifications. The Extended Biotic Index (EBI; Ghetti, 1986), evaluating the overall water quality as the sensitivity of some key groups of macrobenthic fauna to pollution was assessed on the same day or within several days of the fish collections. All fish caught were identified according to Kottelat and Freyhof (2007) and then measured (total length, ±1 mm) and weighed (±1 g) following Anderson and Neumann's method (1996).

> *STATISTICAL ELABORATIONS*

The density of each fish species in all sample sites was calculated (number of specimens by m^2) and related to the environmental parameters by means of Canonical Correspondence Analysis (CCA) a direct gradient analysis method that allows to examine the relationships among multivariate ecological data matrices (ter Braak, 1986) The fish matrix included densities of all species and 368 observations (sampling stations). The environmental matrix included 24 variables (environmental parameters) and 368 observations (Table I). All variables (N) were transformed (ln $(N + 1)$) and standardized (Brown and Austen, 1996).

In a second part, the Quality Integrity Index (IIQUAL; Bianco, 1990), estimating the ratio between the number of native species and the total number of species (native and exotic), was calculated to evaluate the degree of integrity of fish community in each sampling sites considered

Finally, to focus on the differences in ecological preferences between *S. lucumonis* and *S. squalus*, the mean values of IIQUAL, total number of species and all the environmental parameters (Table I) estimated for the two species, were compared by analysis of variance (ANOVA)

All statistical elaborations were accomplished by means of two software: CANOCO 4.5 for CCA and STATISTICA 6.0 for all other tests.

Table II
Summary statistics for Canonical Correspondence Analysis (CCA): eigenvalues of axes 1 and 2 expressed as percentage of total variance.

	Axis 1	Axis 2
Eigenvalues	0.581	0.293
Species-environment correlations	0.81	0.706
Cumulative percentage variance of species data	8.9	13.4
Cumulative percentage variance of species-environment relation	32.4	48.7

RESULTS

S. lucumonis was caught in 60 of the 162 sampling sites investigated while *S. squalus* in 93. Moreover in 55 of the 60 stations, *S. lucumonis* lived in copresence with *S. squalus*. Specifically *S. lucumonis* was present in the upper part of the main rivers (Tiber, Paglia, Nestore and Chiascio) but only in one sampling station of Nera river (Figure 1). *S. squalus* was scarcely present in the Nera river too while it is widely distributed across the Tiber River basin colonising with continuity both the main rivers and the tributaries (Figure 1).

The first axis of the CCA explained 324% of the overall variability while the second axis was less informative (16.3%) (Table II). By the analysis of the canonical and correlation coefficients of the environmental variables with axes of CCA (Table I and Figure 2a), most of these variables resulted significantly correlated with the first axis that well describes the changes occurring along the longitudinal gradient. Specifically, a progressive worsening of water quality (increase of nitrate, nitrite, ammonia, sulphates and chlorides and decrease of EBI and dissolved oxygen) was associated with the decrease of altitude, canopy cover, instream cover, flow rate, current speed and the raise of conductivity and water temperature.

The distribution of fish species in the CCA plot (Figure 2b) well describe their succession along a river from mountain to valley. Moreover the CCA plot well described the strong relationship between the longitudinal evolution of the rivers and the integrity of fish communities: while the river size increases and water quality worsens, the number of species and especially those of exotic origin increases in the fish assemblage (Figure 2b).

Considering the location of *S. lucumonis* and *S. squalus* in relation to axes of CCA, some differences emerged: while the position of the two chubs was similar according to axis 1, their position was opposite in relation to axis 2. More specifically, considering the association of the environmental variables with the second axes, *S. lucumonis* differed from *S. squalus* by a stronger preference for the middle-upper part of watercourses (characterised by a lower flow rate, a short distance from the source and a smaller watershed area and width) (Figure 2a). In addition, the position of *S. lucumonis* in the CCA (Figure 2b) appeared to be more similar to that of the species characteristic of the middle-upper zone (*Barbus plebejus*, *Barbus tyberinus, Cobitis bilineata Padogobius bonelli, Padogobius nigricans, Rutilus rubilio*) with which, therefore, the species seemed to share the same environmental preferences. On the contrary, the position of *S. squalus*, at the cross of the two axes, indicated that this species showed a higher ecological plasticity than *S. lucumonis* and adapting to inhabit different kinds of environments (Figure 2b). Moreover, *S. squalus* was localised in the square of the CCA plot shared by a high number of species (also those typical of downer-part of the river and most of them of exotic origin) (Figure 2b).

The results of ANOVA (Table III) confirmed the differences emerged by CCA: *S. lucumonis* resulted to inhabit the river zone with a smaller distance from the source, watershed area, mean width, a higher number of fish species and IIQUAL than *S. squalus*; the differences between the mean values for the two species for these parameters resulted highly statistically significant ($p < 0.001$) (Table III).

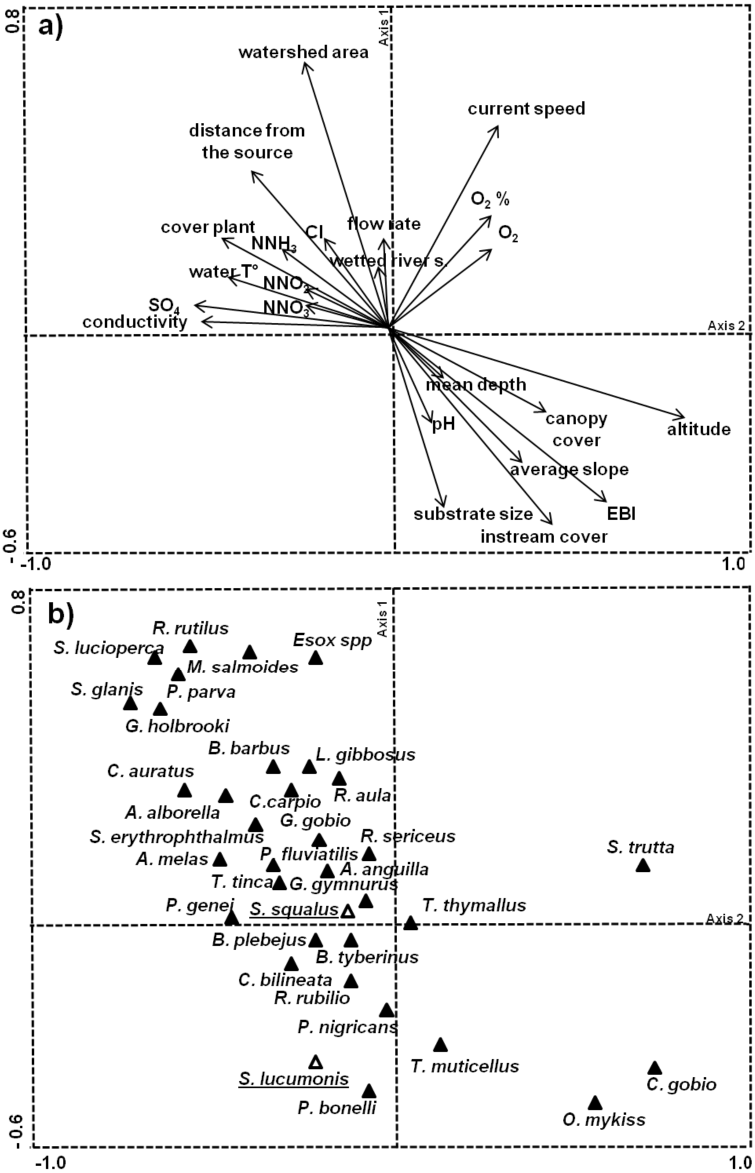

Figure 2
Canonical Correspondence Analysis (CCA): (a) plot of the environmental matrix showing environmental variables; (b) plot of the fish matrix with densities of all fish species. The length of the arrow is proportional to the rate of change: a long arrow indicates that change is strongly correlated with the ordination axes.

DISCUSSION

Reports about declining of *S. lucumonis* (Bianco and Ketmaier, 2001) and the listing of the species in the IUCN red list as endangered are of concern. Because of the lack of information on *S. lucumonis*, the increase of basic knowledge on its population biology and ecology could be very useful for the management and conservation of this endemic species.

In the study area the species is distributed in the upper part of the main rivers (Tiber, Paglia, Nestore and Chiascio) and of their tributaries with the exclusion of Nera river whose water are more suitable for salmonid species (Lorenzoni *et al*., 2010). In the Tiber river basin so as in other parts of its area of distribution (Bianco and Ketmaier, 2003), *S. lucumonis* often lives in copresence with *S. squalus* and, to date, there are no evidences that the decreasing of *S. lucumonis* could be attributed to any competition with *S. squalus* On the contrary the main

Table III
Mean values of variables considered to detect the main ecological differences between brook chub and cavedano chub and results of ANOVA (in bold value of $p < 0.05$).

	MEAN VALUE		ANOV	
VARIABLES	*S. lucumonis*	*S. squalus*	*F* value	*p* value
Distance from the source (km)	22.987	35.103	7.999	**0.005**
Watershed area (km^2)	186.247	467.620	8.691	**0.003**
Altitude (m a.s.l)	250.919	237.041	1.606	0.206
Average slope (%)	2.009	1.995	0.006	0.936
pH	8.132	8.126	0.029	0.865
Conductivity (μS·s^{-1} at 25 °C)	686.984	674.232	0.323	0.570
Nitrate (mg·l^{-1} as N)	2.779	2.386	0.733	0.393
Nitrite (mg·l^{-1} as N)	0.061	0.060	0.002	0.963
Ammonia(mg·l^{-1} as N)	0.281	0.375	0.208	0.648
Sulphates (mg·l^{-1} as S)	68.189	72.132	0.230	0.632
Chlorides (mg·l^{-1} as Cl)	25.416	26.410	0.146	0.703
Water temperature (°C)	14.644	15.572	2.949	0.087
Dissolved oxigen (mg·l^{-1})	9.146	9.125	0.007	0.935
Dissolved oxigen (%)	89.779	91.127	0.255	0.614
E.B.I (index 0-7)	7.470	7.294	1.581	0.210
Mean width (m)	7.131	9.079	6.146	**0.014**
Mean depth (m)	0.360	0.375	0.249	0.618
Current speed (m·s^{-1})	0.204	0.235	1.472	0.226
Flow rate (m^3·s^{-1})	0.690	1.508	1.420	0.234
Wetted river section (m^2)	1.986	2.489	2.165	0.142
Canopy cover (index 0-4)	2.132	1.882	2.164	0.142
Instream cover (index 0-4)	2.314	2.244	0.298	0.586
Cover plant (index 0-4)	0.975	0.990	0.014	0.905
Substrate size (index 0-7)	5.268	4.949	1.886	0.171
IIQUAL (index 0-1)	0.744	0.690	4.753	**0.030**
Total number of species	5.724	6.467	5.303	**0.022**

threats for *S. lucumonis* are identified in the modification of habitat and the introduction of non-native species (Crivelli, 2006).

In the study, the fish assemblage was related to the main environmental variables that characterise the river section and influence the fish community (Godinho *et al*., 2000; Lorenzoni *et al*., 2006; Ferreira *et al*., 2007). The results clearly marked the different composition of the fish assemblage in the various sections of the river in accordance with the hypothesis of Huet (1954, 1962) on the succession of fish species along a river from mountain to valley. The CCA underlined some divergences in the fish assemblages selected by *S. lucumonis* and *S. squalus*: the first species showed a stronger association with the fish community typical of the middle-upper part of the river that (with the exception of the translocated *Padogobius bonelli*) are all native of the Tiber River basin; on the other side, *S. squalus* was localised nearest to those species distinctive of the middle-down part, mostly of exotic origin, and whose negative impact on the well-being of native fish community of Tiber River Basin was already confirmed (Giannetto *et al.*, 2012a) These differences were also confirmed by ANOVA that explained as *S. lucumonis* was significantly related to a higher value of IIQUAL and a lower number of species than *S. squalus*.

The CCA and ANOVA highlighted the presence of some important ecological differences between the two species of chub in the watercourses of Tiber River basin: specifically, *S. lucumonis* showed a stronger preference for small watercourses with a low flow rate and a shorter distance from the source, while *S. squalus* can be found also in lentic ecosystems and in stretches of lowland rivers in accordance with the results of Lorenzoni *et al*. (2011).

Further researches, focused on microhabitat use of the two species, are encouraged to enlarge the knowledge on their ecology and to underline if, for example, when the species live in copresence, they differentiate their microhabitat on the same sampling sector.

Summarising, the preliminary results of this research allowed to investigate the role of the environmental variables in the fish communities of Tiber River basin and confirmed the significant function of the small watercourses as refuge zones for the native community as well as their important function for the conservation of fish biodiversity (Lorenzoni et al., 2006). Ferreira et al. (2007), analysing the regional and local environmental correlates of native fish fauna in Iberian peninsula, found that *S. alburnoides* and *S. pyrenaicus* – two Iberian endemic cyprinids – responding primarily to some local variables (such as mean width, mean depth or instream cover) can be more vulnerable to immediate disturbances of habitat. This confirmed that the preservation of small watercourses is essential for the conservation of those endemic and endangered species – such as *S. lucumonis*, – and for the other native species; to this aim, the management of these river sectors should focus on the control of translocated and exotic species as well as on maintenance of water and habitat qualities.

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REFERENCES

Anderson R.O. and Neumann R.M. 1996. Length, weight, and associated structural indices. *In* Murphy B.R. and Willis D.W. (ed.), Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland, 447–481.

Bianco P.G., 1990. Proposta di impiego di indici e di coefficienti per la valutazione dello stato di degrado dell'ittiofauna autoctona delle acque dolci. *Riv. Idrobiol., 29*, 130–149.

Bianco P.G. and Keitmier V., 2001. Anthropogenic changes in the freshwater fish fauna of Italy, with reference to the central region and *Barbus graellsii*, a newly established alien species of Iberian origin. *J. Fish. Biol., 59A*, 190–208.

Bianco P.G. and Ketmaier V., 2003. Threatened fishes of the world: *Leuciscus lucumonis* Bianco, 1983 (Cyprinidae). *Environ. Biol. Fish., 68,* 370.

Bianco P.G. and Taraborelli T., 1984. *Leuciscus lucumonis* Bianco, 1983 nel bacino del Tevere, e altri reperti di pesci d'acqua dolce in Italia (Pisces, Cypriniformes). *Natura 75,* 110–116.

Brown M.L. and Austen D.J., 1996. Data management and statistical techniques. *In*: Murphy B.R. and Willis D.W. (eds.), Fisheries Techniques, American Fisheries Society, Bethesda.

Crivelli A.J., 2006. *Squalius lucumonis*. *In*: IUCN 2011. IUCN Red List of Threatened Species. Version 2012.1. <www.iucnredlist.org>. Downloaded on 10 December 2012.

Ferreira M.T., Sousa L., Santos J.M., Reino L., Oliveira J., Almeida P.R., Cortes R.V., 2007. *Ecol. Freshw. Fish. 16*, 504–514.

Gandolfi G., Zerunian S., Toricelli P., Marconato A., 1991. I pesci delle acqua interne italiane, Istituto poligrafico e Zecca dello Stato, Roma, 291 p.

Ghetti P.F., 1986. I macroinvertebrati nell'analisi di qualità dei corsi d'acqua. Bertelli, Trento.

Giannetto, D., Carosi A., Franchi E., Pedicillo G., Pompei L. and Lorenzoni M., 2012a. Assessing the impact of non-native freshwater fishes on native species using relative weight. *Knowl. Managt. Aquatic Ecosyst.* 404, 03.

Giannetto, D., Franchi E., Pompei L., Porcellotti S., Tancioni L., and Lorenzoni M., 2012b. Proposed empirical standard weight equation for brook chub *Squalius lucumonis. N. Am. J. Fish. Manage. 32*, 428–435.

Godinho F.N., Ferreira M.T. and Santos J.M., 2000. Variation in fish community composition along an Iberian river basin from low to high discharge: relative contributions of environmental and temporal variables. *Ecol. Freshw. Fish., 9*, 22–29.

Huet M., 1954. Biologie, profils en long et en traverse des eaux courantes. *Bull Fr. Pêche Piscic.*, 175 41–53.

Huet M., 1962. Influence du courant sur la distribution des poissons dans les eaux courantes. *Revue Suisse d'Hydrol.*, *24*, 412–432.

Lorenzoni M, Ghetti L. and Mearelli M., 2006. Native and exotic fish species in the Tiber River watershed (Umbria - Italy) and their relationship to the longitudinal gradient. *Bull. Fr. Pêche Piscic.*, 382, 19−44.

Lorenzoni M., Ghetti L., Carosi A., Dolciami R., 2010. La fauna ittica e i corsi d'acqua dell'Umbria. Regione Umbria, Perugia, 300 p.

Lorenzoni M., Carosi A., Pedicillo G., Pompei L., Rocchini E., 2011. Reproductive properties of the chub *Leuciscus cephalus* (Linnaeus, 1758) in the Assino Creek (Umbria, Italy). *Knowl. Managt. Aquatic Ecosyst.,* 409, 1–10.

Kottelat M., Freyhof J., 2007. Handbook of European Freshwater Fishes, Cornol: Publications, Kottelat.

Mearelli M., Giovinazzo G., Lorenzoni M., Petesse M.L. and Carosi A., 1995. Zonazione ittica dei corsi d'acqua del bacino del fiume Tevere. *Atti S.It.E.* 16, 669–671.

Mearelli M., Lorenzoni M., Carosi A., Giovinazzo G. and Petesse M.L., 1996. Carta Ittica della Regione Umbria, Regione Umbria, Università di Perugia. Perugia.

Moran P.A.P., 1951. A mathematical theory of animal trapping. *Biometrika, 38*, 307–311.

Perea S., Böhme M., Zupanèiè P., Freyhof J., Šanda R., Özuluð M., Abdoli A. and Doadrio I., 2010. Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. *BMC Evolutionary Biology* 10, 265.

Pompei L, Carosi A., Pedicillo G., Rocchini E. and Lorenzoni M., 2011. Age and growth analysis of the chub, *Squalius squalus* (Bonaparte, 1837), in the Assino Creek (Umbria, Italy). *Knowl. Managt. Aquatic Ecosyst.*, 400, 09.

ter Braak C.J.F., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology, 67*, 1167–1179.

Zerunian S., 2002. Condannati all'estinzione. Agricole (Ed), Bologna.

Zippin C., 1956. A n evaluation of the removal method of estimating animal populations. *Biometrics*, *12,* 163–189.