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Reproductive properties of the chub *Squalius squalus* (Bonaparte, 1837) in the Assino Creek (Umbria, Italy)

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ABSTRACT

Key-words: *reproduction, sex ratio, age at maturity, fecundity, gonado-somatic index*

This study investigated the reproductive properties of the population of chub *Squalius squalus* (Linnaeus, 1758) in the Assino creek, one of the largest tributaries of the upper course of the River Tiber. A total of 547 specimens were caught monthly from March 2008 to May 2009. The age composition of the sample ranged between 0+ and 11+. The sample was made up of 64.54% males and 35.46% females; sexual maturity was attained in both sexes in the second year of life. Analysis of the gonado-somatic index (GSI) and of the stage of gonad maturation throughout the year suggests that the reproductive period of the population extends over a long time (April–July). The relationship between total length and fecundity was $F = 5.466\mathrm{TL}^{2.312}$. The mean number (±SE) of eggs produced by each female was 15 351 ± 1705 and increased with the size and age of the specimens; with regard to relative fecundity, an average value (±SE) of 53 553 ± 4098 eggs · kg^{-1} was observed. The mean diameter (±SE) of ripe eggs was 0.117 ± 0.004 cm. This study sheds light on some important characteristics of the reproductive biology of the chub that have not been fully investigated in Italian populations.

RÉSUMÉ

Caractéristiques de la reproduction du chevesne *Squalius squalus* (Bonaparte, 1837) dans le ruisseau Assino (Ombrie, Italie)

Mots-clés : *reproduction, sex-ratio, âge à la maturité, fécondité, indice gonado-somatique*

Ce travail a étudié les caractéristiques de la reproduction de la population du chevesne, *Squalius squalus* (Linnaeus, 1758), dans le ruisseau Assino, l'un des principaux affluents du cours supérieur du Tibre. Au total 547 spécimens ont été capturés du mois de mars 2008 à mai 2009. La composition par âge de l'échantillon variait entre 0+ et 11+. L'échantillon était composé de 64,54 % de mâles et 35,46 % de femelles ; la maturité sexuelle est atteinte chez les deux sexes dans la deuxième année de vie. L'analyse de l'indice gonado-somatique (GSI) et du stade de maturation des gonades au cours de l'année suggère que la période de reproduction de la population s'étend sur une longue période (avril–juillet). La relation entre la longueur totale et la fécondité était $F = 5.466\mathrm{TL}^{2.312}$. Le nombre moyen (±SE) des œufs produits par femelle était 15 351 ± 1705 et a augmenté avec la taille et l'âge des spécimens ; pour la fécondité relative, une valeur moyenne (±SE) de 53 553 ± 4098 d'œufs·kg^{-1} a été observée. Le diamètre moyen (±SE) des œufs mûrs était 0, 117±0, 004 cm. Cette étude met en lumière quelques caractéristiques importantes de la biologie reproductive du chevesne qui n'ont pas été entièrement étudiées dans les populations italiennes.

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INTRODUCTION

The chub *Squalius squalus*, is one of the most common freshwater fish in Italy; it is found throughout the country, except for the islands, with populations so numerous that in many ecosystems it is the dominant species in terms of biomass (Zerunian, 2004). It is one of the native species that characterize the fish communities of the "barbel zone" which is typical of the intermediate stretches of the River Tiber and the most representative of the Tiber river basin (Lorenzoni *et al.*, 2006). Despite its abundance little substantial information is available on chub biology in Italian watercourses (Vitali and Braghieri, 1984); more specifically, information on its reproductive biology are scant and generic (Gandolfi *et al.*, 1991; Bianco and Santoro, 2002).

The present study was carried out to investigate some reproductive characteristics of *Squalius squalus* in the Assino creek. This 24 km stream, which is one of the largest tributaries of the upper course of the River Tiber, has a markedly torrential regime, with maximum rainfall in autumn and minimum in summer. Environmental conditions are not optimal for the fish fauna owing to the obvious effects of pollution (Lorenzoni *et al.*, 2007). Nevertheless, the creek is of considerable reproductive value being utilized as a spawning area by the fish that come from the River Tiber.

The study of life-history traits of fish species is essential to the analysis of population performance and thus to both theoretical ecology and fisheries management (Winemiller and Rose, 1992) The growth and condition of chub in the Assino creek have already been investigated (Pompei *et al.*, 2011). The present research therefore aimed to supplement our knowledge of the biology of the same population by adding data on some important aspects of reproductive biology, such as age at maturity, reproductive period, gonado-somatic Index and fecundity.

MATERIALS AND METHODS

Samplings were conducted from March 2008 to May 2009 using electrofishing. The samplings were carried out once a month in the same area, localized in the Assino creek, near the confluence on the Tiber river.

A total of 547 specimens were captured and transported to the laboratory where total length (TL, in cm) and weight (W in g) were measured to the nearest 0.1 cm and 0.1 g, respectively.

For age assessment scales were removed from the left side of the fish, above the lateral line, near the dorsal fin (De Vries and Frie, 1996) and stored in ethanol (33%). Subsequently, specimens' ages were determined by means of a scalimetric method (Bagenal and Tesch, 1985). Fish were sacrificed by administering an overdose of anesthetic acetone chloroform (2,2,2,-trichloro-2-methyl-propanol) and were dissected to determine the sex through macroscopic observation of the gonads.

In the statistical analyses that follow, fishes caught in the same month, even if in different year (2008 or 2009) were pooled.

The sex ratio was investigated both by disaggregating the sample by age group and considering only the months of the breeding season (April–July); deviation from the 1:1 null hypothesis was tested statistically by means of chi-square (χ^2) analysis.

The gonads were removed and weighed to the nearest of 0.01 g (Wg). The reproductive state of all specimens was assessed according to the Nikolsky scale (Bagenal and Tesch, 1985). Age at maturity was defined as the age at which 50% of specimens in an age-group were mature (Şaşi, 2004).

The spawning period was determined by identifying monthly changes in the gonado-somatic index (GSI) which was calculated from gonad samples taken monthly from both females and males by using the formula: $GSI = 100(Wg/W)$ (Ricker, 1975). The differences between sexes in mean GSI values calculated for all males and females on the year round were analyzed by means of U Mann-Whitney test.

The ovaries of 48 females of various sizes (15.9–47.2 cm) at stages IV and V of gonad development *i.e.* when they have achieved their maximum weight, were excised and subsamples

Table I
Age composition of Squalius squalus population in the Assino creek expressed as the number (n) and percentage (%) of male and female specimens in each age-class in all year around. The sex ratio was also examined by taking into account the only months of the spawning season (April–July). Deviations from unity in the sex ratios (males:females) were tested by chi-square test (χ^2); values of $p < 0.05$ were considered significant.

Age class	Males		Females		Sex ratio	χ^2	*p*
	n	%	*n*	%	(*M*:*F*)		
1+	11	84.62	2	15.39	5.50:1	6.23	$p < 0.05$
2+	73	72.28	28	27.72	2.61:1	73.02	$p < 0.05$
3+	114	79.72	29	20.28	3.39:1	50.52	$p < 0.05$
4+	20	41.67	28	58.33	0.71:1	1.33	$p > 0.05$
5+	22	48.89	23	51.11	0.96:1	0.02	$p > 0.05$
6+	11	47.83	12	52.17	0.92:1	0.04	$p > 0.05$
7+	2	22.22	7	77.78	0.29:1	2.78	$p > 0.05$
8+	–	–	4	100	0:1	4.00	$p < 0.05$
9+	–	–	4	100	0:1	4.00	$p < 0.05$
10+	–	–	1	100	0:1	1.00	$p > 0.05$
11+	–	–	1	100	0:1	1.00	$p > 0.05$
All	253	64.54	139	35.46	1.82:1	33.15	$p < 0.05$
Month							
April	40	60.61	26	39.39	1.54:1	2.97	$p > 0.05$
May	46	57.50	34	42.50	1.35:1	1.8	$p > 0.05$
June	28	63.64	16	36.36	1.75:1	3.27	$p > 0.05$
July	14	70.00	6	30.00	2.33:1	3.2	$p > 0.05$
All	128	60.95	82	39.05	1.56:1	10.08	$p < 0.05$

of eggs were taken from the front, middle and back parts of the ovaries and mixed. The subsamples thus obtained were weighed to the nearest 0.01 g and preserved in Gilson's Fluid (Bagenal and Braum, 1978) The diameters of five randomly chosen eggs of the subsample were measured by means of the software Image J. The mean value (δ in cm) of the five diameters measured for each female was used in the subsequent analysis.

The eggs in the subsamples were counted under a lens. Individual fecundity, represented by the total number of eggs in each ovary (F), was calculated for 26 females ranging from 19.6 cm to 43.6 cm using the formula $F = (Wgn)/w$, where n is the number of eggs counted in the subsample and w is the weight of the subsample.

For the same females, relative fecundity (RF) was expressed as the number of eggs produced (F) per unit of body weight (W): $RF = 1000(F/W)$.

In order to analyze how the mean diameter of eggs, fecundity and relative fecundity changed with the increase of length and age of the females, the following regressions were calculated: $\delta = a + bTL$, $\delta = a + b$ Age; $F = aTL^b$, $F = a$ Age^b; $RF = a + bTL$, $RF = a + b$ Age.

RESULTS

The age composition of the specimens from the Assino creek ranged between 0+ and 11+ age-classes. The overall sample comprised 155 immature specimens, 253 males and 139 females. Sex distribution frequency (Table I) showed a marked deviation from the expected 1:1 ratio; the sex ratio observed in the total sample was 1.82:1 (*M*:*F*) the difference being highly significant on chi-square analysis ($\chi^2 = 3315$, $p < 0.05$). However the sex ratio changed significantly with age. In the younger age-classes it was heavily tilted in favor of males [(1 + (5.50:1), 2 + (261:1), 3 + (393:1)], while among older specimens, females predominated; indeed, all specimens that had lived through more than 7 winters were females. Examining the sex ratio only during the months of spawning season (April–July) was evident once

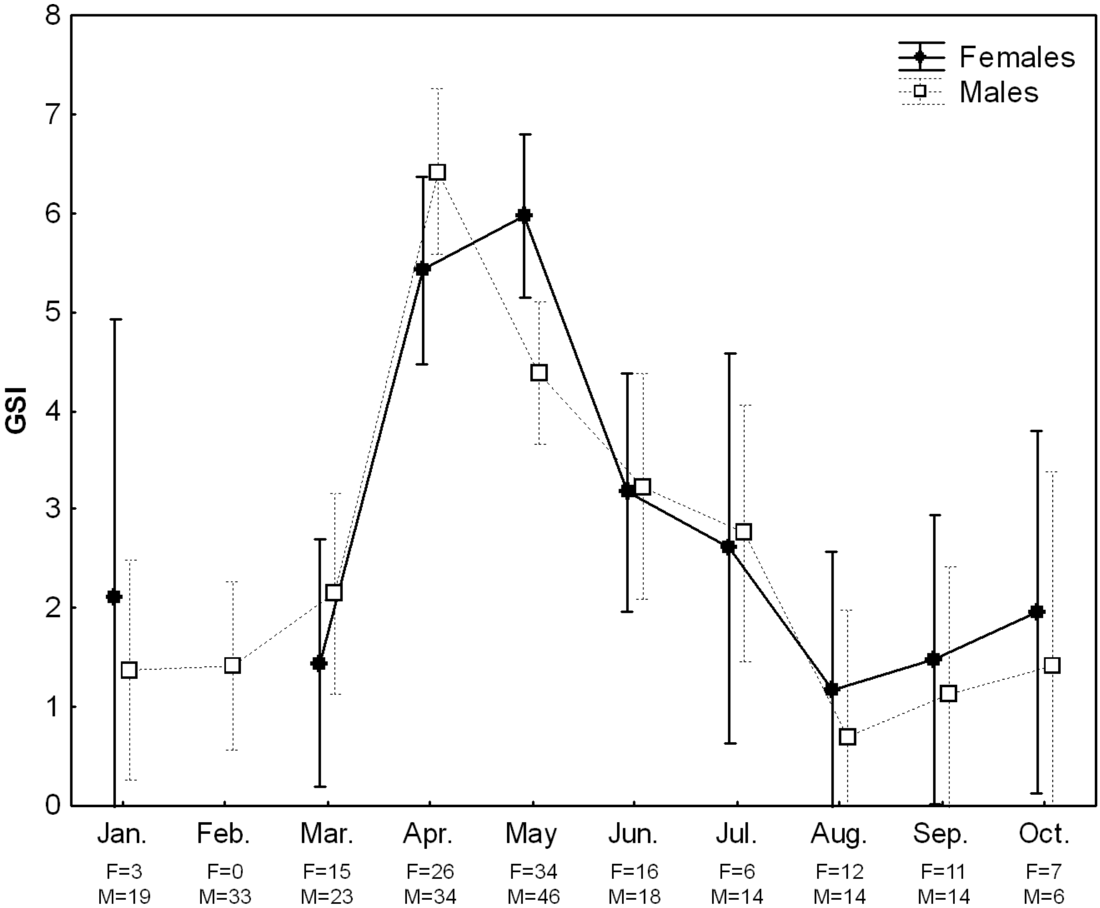

Figure 1
Monthly variations in mean GSI values in male (dotted line) and female (solid line) specimens of Squalius squalus from the Assino creek vertical bars indicate 95% confidence intervals. The number of male (M) and female (F) used in the analysis for each month are indicated.

again as the males were always more numerous than females, with differences that are significant on chi-square analysis when considering the whole reproductive period ($\chi^2 = 10.08$, $p < 0.05$).

Sexual maturity (Table II) was reached in both sex at two years, when 79.17% of males and 66.67% of females were mature. None of the females in the 1+ age-class was mature, whereas 27.27% of males in the same age-class had already attained sexual maturity.

The mean GSI value (±SE) was 3.713 ± 0.324 in females, varying between a minimum of 0.408 and a maximum of 16.000, while in males it was 3.047 ± 0.177, varying between 0.374 and 10.090. The differences between the sexes were not significant on *U* Mann-Whitney test ($Z = 1.366$, $p > 0.05$).

Monthly changes in GSI values were also analyzed (Figure 1). From March onward GSI increased sharply in both sexes, reaching its highest mean values (±SE) in April for males ($GSI = 6.420 \pm 0.423$) and in May for females ($GSI = 5.977 \pm 0.423$). Subsequently, mean GSI values declined until August, when the lowest minimum value was recorded for both sexes (males: $GSI = 0.696 \pm 0.65$; females: $GSI = 1.174 \pm 0.712$).

Analysis of the stage of gonad maturation throughout the months (Figure 2) showed that in April most specimens, both male and female, had ripe gonads (stage IV). In May more than 50% of females were reproducing (stage V), whereas almost 30% had already completed egg spawning (stage VI); reproducing males accounted for 26.09% of the total, but a small percentage of the sample (2.27%) already had stage V gonads in April. Reproduction continued

Table II
Percentage (%) of immature and mature individuals in male and female samples of the Squalius squalus population in the Assino creek, broken down by age-class.

Age class	Males		Females	
	Immature %	Mature %	Immature %	Mature %
1+	72.73	27.27	100	0
2+	20.83	79.71	33.33	66.67
3+	0.88	99.12	6.9	93.1
4+	0	100	0	100
5+	0	100	0	100
6+	0	100	0	100
7+	0	100	0	100
8+	–	–	0	100
9+	–	–	0	100
10+	–	–	0	100
11+	–	–	0	100

until July, when 33.33% of females and 50% of males still had stage V gonads; by August, it could be regarded as completed, as the gonads in both sexes were mostly quiescent (stage II) or were already maturing (stage III).

Egg number varied from a minimum of 1606 to a maximum of 35 981; positive correlation was found by relating eggs number with total length ($r = 0.822$), as well as with age ($r = 0.795$) (Figure 3a, 3b) meaning that fecundity increased with the size and the age of females; both these correlations were statistically significant ($p < 0.05$).

Mean relative fecundity (±SE) was 53 553 ± 40 98 eggs · kg^1 and displayed a fairly broad range, from a minimum of 14 640 to a maximum of 111 724 eggs · kg^{-1}. The relationship between *TL* and *RF* is represented by the equation $RF = 104\,807.352 - 1743.117TL$ ($r^2 = 0.366$), while the relationship between age and *RF* was: $RF = 88\,753.362 - 6690.124$ Age ($r^2 = 0.345$). *RF* decreased with the size and the age of the specimens as shown by correlation analysis: the number of eggs per unit of body weight correlated negatively with length ($r = -0.605$; $p < 0.05$) and age ($r = -0.587$; $p < 0.05$).

The diameter of mature eggs (δ) in the spawning season varied considerably, ranging from 0.059 to 0.163 cm; correlation analysis indicated the existence of a positive relationship both with *TL* ($r = 0.491$; $p < 0.05$) and with age ($r = 0.518$; $p < 0.05$) (Figure 4a and 4b), indicating an upward trend in eggs size as the length and age of the specimen increased.

DISCUSSION

In this study a total of 547 specimens of *S. squalus* from the Assino creek were examined. The sex ratio was 1.82:1 (*M*:*F*), differing significantly from 1:1. Generally, the ratio of males to females is higher in the early stages of life, while at later stages the situation is reversed, with females becoming more abundant (Nikolsky, 1963). This is also consistent with the characteristics of *S. squalus*, in that females live longer, prevailing in the age-classes above 6 years (Gandolfi *et al.*, 1991). This situation was confirmed in the present study, as the sex ratio strongly favored males in the younger age-classes and females in the older age-classes; indeed, all specimens that had lived through more than 7 winters were females. Similar patterns have been found in many other studies (Öztaş and Solak, 1988; Altindağ, 1996; Ekmekçi, 1996; Bianco and Santoro, 2002; Erdoğan *et al.*, 2002; Koç *et al.*, 2007) conducted in Europe on species belonging to *Squalius cephalus complex*, of which *Squalius squalus* represents the only species present in Italy. Since information on the reproductive biology of Italian chub is scant and generic, the results yielded by this research were compared with those of previous studies on *Squalius cephalus* in Europe.

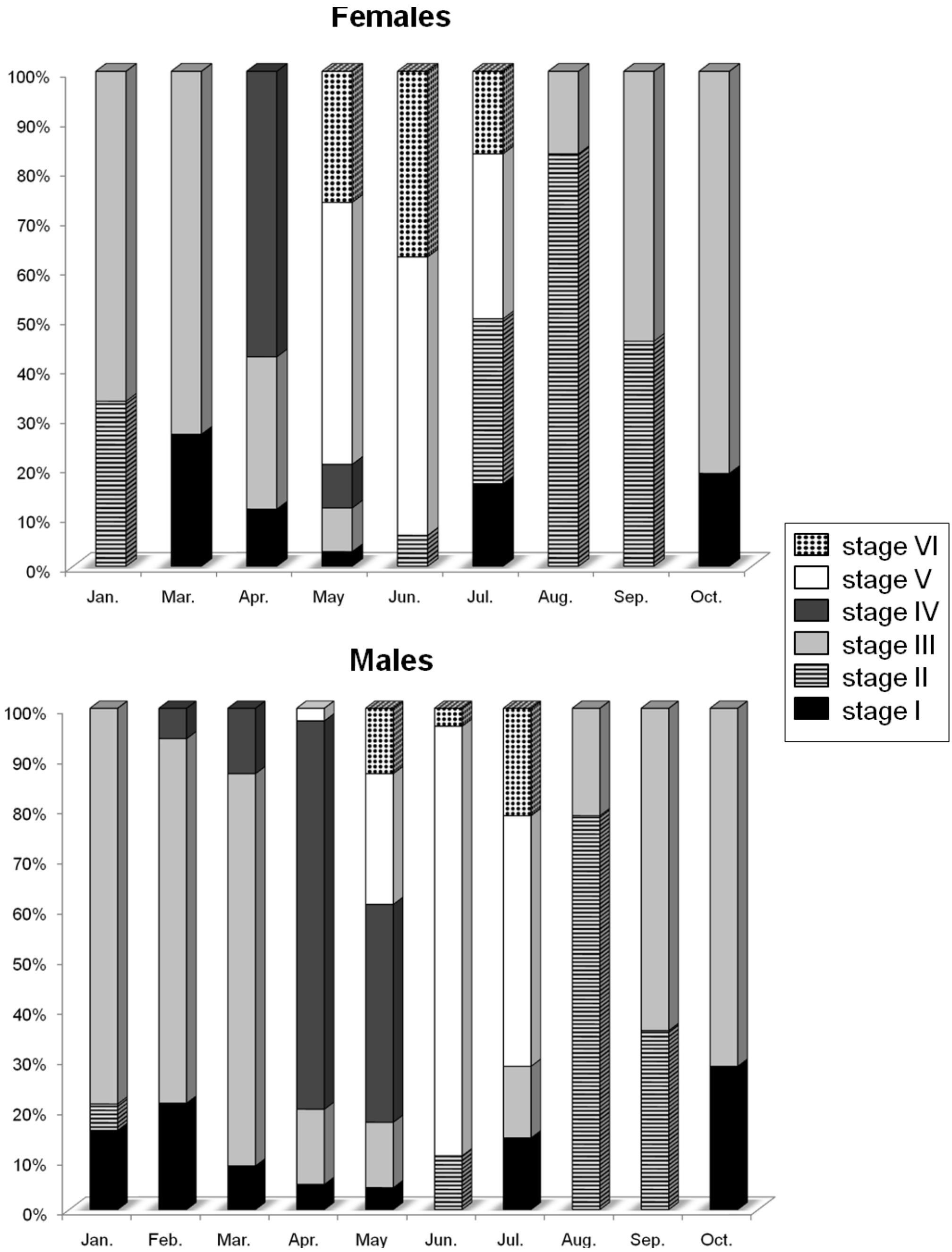

Figure 2
Relative frequency of reproductive stages in the different sampling months in male and female specimens of Squalius squalus from the Assino creek. Reproductive stages: I = immature-inactive; II = immature-developing; III = maturing; IV = mature; V = ripe running; VI = spent.

This imbalance in the sex ratio could also be attributed to the behavior of males in the breeding season. As mentioned above, the Assino creek serves as a reproductive area for chub that migrate from the Tiber river. Males probably spend more time in the creek, swimming up first and waiting for the females to arrive, while females immediately leave the breeding site once they have laid their eggs.

Sexual maturity is reached after the second winter of life (2+ age-class) in both sexes. This situation is similar to that reported for other populations in Southern Europe (Turkey and Greece) (Altindağ, 1996; Ünver, 1997; Şaşi, 2004; Froese and Pauly, 2010) By contrast, sexual

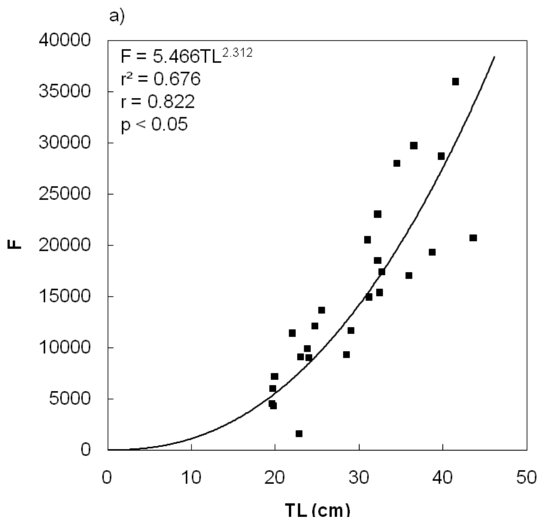

Figure 3
Relationship between eggs number (F) and total length (TL in cm) (a) and between eggs number and age (b) in a subsample of females (n = 26) of Squalius squalus from the Assino creek.

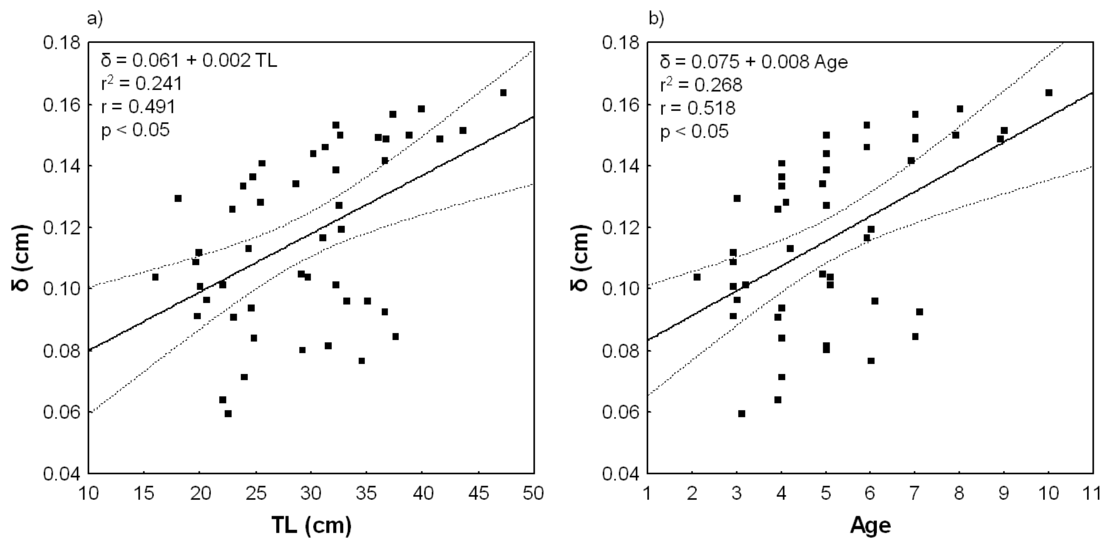

Figure 4
Correlation between eggs diameter (δ in cm) and total length (TL in cm) (a) and between eggs diameter and age (b) in a subsample of females (n = 48) of Squalius squalus from the Assino creek. Dotted lines indicates 95% confidence intervals.

maturity has been seen to occur later in Northern Europe (Mann, 1976; Froese and Pauly, 2010).

Analysis of GSI and of the stage of gonad maturation throughout the year suggests that the reproductive period of the population extends over a long time (April–July), with males maturing slightly earlier than females. The ovaries of these females did not show eggs at different stages of maturation, but contained batches of large yolky oocytes (vitellogenic oocytes) (Bagenal, 1978) that would probably have been spawned in the next few days. Although the chub adopts a multiple spawning strategy in many habitats (Bolland *et al.*, 2007), in the Assino creek it displays the synchronous ovogenesis that is characteristic of monospawner fish, in which each female spawns only one batch of eggs per season (Kestemont and

Philippart, 1991). Therefore, the long reproductive period was not due to the asynchronous ovogenesis characteristic of multispawner fish, whereby each female spawns repeatedly over several months (Kestemont and Philippart, 1991), but rather to the fact that some females spawn earlier and others later in the season (Vila-Gispert and Moreno-Amich, 2000).

The spawning season appears to be similar to that observed by some authors in Turkey (Şen, 1988; Kalkan *et al.*, 2005) while it starts earlier and lasts longer than the period reported by several other authors (Mann, 1976; Ünver, 1997; Şaşi, 2004; Koç *et al.*, 2007; Şen and Saygin, 2008). This could represent an alternative strategy that offers advantages at the population level; in highly variable environments like the Assino creek, rapid changes in conditions could affect the reproductive event, if it lasts a short time. Protracted spawning events result in the production of young-of-the-year chub from the spring until the summer (Bolland *et al.*, 2007); this is confirmed by the significantly intra-population *TL* variation in the back-calculated lengths of chub at age 1 (Pompei *et al.*, 2011).

In *S. squalus*, eggs number correlates significantly with fish length and age larger and older fish being more fecund (Libovarsky, 1979; Erk'akan and Akgül, 1986; Ekmekçi, 1996; Karatas and Akyurt, 1997; Erdogan *et al.*, 2002). Relative fecundity displays a marked downward trend as a function of the length and age of the females while egg diameters in females captured just prior to spawning increase with length and age This implies that the quality of eggs tends to improve with growth at the expense of quantity; larger eggs with more abundant yolk will give rise to larger larvae, thus improving their chances of survival (Mann, 1991).

Many aspects of the biology of the *S. squalus* population in the Assino creek – such as the high reproductive investment of both sexes, the early achievement of sexual maturity and the increase in egg diameter with age and size – can probably be ascribed to the overall strategy developed by the species in order to fit the particular ecological conditions present in the creeks of central Italy. Mediterranean rivers and streams are regarded as highly variable ecosystems because of variations in water flow, water temperature and resource availability throughout the year and environmental variability influences most of the life-history parameters of the fish inhabiting these ecosystems (Wootton, 1984; Bruton, 1989; Fernandez-Delgado and Herrera, 1995; Vasiliou and Economidis, 2005). In these environments, which are characterized by the presence of mortality-independent factors, the fitness of individuals may be secured primarily by engaging in reproductive strategies that maximize reproductive success. An reselected species will have a life-history that is characterized by high energy investment in reproduction, low age at maturity, a high rate of unpredictable mortality and a short lifespan Such species are more likely to be found in fluctuating or harsh environments (Mac Arthur and Wilson, 1967; Balon, 1979; Bruton, 1989).

However the chub is not exclusively associated with this type of environment; it is also found in lentic ecosystems and in stretches of lowland rivers, where environmental conditions are far less strict. Several authors have pointed out that fish inhabiting a wide variety of environments are able to adjust some of their lifehistory traits in response to ecological conditions (Hutchings and Myers, 1994). Thus, in the chub of the Assino creek the reduction in relative fecundity and the increase in egg diameter during aging may be regarded as an adaptation that would confer greater plasticity on its biological characteristics. Species can display trade-offs between the number of offspring and their quality (Fleming and Gross, 1990); a smaller number of offspring may be compensated for by an increased parental investment in their quality and a longer lifespan (Lappalainen *et al.*, 2008). This strategy would enhance post-hatching survival in conditions that can sustain a large number of adults.

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