

Fecundity and annual course of maturation in spirlin, *Alburnoides bipunctatus*

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Received 21 September 2006; Accepted 11 December 2006

Abstract. A total of 79 females of spirlin *Alburnoides bipunctatus* from the Rudava stream (small lowland stream, Western Slovakia) were examined to obtain data on fecundity and annual oocyte development cycle. Within spawning season (mid-April to early July), the reproduction resources seemed to be allocated in its former half more intensively (more yolked eggs present in ovaries compared to the rest of spawning season). For the mean SL = 76 mm and eviscerated body weight = 7.2 g, mean absolute fecundity of females in pre-spawning phase (n=13) was 3020 eggs (SD = 923; 2695 yolked eggs, SD = 1054), and mean relative fecundity 430 eggs/g (SD = 81; 373 yolked eggs, SD = 100). Number of eggs in the right and the left ovary differed significantly in 63.3 % of females, with a maximum difference of 1405 eggs. Two clues, namely 1) no gap in distribution between immature, unyolked oocytes (present in an ovary throughout the whole year), and yolked (vitellogenic) oocytes, 2) no increase in mean diameter of yolked oocytes towards the end of spawning season, suggest that spirlin is a species with indeterminate fecundity.

Key words: spawning season, resource allocation, size class, distribution

Introduction

Spirlin (*Alburnoides bipunctatus* Bloch, 1782) is reported as a “nearly threatened” species in the Red list of lampreys and fishes of Slovakia (H e n s e l & M u ž í k 2001), and it is also protected by national legislation. With respect to reproduction and/or life-history of this species, most attention has been paid to its early ontogeny, and descriptions of environmental factors affecting spawning and spawning behaviour (P e ň á z 1976, 1995, B l e s s 1994, 1996, 2001). However, little information on the species’ fecundity and annual cycle of oocyte development is available.

Spirlin is reported to belong to batch spawners (fractional, portional, heterochronal; P a p a d o p o l & C r i s t o f o r 1980, B l e s s 1996, 2001, B r e i t e n s t e i n & K i r c h h o f e r 2000). It seems that in studies of fecundity in fishes, it is essentially important to start with a correct assumption on whether the species fecundity is determinate or indeterminate. For example D i c k e r s o n et al. (1992), who regarded the fecundity of chub mackerel as indeterminate, found out fecundity almost five times higher (though covering only peak months of the reproductive season) than M a c G r e g o r (1976) who estimated fecundity for the entire reproductive season.

In fact, any attempt to estimate the standing stock of yolked oocytes prior to the onset of spawning season (when the fecundity of a batch spawner is evaluated) only makes sense in case of fish species with determinate fecundity sensu H u n t e r et al. (1985), i.e. if no

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additional yolked oocytes to be released emerge, and the number of yolked oocytes only decreases. In other words, the number of eggs for all batches to be spawned in the following spawning season (disregarding atretic losses) is fixed even before the first batch is released. On the other hand, there is no such a pre-fixed number of eggs in fishes with indeterminate fecundity: immature unyolked oocytes advance continuously into yolked ones throughout the spawning season, and finally may be spawned as mature eggs (H u n t e r et al. 1985). The problem is that the presumption about a seasonally determined fecundity is often false, which may lead to serious bias in biomass estimation (H u n t e r & M a c e w i c z 1985).

Recently, the assumption of indeterminate fecundity has been confirmed for a number of brackish and marine fish species, e.g. northern anchovy *Engraulis mordax* (H u n t e r & G o l d b e r g 1980), chub mackerel *Scomber japonicus* (D i c k e r s o n et al. 1992), goldlined seabream *Rhabdosargus sarba* (H e s p et al. 2004), anchovy *Engraulis encrasicolus* (M o t o s 1996), European hake *Merluccius merluccius* (M u r u a et al. 1998).

However, there has been little information on freshwater fish species in this respect, though, especially for batch spawners with prolonged reproductive season in tropical or temperate climate, indeterminate fecundity may be typical (H u n t e r et al. 1985), as has been proved, for example, for *Notropis lutrensis* (G a l e 1986). Therefore, the aim of this study is to examine fecundity in spirilin, to analyse the annual course of its maturation, and to outline the problems associated with testing the assumption of determinate or indeterminate fecundity.

Material and Methods

In 2002, 18 samples of spirilin females were collected by portable electrofishing gear from the same location on the Rudava stream (a small lowland stream, western Slovakia, r. km 17),

Table 1. The dates of samplings, intervals between samplings, water temperature and number of collected females of spirilin in the Rudava stream.

sample No.	date of sampling (all samples – year 2002)	days after previous sampling	water temperature	No. of females
1	13 February	-	5	4
2	20 March	35	8	4
3	3 April	14	11	4
4	17 April	14	11.5	3
5	2 May	15	15	5
6	7 May	5	13.5	5
7	17 May	10	15.5	5
8	24 May	7	16	5
9	30 May	6	14	5
10	7 June	8	15	5
11	12 June	5	14.5	5
12	25 June	13	17	5
13	3 July	8	17	5
14	6 August	34	17	4
15	27 September	52	11	5
16	17 October	20	10	5
17	22 November	36	10	2
18	16 December	24	2.5	3

on a monthly basis, except for spawning season, when sampling took place more often (Table 1). In total, 79 specimens were examined. During each sampling, water temperature was recorded. Because of the conservation status of spiralin in Slovakia, five mature females maximum were collected for each sample (Table 1).

All samples were preserved in 4% formaldehyde solution, and then standard length (SL) was measured to the nearest 1 mm, and eviscerated body weight weighed to the nearest 0.1 g. Gonadosomatic index (GSI) was calculated from eviscerated female body weight following the routine formula (e.g. Holčík & Hense1 1972). Fecundity was evaluated using the gravimetric method (Holčík & Hense1 1972). The regression equations for absolute fecundity as a function of eviscerated female weight and SL were plotted for females in pre-spawning phase ($n = 13$). Right and left ovaries were processed separately. From each ovary, a tissue sample from the middle part, covering the whole cross-section was extracted, equaling 30 – 50% of the total ovary weight and weighed to the nearest 0.001 g. Since the number of oocytes in the right and left ovary differed considerably, a confidence interval (Miller 2001) was calculated for each female, to find out whether the difference is significant. Differences between right and left ovary at the population level (i.e. whether the number of oocytes is higher in the right or the left ovary for the population as a whole) were tested using a paired t – test. To evaluate the expected decrease in relative fecundity of females (yolked oocytes only) collected in course of full spawning season (May 2 – June 25, 2002), ANOVA and Scheffe tests were used.

Since the shape of a typical preserved oocyte was not spherical, in the following text, the term “diameter” denotes the maximum range of each oocyte. To determine the extent of shrinkage of oocytes preserved in formaldehyde, 50 fresh oocytes were measured in diameter and stored subsequently in 4% formaldehyde solution for 14 days, and then remeasured again. No statistically significant difference was found (paired t -test; $P = 0.15$), and thus, subsequently, only fixed oocytes were analyzed. For the diameter analysis, 50 randomly chosen oocytes from each (right and left) ovary were measured using an ocular micrometer. Opaque eggs larger than the mean size, i.e. 0.65 mm (0.56–0.73 mm), were considered “yolked”, and the number of yolked oocytes was calculated based on their proportion in the samples of oocytes examined. Egg size groups, inflection points and empty intervals were then identified from the scatterplots, following the oocyte size distribution (e.g. Fig. 1).

Results

The individuals examined ranged from 52 mm to 92 mm SL (mean = 76 mm, SD = 0.8, $n = 79$), attaining weight from 2.6 to 13.8 g, (mean = 7.1 g, SD = 2.1). No significant difference in SL and weight among all 18 samples was found (SL: ANOVA, $P = 0.76$, weight: Kruskal-Wallis test, $P = 0.40$).

Macroscopical indication of spawning in progress (spawning tubercles, leaking gonadal products) was apparent only in fish collected from May 2 to June 25 (water temperatures 15 °C and 17 °C, respectively). However, the analysis of egg diameter distribution indicated quite protracted spawning season, lasting approximately from mid-April (April 17, see Fig. 1a) to early July (July 3, see Fig. 1b).

Absolute fecundity of all studied females ranged from 975 to 5206 eggs (0 to 4892 yolked eggs), relative fecundity from 176 to 586 eggs/g (0 to 560 yolked eggs/g). A positive linear regression was found both between absolute fecundity and SL and absolute fecundity and eviscerated body weight ($P < 0.01$ and $P < 0.001$, respectively, $n = 13$). However,

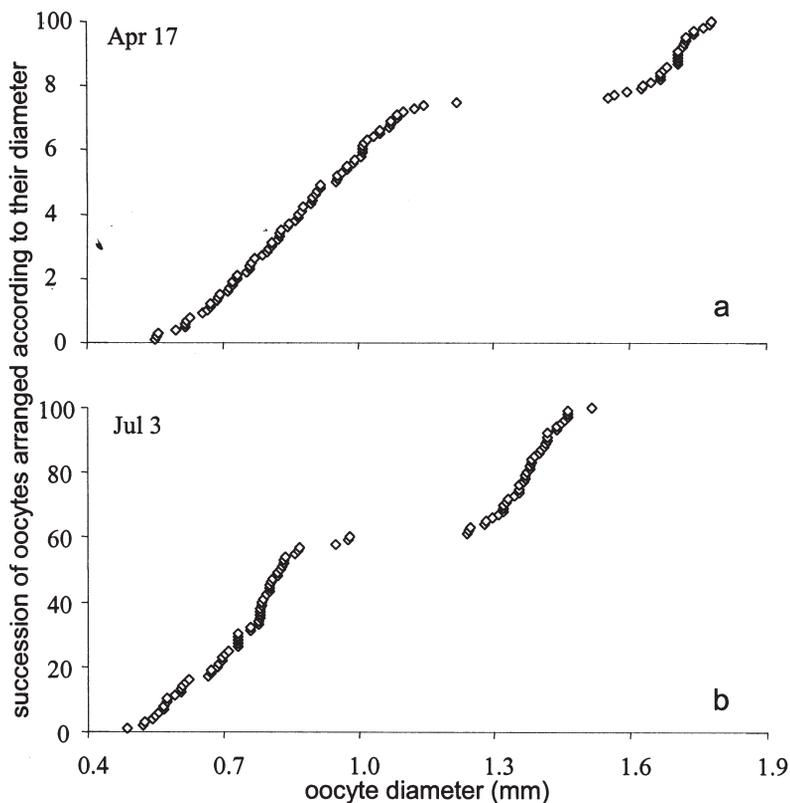


Fig. 1. An example of oocyte size distribution scatterplot of spiralin females (Rudava stream) collected at the beginning (a) and the end (b) of spawning season, both capable to release the spawning batch.

according to coefficients of determination, eviscerated body weight explained the number of oocytes in an ovary slightly better than SL ($r^2 = 0.66$ and $r^2 = 0.52$, respectively; Fig. 2).

GSI ranged from 1.6 to 36.4. Seasonal variation in absolute and relative fecundity was most obvious when only the yolked eggs were considered, with a sharp decrease after the spawning period, and the same pattern was observed in GSI (Table 2, Fig. 3). Number of eggs in the right and the left ovary differed significantly in 63.3 % of females, with mean difference 215 eggs (SD = 216) and maximum difference 1405 eggs. However, it is not possible to conclude which of the ovaries (left or right) contained more eggs, because the ovary size was not firmly associated with its left/right position, and moreover, taken the population sample as a whole, the number of eggs in the ovaries did not differ (paired t-test, $P = 0.75$).

Two different patterns in mean relative fecundity were obvious during the full spawning season (May 2 – June 25). In four subsequent May samples, the mean relative fecundity of yolked oocytes was decreasing considerably from sample to sample (ANOVA, $P < 0.001$), whereas at the end of May (May 30) and in June it appeared to stabilize at more-less constant level (ANOVA, $P = 0.05$, Fig. 4).

The diameter of all measured oocytes ranged from 0.20 mm to 1.96 mm (the smallest eggs were translucent and did not contain yolk, the large eggs were yellowish and filled with yolk), with the mean 0.95 mm, whereas the mean diameter of yolked (vitellogenic) oocytes varied seasonally from 0.68 mm to 1.20 mm (Fig. 5).

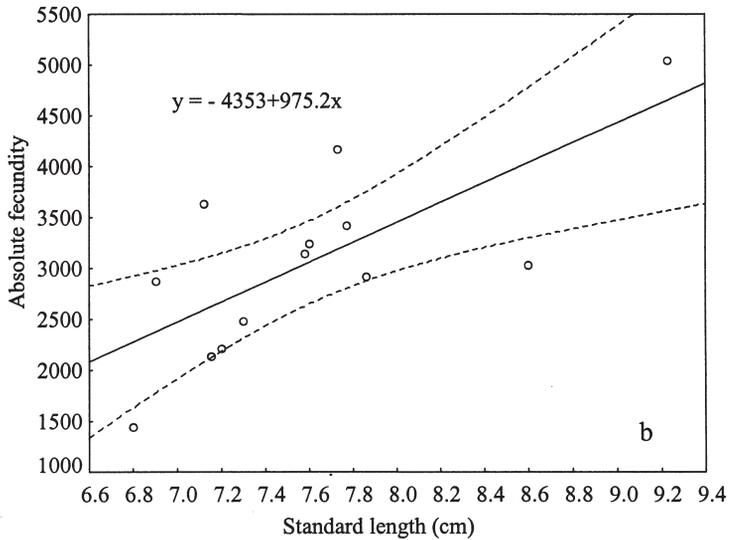
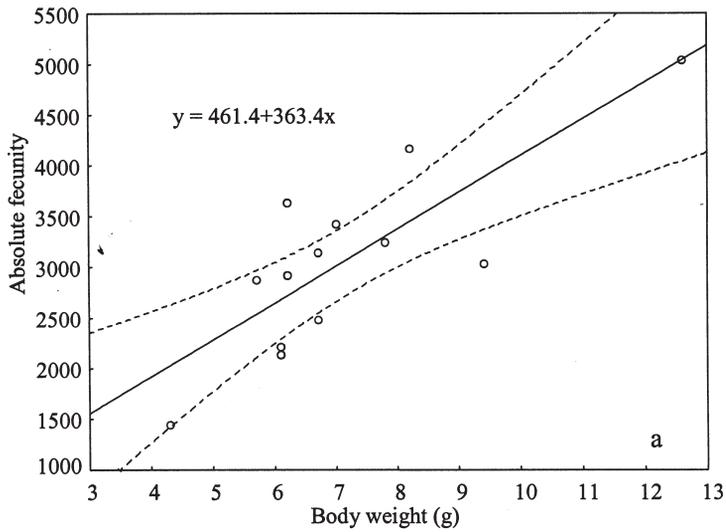


Fig. 2. Regression equations and plots of absolute fecundity (y-axis) against eviscerated body weight (a) and SL (b; both x-axis) with 95% confidence interval delineated.

There was no gap in the size-distribution between the standing stock of oocytes (immature, present in ovary within the whole year) and yolked oocytes. The size-distribution plots (Fig. 6) suggest that, in general, the reproductive cycle of spiralin females can be divided into 4 phases:

Pre-spawning phase

The samples collected from February to April can be characterized by gradual ripening of ovaries as the spawning season approaches. The mean diameter increases from 0.81 mm in December to 1.06 mm in early April (Fig. 5). The “gap” in the oocyte size-distribution

Table 2. Gonadosomatic index (GSI), absolute (AF) and relative (RF, number of eggs/g) fecundity of spiralin from the Rudava stream according to the phase of reproduction cycle. (_a = all oocytes included, _y = only yolked oocytes included).

Season:	pre-spawning	spawning	post-spawning	resting
Dates of sampling:	February – April 17 (1 female)	April 17 (2 females) – July 3 (1 female)	July 3 (4 females) – August	September – December
n	13	43	8	15
AF _a	min	1445	975	1429
	max	5043	3808	3280
	mean	3020	2348	2147
	SD	923	757	751
RF _a	min	323	176	211
	max	586	583	550
	mean	430	332	355
	SD	81	99	117
AF _y	min	1084	810	0
	max	4892	3580	1580
	mean	2695	2180	515
	SD	1054	725	629
RF _y	min	242	160	0
	max	551	560	330
	mean	373	307	98
	SD	100	91	131

(that appeared for the first time in December; see below) moves towards higher values. The oocytes grow gradually (Fig. 6a, b, c).

Spawning phase

Due to asynchronous spawning, the fish collected during spawning season show diverse stages of oocyte development. According to the state of readiness for spawning, females of spiralin could be divided into three subgroups:

The first subgroup consisted of females prepared for immediate or soon spawning (Fig. 6d). These females contained 2 or 3 distinguishable oocyte size-classes, separated by an empty (or with only a few scattered oocytes) interval in the oocyte size-distribution, the „gap“, and/or by an inflection point. The largest size-class (I) included the most advanced oocytes, which were separated from the other oocytes by a distinct gap (Fig. 6d). The diameter of this size-class of oocytes ranged from 1.33–1.59 mm (mean = 1.46 mm, SD = 0.07, n = 24) to the maximum values, i.e. 1.8 mm, approximately. Presumably, the most advanced size-class contained one single spawning batch. If we adopt this assumption, then the spawning batch consisted of 19 to 51% (mean = 35.3 %, SD = 8.63, n = 24) of total number of oocytes present in the ovary. In the size-class II, the diameter of the largest oocytes ranged between 1.01–1.33 mm (mean = 1.13 mm, SD = 0.06, n = 24). This size-class comprised of 31–68% (mean = 46.31, SD = 10.55, n = 16) of all oocytes present in the ovary and represented the recruitment pool for the next spawning batch, i.e. these oocytes could be potentially spawned within the given spawning season. In some females, oocytes of size-class III were also apparent. This size-class consisted of oocytes with diameter up to

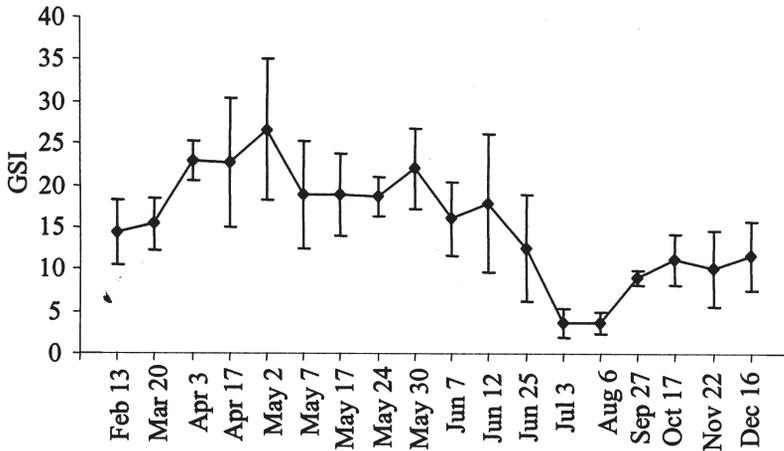


Fig. 3. Seasonal variation in the mean GSI (\pm SD) of spiralin females from the Rudava stream.

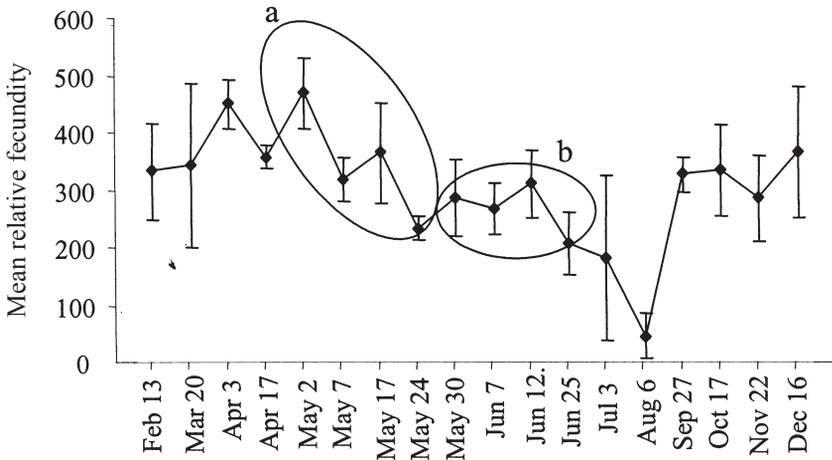


Fig. 4. Seasonal variation in the mean relative fecundity (eggs/g, \pm SD) of spiralin females from the River Rudava (only yolked oocytes included). The ovals highlight decreasing tendency in the first half of the full spawning season (a) and the flat trend in its rest (b).

0.63–0.9 mm (mean = 0.75 mm, SD = 0.06, n = 16) and comprised 6 – 20 % (mean = 11.8 %, SD = 4.20, n = 16) of the oocytes present in the ovary during spawning season (Fig. 6d). This class was not clearly distinguishable in every female collected during the spawning season; however, the existence of this class is also supported by a characteristic look of ovary in females during the post-spawning phase (see below).

The distribution of oocytes in the ovaries of females of the second subgroup indicates that the most advanced oocytes (size-class I) have been already released and the next batch has not been yet recruited (Fig. 6e). In other words, the oocytes of size-class I are absent, and therefore, the maximum size of the oocytes present in ovaries does not exceed 1.07–1.2 mm (mean = 1.16 mm, SD = 0.05, n = 6), which, at the same time represents the edge of the distinct gap between the size-classes I and II described in females of the previous subgroup (see above).

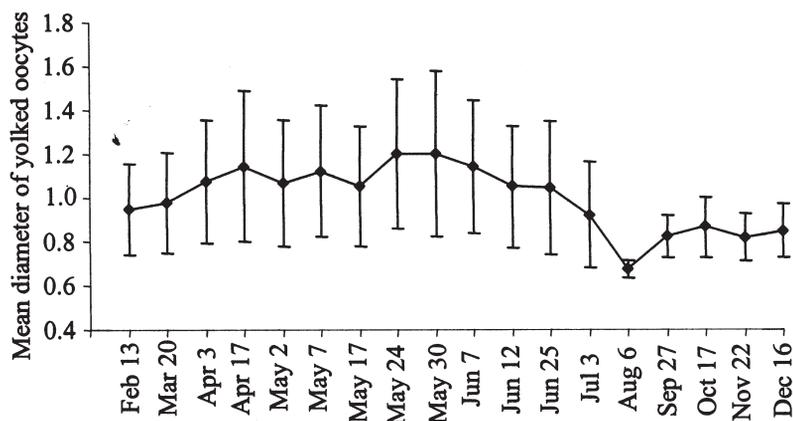


Fig. 5. Seasonal variation in the mean diameter of oocytes (\pm SD) of spiralin females from the Rudava stream (in mm).

Females of the third subgroup were very similar to those of the first subgroup but these were in a less advanced state of ripeness (in terms of oocyte size-class distribution), and thus not prepared for immediate spawning. The gap between the size-classes I and II, so obvious in females of the first subgroup, was shifted towards lower values (mean min. = 0.98, SD = 0.07, mean max. = 1.23, SD = 0.1, n = 14) and not as distinct (i.e. more oocytes scattered in the gap, which was typically empty in the first category). The maximum oocyte diameter attained 1.4 – 1.6 mm, and the most advanced oocyte size-class I seemed just like being recruited from oocytes of the size-class II defined in the first subgroup of females (Fig. 6f).

Post – spawning phase

Spiralin collected after the spawning season (i.e. in July and especially in August) demonstrated different characteristics compared to those sampled during the spawning season. In July, 3 out of 5 fish ovaries still contained yolked oocytes, however, only one female was likely to release one more spawning batch (Fig. 1b), whereas the others appeared already undergoing atretic process (Fig. 6g). The mean diameter decreased by about 0.3 mm compared to the last June sample (June 25).

In August, virtually none of the ovaries analyzed contained yolked oocytes, and the mean diameter was even 0.48 mm lower than that of the sample from June 25 (Fig. 5). The absence of yolked oocytes suggests the continuing process of atresia and thus we consider the remaining oocytes “standing stock of oocytes” present in the ovary of spiralin throughout the year (Fig. 6h). In some females, this size class of the smallest oocytes could also be recognized (delineated by an inflection point) in samples collected during spawning season (Fig. 6d).

Resting phase

In September (Fig. 6i), the mean diameter of oocytes increased by 0.28 mm compared to the previous month but it remained more-less constant in the subsequent period (October, November and December; Fig. 6j, k, l), and no significant increase was apparent until April (Fig. 5). However, the formation of the gap in oocyte size-distribution was first observable in December (Fig. 6l).

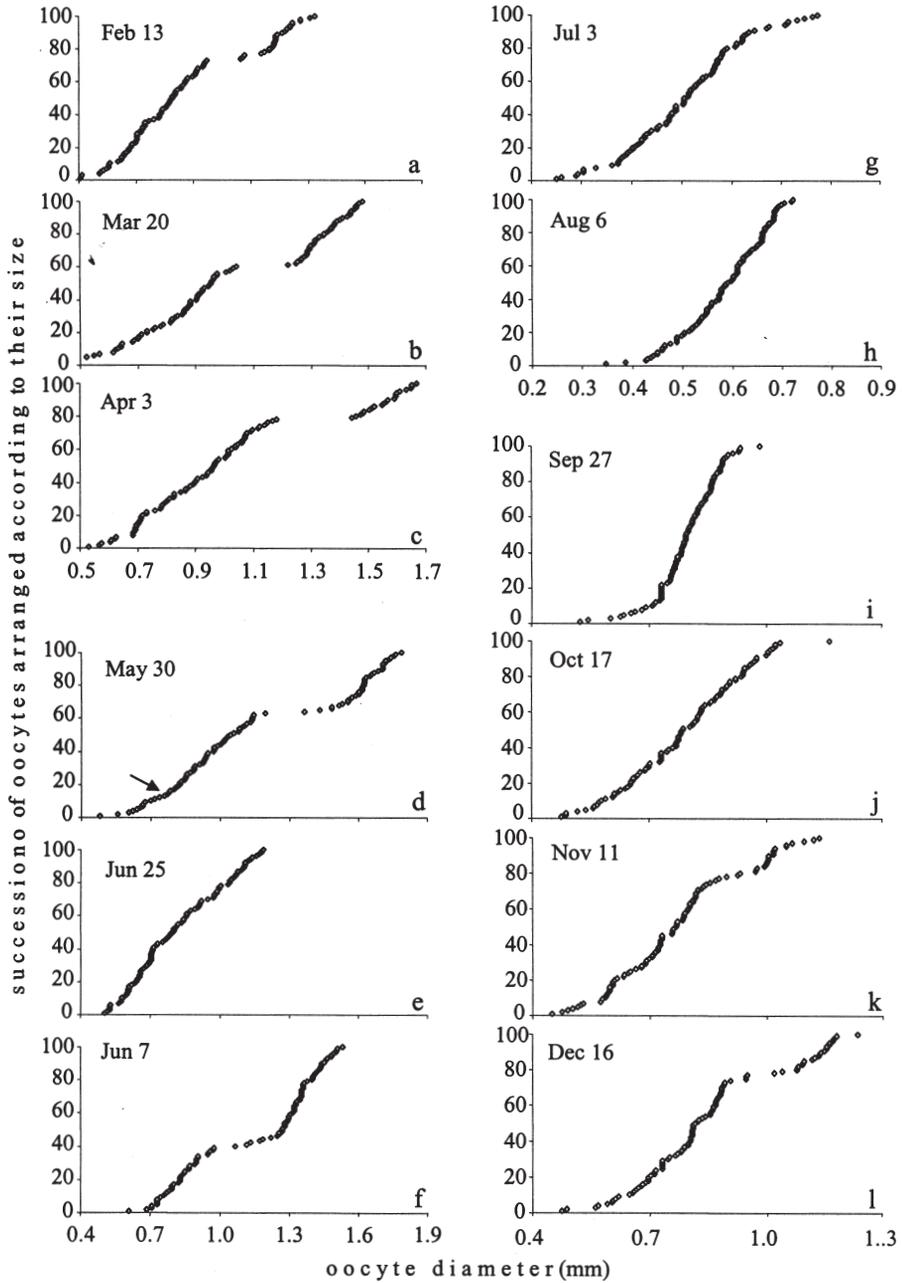


Fig. 6. Examples of oocyte size distribution (in mm, x-axis). The oocytes are size-arranged and numbered (y-axis): females in the pre-spawning phase (a, b, c). Females in spawning phase: A female in the state of full ripeness, ready to release the spawning batch. The arrow marks the inflection point separating the oocyte size classes II and III (first subgroup; d). A female soon after having released her spawning batch (second subgroup; e). A female recruiting the next batch (third subgroup; f). Females in post-spawning phase: In July, ovaries still contained a few yolked oocytes (g) whereas in August, only the standing stock of oocytes was present (h). Females in the resting phase: the oocytes grew slowly and approached the characteristic look of oocyte size-distribution of the females in pre-spawning phase (i, j, k, l).

Discussion

Absolute fecundity of spiralin from the River Rudava varied within a range similar to that of the samples from the river Radimna in Romania, where it attained 1581 – 6110 eggs (Papadopol & Cristofor 1980). In contrast, spiralin *Alburnoides bipunctatus fasciatus* (Nordman, 1840), which seems to be a synonym for *Alburnoides bipunctatus* (Bloch, 1782), (see Kottelat 1997), from the Oltu stream (Turkey, $n = 58$) has been reported to attain mean absolute fecundity 13 135 eggs (Yildirim et al. 1999). Even if the bigger mean size of the Turkish females is considered (mean fork length 10.3 cm, mean weight 18.9 g), the difference between the two populations is striking – mean absolute fecundity of spiralin from the Oltu stream appears to be 4.35-times higher than that of spiralin from Rudava.

Although the difference in the number of oocytes between ovaries, such as found in some females of spiralin examined, is not typical for cyprinid fishes, it has been recorded previously, e.g. in ziege *Pelecus cultratus* (Balon 1956). Nevertheless, evaluation at the whole sample level (no significant difference) suggests that this asymmetry is a subject of individual variation in ovary growth rate rather than adaptation.

Our oocyte diameter distribution analysis indicate that the reproductive season of spiralin from the Rudava stream is quite protracted (late April – early July), a reproduction feature typical for batch spawners. This finding is consistent with observations made in the laboratory, where outdoor conditions were simulated (Bless 1996), and also with field data of Dyk (1952) and Peňáz (1995) gathered from a similar geographical range. It appears, that within the spawning season, most of reproduction resources are allocated in its first half (Fig. 4). This may be due to more favorable environmental conditions caused by an increased water flow, especially in early or middle spring, typical for lowland rivers in the area, where our study was carried out. Being a litophilous species (Balon 1975, 1981, Holčík 1989), spiralin is not capable to use flooded riparian vegetation as spawning substrate, but rather thrives on increased food availability and suitable nursery habitat for offsprings (Dewey & Jennings 1992).

According to the concept of fecundity determination, indeterminate fecundity is supposed to be especially characteristic for batch spawning fish species with prolonged spawning season (Hunter et al. 1985). Being restricted by determinate fecundity hardly bears any advantage for a batch spawner, rather opposite, it may reduce its capability to profit from potential increase in food availability during spawning season, and to convert it into its own reproduction success. Therefore, from an evolutionary point of view, batch spawners with determinate fecundity may represent an intermediate step between total spawners (releasing all eggs in a single batch) and batch spawners with indeterminate fecundity.

With regards to the methods used in our study, three points, that may potentially suggest the existence or non-existence of seasonally determined fecundity in spiralin, should be discussed. Firstly, it is the gap in distribution between immature, unyolked oocytes (present in an ovary throughout the whole year), and yolked (vitellogenic) oocytes, which indicates that the fecundity of a species is seasonally determined (e.g. Hunter et al. 1985, 1989, Murua et al. 1998). Even at the end of spawning season (late June and early July, when the gap between immature and yolked oocytes should be the most apparent if spiralin had determinate fecundity) no such a gap forming was observed. This is not to say that no gap in size-distribution of oocytes was found, however, there is no doubt that this gap separates two size-classes of yolked oocytes – spawning batch (size-class I) and its recruitment pool (size-class II, in some cases also size-class III) .i.e. it is not the gap (“hiatus”) sensu Hunter et

al. (1985, 1989). The oocytes below the gap (i.e. size-class II) do not occur in the ovaries all year round (Fig. 6g, h). Moreover, the presence of females of the second subgroup (see above) indicates that oocytes of the size-class I represent just a single spawning batch. It seems that spiralin's most advanced oocyte size-class I corresponds to "hydrated" oocyte group sensu Hunter et al. (1985), though they totally lack translucent appearance and hence are not "easily recognized" (Murua et al. 1998). On the other hand, the lack of gap is not a strong evidence for indeterminate fecundity, whereas its presence is strong evidence for determinate fecundity (Murua et al. 1998).

Secondly, as the spawning season proceeds, for batch spawners with determinate fecundity, an increase in mean diameter of yolked oocytes should be characteristic (Hunter et al. 1992). Our results show both increase and decrease (Fig. 5). The absence of increasing tendency may be 1) accidental due to a low number of samples, 2) a consequence of fluctuation due to asynchronous spawning of spiralin, or 3) caused by a recruitment of new yolked oocytes from the "standing stock" of unyolked oocytes, i.e. the indeterminate fecundity feature.

Thirdly, in fish species with determinate fecundity the number of yolked oocytes should gradually decrease with the ongoing spawning season (Hunter et al. 1992). But, logically, when approaching the termination of spawning, even fish with indeterminate fecundity may reduce the number of their oocytes (otherwise there would be no reason to stop spawning). In our study, the mean relative fecundity of yolked oocytes in samples collected later in the spawning season seemed to decrease or rather fluctuate. Nevertheless, we presume that the decreasing number of yolked oocytes during spawning season would be only a very little evidence for determinate fecundity unless it was really striking. Summing up, the absence of the gap, and no increase in mean diameter of the oocytes supports the idea that spiralin has indeterminate fecundity.

Acknowledgements

We would like to thank to S. Katina for suggestions on the statistical processing of data. We also thank to S. Sírýová and J. Toměček for field assistance.

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