

## Similarities and differences in the reproductive biology of loaches (*Cobitis* and *Sabanejewia*) under laboratory conditions

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**Abstract.** Loaches from 12 populations (two *Sabanejewia*, one *Iberocobitis*, two *Bicanestrinia* and seven *Cobitis* s.str.) were bred under comparable conditions in aquaria. Both *Sabanejewia* spread very small (diameter 1.0–1.2 mm), sticky eggs all over the aquarium, while all *Cobitis* spawned into the most dense vegetation. Eggs of *Cobitis* s.str. and *Bicanestrinia* were large (2.0–3.4 and 2.0–2.5 mm, respectively) and unsticky. The eggs of *Iberocobitis* were intermediate sized (1.7 mm) and slightly sticky. Significant differences in egg size were found between most species. Eggs diameter was correlated to size of hatchlings (Spearman rank coefficient 0.85,  $p < 0.001$ ). The mean number of eggs per spawning act ranged between species from 246 to 2058 and the number of spawning acts per female from 1 to 18. In at least three species a change of adult coloration during spawning occurred. Spawning behaviour was identical in at least four species of *Cobitis*. The largest differences in reproductive biology were found between genera and the second largest between subgenera. Some characters were species-specific. Consequently, features of reproductive ecology may characterise taxonomic units of loaches.

**Key words:** mating behaviour, spawning substratum, egg size, early life stages

### Introduction

Loaches one of the most important features within the life-history of fish. Therefore, each species has developed its specific characteristics in reproductive biology (Blon 1975). These specific adaptations have a great impact on the ecology of the species. Besides these highly species-specific characteristics, some general features of reproductive biology are usually shared by groups of related species. On the other hand, the reproductive guilds may be similar because of a similar ecology of non-related species.

In the present study, some features of reproductive biology of different species of spined loach are compared in order to identify the general pattern that is common to genera or subgenera. The species-specific differences mark differences in the ecology of the species.

### Material and Methods

Loaches of the genera *Cobitis* and *Sabanejewia* from 12 populations were bred under comparable conditions in aquaria. The origin of the fishes as well as the number of females is listed in Table 1. All females were well fed and nearly of maximum size. The fish from Lake Müggelsee belonged to a diploid-polyploid complex, while the fish from the R. Hunte karyotypically were *C. taenia* (Ráb, pers. com.). Based on the pigmentation pattern, spined loach from Bavaria resembled *C. elongatoides*. The taxonomic state of *Cobitis* from the Rhine basin and from China remains unsolved (Nalbant, pers. comm.).

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One or two pairs were kept per tank to insure an individual recognition of the animals. Details of the methods for breeding the fish and measuring eggs and hatchlings are given by B o h l e n (1999). The diameter of yolk and chorion and the length of the hatchlings were compared using one-way ANOVA (SPSS-software,  $p < 0.05$ ). Spawning was observed by eye in *C. taenia*, *C. bilineata*, *C. paludica* and *C. fahireae*. In *S. vallachica* and *Cobitis* sp. from China specimens were observed immediately after spawning. In these species, a comparison of the coloration between spawning and non-spawning condition was possible. Species were checked for a preference of spawning substratum by counting the eggs released in different areas of the aquarium.

## Results

Spawning was similar in all *Cobitis* species that were observed by eye. All males in the tank followed the female, the female penetrated into dense vegetation, stopped and one male embraced the female. The female started swimming and the circle began again, often with another male embracing the female. However, some differences in the intensity of male courtship were observed between species. Males of *C. bilineata* often stayed at the spawning substratum and waited for the female, while males of *C. paludica* followed the female vigorously without leaving her. Courtship intensity of male *C. taenia* and *C. fahireae* was intermediate.

In one occasion a sneaking behaviour was observed in *C. bilineata*. In a video-taped spawning a male could be watched passing very quickly by a couple in embrace. When its anal opening was close to the couple, it directed the ventral side of the body toward the couple and obviously released sperm.

For oviposition, the female swam into a bunch of moss placed in each aquarium. Nearly all eggs of *Cobitis* were found in this dense vegetation (Table 2). In contrast, eggs of *S. sp.* and *S. vallachica* were found in a similar proportion on sandy bottom, on gravel bottom, in moss, attached to large stones and in empty boxes. Eggs of *Sabanejewia* were smaller than eggs of *Cobitis* and were sticky. Within the genus *Cobitis*, only the eggs of *C. paludica* were sticky. The size of the eggs and the hatchlings differed significantly between most of the populations (Table 2). A clear correlation between egg diameter and size of hatchlings was found (Spearman rank coefficient 0.85,  $p < 0.001$ ). Since the eggs of *Sabanejewia* were scattered in the aquarium and stuck to dirt and sand, it was not possible to collect eggs quantitatively. In *Cobitis*, the egg number could easily be estimated (Table 2). A change of coloration was observed in *C. bilineata* and *C. fahireae*. During the reproductive season, male *C. bilineata* bore striped second and fourth Gambetta zones (= L 3 and L 5 of T a k e d a & F u j i e 1945), while during the rest of the year they were spotted like the females. In *C. fahireae*, the males became very intensely pigmented, while ripe females appeared paler. In *C. paludica*, a change of coloration was observed only during spawning activity. The female became very intensely pigmented and the males became pale. This spawning coloration disappeared within a few hours after spawning activity. During the day prior to spawning, male *Cobitis* increased their swimming activity, whilst the females were mostly buried in the sand.

The embryonic and larval development in all investigated species of *Cobitis* was similar to the descriptions by K o c h a n o v a (1956). In all *Cobitis* species, the embryos developed long external gill filaments, a rich vascularisation of the fin fold as well as a broad head (Fig. 1.A). Coloration and pigmentation of the embryos varied between species. For example, in *C. bilineata* two types of pigmentation intensity were observed (B o h l e n 1998), *C. taenia* was the only

**Table 1.** Taxonomic state, origin and number of females under observation of spined loach species considered in the present study.

Species	Subgenus	Locality	River system	Number of females
<i>Sabanejewia spec.</i>	-	R. Stryy, south-west Ukraine		4
<i>S. vallachica</i>	-	R. Ialomitza, central Rumania		3
<i>Cobitis paludica</i>	<i>Iberocobitis</i>	R. Murtiga, central Spain		2
<i>C. taenia</i>	<i>Cobitis s. str.</i>	R. Hunte, north-west Germany		22
<i>C. sp. from Rhein</i>	<i>Cobitis s. str.</i>	R. Rhine, western Germany		1
<i>C. sp. from Müggelsee</i>	<i>Cobitis s. str.</i>	L. Müggelsee, north-east Germany		3
<i>C. sp. from Bavaria</i>	<i>Cobitis s. str.</i>	pond near Erding, south Germany		1
<i>C. bilineata</i>	<i>Cobitis s. str.</i>	L. Garda, northern Italy		8
<i>C. sp. from China</i>	<i>Cobitis s. str.</i>	pet shop, "from China"		2
<i>C. fahireae</i>	<i>Cobitis s. str.</i>	Channel at Bergama, western Turkey		3
<i>C. turcica</i>	<i>Bicanestrinia</i>	L. Beysehir, Anatolia, Turkey		4
<i>C. simplicispina</i>	<i>Bicanestrinia</i>	Spring at Kirk göz, southern Turkey		2

**Table 2.** Features of the reproductive biology of different species of spined loach: In the first row the mean \* SD is given, in the second row the range and in the third row the number of cases.

Species	Spawning substratum	Change of coloration at spawning	Eggs sticky	Diameter yolk (mean $\pm$ SD, n)	Diameter chorion (mm)	Length hatchling (mm)	Eggs per portion (median, range, n)	Portions per female per year (median, range, n)
<i>S. sp.</i>	spreading without preference for substratum		yes	0.79 $\pm$ 0.03 0.73 – 0.90 n = 30	1.15 $\pm$ 0.05 1.05 – 1.26 n = 30	3.67 $\pm$ 0.37 3.14 – 4.22 n = 40	<100 to a few hundred n = 6	
<i>S. vallaichica</i>	spreading without preference for substratum	no	yes	0.71 $\pm$ 0.02 0.66 – 0.74 n = 40	1.03 $\pm$ 0.04 0.94 – 1.09 n = 40	3.19 $\pm$ 0.12 3.00 – 3.37 n = 20	<100 to a few hundred n = 8	
<i>C. paludica</i>	dense vegetation	yes	yes	1.12 $\pm$ 0.05 1.02 – 1.24 n = 100	1.71 $\pm$ 0.08 1.53 – 1.86 n = 120	4.20 $\pm$ 0.22 3.82 – 4.80 n = 158	1019 228 – 2240 n = 12	4.5 3 – 7 n = 4
<i>C. taenia</i>	dense vegetation	no	no	1.10 $\pm$ 0.08 0.53 – 1.29 n = 1440	2.49 $\pm$ 0.24 1.49 – 3.14 n = 1440	4.97 $\pm$ 0.34 4.08 – 5.85 n = 452	278 62 – 1156 n = 106	11 1 – 18 n = 12
<i>C. sp. from Rhein</i>	dense vegetation		no	1.32 $\pm$ 0.04 1.22 – 1.35 n = 20	3.14 $\pm$ 0.14 2.71 – 3.30 n = 20	6.05 $\pm$ 0.08 5.95 – 6.23 n = 20	611 n = 1	
<i>C. sp. from Müggelsee</i>	dense vegetation		no	1.27 $\pm$ 0.11 1.03 – 1.52 n = 60	2.75 $\pm$ 0.22 2.28 – 3.04 n = 66	5.88 $\pm$ 0.30 5.27 – 6.31 n = 20	128 n = 1	
<i>C. sp. from Bavaria</i>	dense vegetation		no		3.13 $\pm$ 0.22 2.81 – 3.56 n = 20	5.63 $\pm$ 0.21 5.33 – 5.98 n = 7	43 n = 1	n = 1
<i>C. bilineata</i>	dense vegetation	yes	no	1.21 $\pm$ 0.09 0.99 – 1.40 n = 160	2.67 $\pm$ 0.30 2.05 – 3.44 n = 160	4.98 $\pm$ 0.23 4.60 – 5.53 n = 74	505 212 – 1650 n = 17	2 2 – 7 n = 6
<i>C. sp. from China</i>	dense vegetation	no	no	1.13 $\pm$ 0.02 1.10 – 1.18 n = 20	1.95 $\pm$ 0.06 1.82 – 2.04 n = 20	4.33 $\pm$ 0.12 4.08 – 4.51 n = 20	2058 - n = 1	n = 2

Table 2. continued

Species	Spawning substratum	Change of coloration at spawning	Eggs sticky	Diameter yolk (mean $\pm$ SD, n)	Diameter chorion (mm)	Length hatching (mm)	Eggs per portion (median, range, n)	Portions per female per year (median, range, n)
<i>C. fahitreae</i>		yes	no	1.29 $\pm$ 0.03	3.21 $\pm$ 0.07		390	2
				1.25 - 1.39 n = 20	3.07 - 3.33 n = 20		171 - 608 n = 2	- n = 1
<i>C. turcica</i>	dense vegetation		no	1.08 $\pm$ 0.04	2.02 $\pm$ 0.04	4.32 $\pm$ 0.07	604	2
				1.03 - 1.15 n = 40	1.94 - 2.16 n = 40	4.18 - 4.46 n = 20	307 - 901 n = 2	n = 1
<i>C. simplicispina</i>	dense vegetation		no	1.21 $\pm$ 0.09	2.49 $\pm$ 0.12		755	
				1.07 - 1.48 n = 20	2.30 - 2.79 n = 40		309 - 1200 n = 2	n = 2

with a ventral row of spots, the embryos of *C. paludica* had only few dark pigments and a slightly orange coloration. Embryos of *Sabanejewia* did not have external gill filaments nor a vascularisation of the finfold (Fig. 1.B). Their heads were laterally compressed as in juveniles and adults, but bore a remarkable transparent bump. The structure and function of this bump remains unclear.

### Discussion

The mating behaviour was similar between the species and in close agreement with the descriptions by K n a c k (1960) and L o d i & M a l a c a r n e (1990). At the present state of knowledge, the mating behaviour with the conspicuous embrace is a common character to

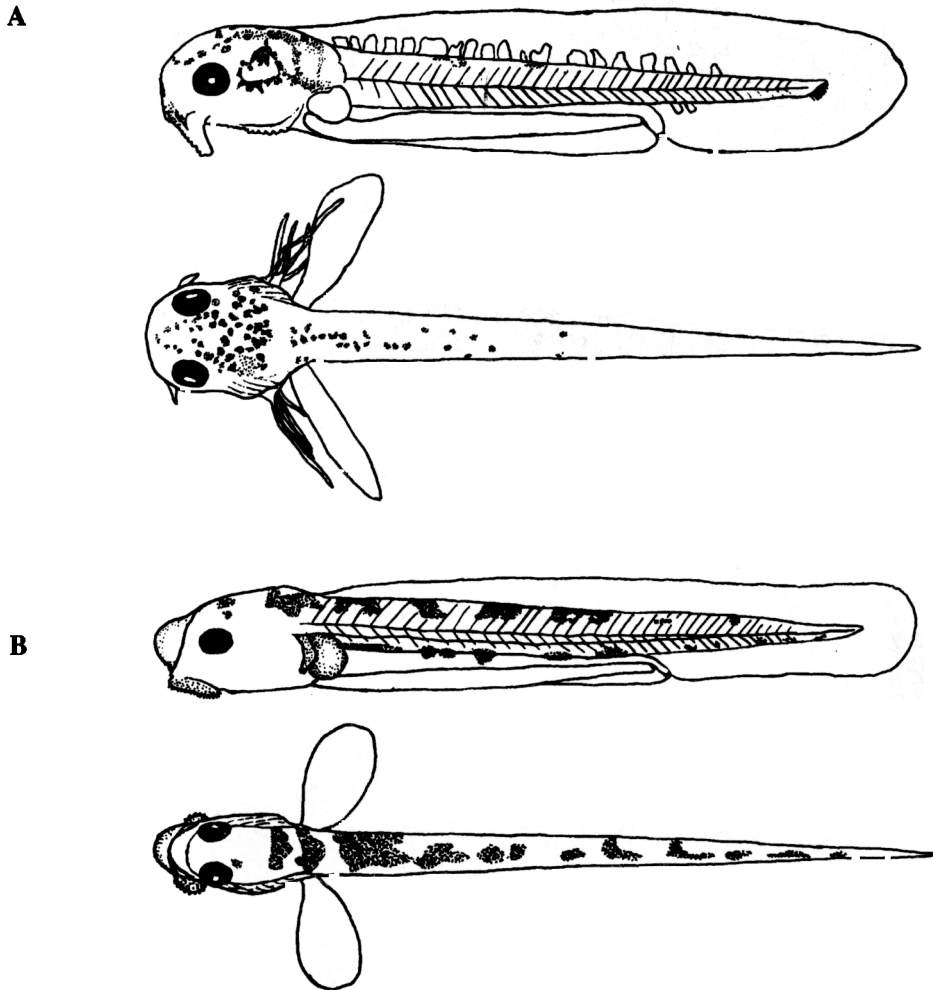


Fig. 1. General appearance of early life stages of *Cobitis* and *Sabanejewia*. (A) late embryonic stage of *C. paludica* (6.1 mm), (B) late embryonic stage of *S. spec.* (5.7 mm).

all species of *Cobitis*. In a similar form it is found in *Misgurnus* (Tsukahara 1948, Knaack 1960) and *Pangio* (Ott 1995). When spawning in *Cobitis* was induced by hormone injection, the females did not show a preference for spawning substratum, but released eggs in the open water and near the bottom (Knaack 1960, Lodi & Malacarne 1990). Oviposition into dense vegetation can be assumed to be the rule in natural spawning. In *Sabanejewia*, no preference for any spawning substratum was found, but most likely the eggs were spread indiscriminately. In the field, eggs of *Sabanejewia* would be exposed to a different microhabitat from those of *Cobitis*. From the stickiness of *Sabanejewia* eggs, this microhabitat would include the surface of hard structures, like gravel or stones.

The sizes of yolk and chorion were shown to be species-specific by Minamori (1952) and Saitoh (1990). On the other hand, Knaack (1962) reported a large variability of these parameters within and between the clutches of different females. Since the experimental animal in the study of Knaack originated from a diploid-polyploid complex, the reported variability may be an effect of the genetic composition of the female. However, in the present study as well as in those by Minamori (1952) and Saitoh (1990), the size of yolk and chorion did not show excessive intraspecific variability, but was characteristic for each species of spined loach. The size of yolk and chorion are mainly an adaptation to the reproductive strategy of the species and the microhabitats of the eggs (Minamori 1952, Park & Kim 1996) and do not necessarily reflect the phylogeny of the investigated species.

The long external gill filaments and the finfold vascularisation in embryos of *Cobitis* are adaptations to enhance oxygen uptake (Kryzanowsky 1934). Long external gill filaments were reported from most Cobitinae studied so far (*Misgurnus*, Grieb 1937, *Lepidocephalichthys*, Ott 1995, *Pangio*, Ott 1995, *Cobitis*, Shimizu et al. 1998). In the genus *Niwaella*, one species has long gill filaments (Kim & Lee 1995), while the second has not (Suzuki 1966). *Sabanejewia* is the first genus known so far to have generally no external gill filaments. Since additional respiratory organs reflect low oxygen supply in the environment of fish, the early life stages of *Sabanejewia* seem to develop in a microhabitat with high oxygen level.

From the reported differences in the reproductive biology as well as from the differences in sexual dimorphism and morphology, it is unlikely that *Sabanejewia* represents the sister-lineage to *Cobitis*, as reported by Sawada (1982) and Banarescu (1991).

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