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Sex ratio and body size in *Cobitis elongatoides* and *Sabanejewia balcanica* (Cypriniformes, Cobitidae) from a thermal spring

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Abstract. The sex ratio of loach fishes from the warm thermal spring Baile Episcopesti in western Romania was investigated. In *Cobitis elongatoides*, the sex ratio was balanced, while in *Sabanejewia balcanica,* males made up 77% of the adult fish. In both species, body size at onset of maturation and maximum length were remarkably smaller than in other populations. Mature males of *C. elongatoides* measured 35 to 51 mm in total length while the largest female reached 69 mm. Males of *S. balcanica* measured 40 to 53 mm and the largest female had a total length of 54 mm. We consider both the dwarfism of the fish as well as the male-biased sex ratio in *S. balcanica* to be caused by the thermal regime in the spring.

Key words: environmental sex determination (ESD), Fisher's principle, cobitine loach, Baile Episcopesti, dwarfism

Introduction

The sex of fishes may be determined by genetic as well as by environmental components (Bull 1983, Conover & van Voorhees 1990, Devlin & Nagahama 2002). Therefore, unusual ecological conditions can create shifts in the sex ratio of fishes. Nowadays, environmental sex determination has become a widely used tool in fish production (Purdom 1993), but little is known about the ecological impact of environmental sex determination in natural populations. According to the sex ratio model of Fisher (1930), animals should produce offspring of a balanced sex ratio. Therefore, a population with an unbalanced sex ratio will be exposed to frequency-dependent selection for the minor sex. In general, a population with an imbalanced sex ratio due to unusual environmental conditions can be considered to be disturbed or maladapted for the given conditions. As shown by Conover & van Voorhees (1990) in the Atlantic silverside, *Menidia menidia*, an unbalanced sex ratio induced by high temperature may become balanced under warm, but stable environmental conditions within a few generations in artificial systems. In thermal springs, the environmental conditions are generally warmer and more stable than in surrounding habitats and effluent rivers. Therefore, Fisher's principle should apply in a similar manner as in the experiments of Conover & van Voorhees (1990). If the sex ratio of an immigrating fish species were biased at first by the environmental conditions, it would be expected to rebalance in time.

In the present study, we report on a population of *Sabanejewia balcanica* with an apparently male biased sex ratio from a thermal spring and compare the observations with the predictions from the experiments of Conover & van Voorhees (1990).

Study Area

The Baile Episcopesti thermal spring is located near Oradea, in western Romania. It consists of a round spring pool with a diameter of approximately 30 m and a maximum depth of about 1 m. An effluent creek connects the spring with the River Crisul Repede, a left tributary of the River Tisza. The temperature of the water usually is 28–40°C in the pool and 20–23°C in the creek (M ü l l e r 1958). The minimum temperature of the pool during winter is 25°C (B ă n ă r e s c u , pers. comm.). Some of the spring biota, like the endemic snail *Melanopsis parreysi*, are thought to represent tertiary relicts (M ü l l e r 1958). The spring contains also an endemic fish species, *Scardinius racovitzai*. The warm stenothermic plant *Nymphaea lotus* has its only European point of occurrence in the spring.

P a u c a & V a s i l i u (1933) recorded the following fish species from the spring: *Rutilus rutilus* (most probably misidentified specimens of *S. racovitzai)*, *Cyprinus carpio*, *Gobio* sp., *Barbus petenyi*, *Rhodeus amarus*, *Squalius cephalus* and *Cobitis elongatoides*. In 1953, M ü l l e r (1958) found the same species in the pond plus three further species in the effluent creek: *Vimba vimba*, *Chondrostoma nasus* and *Sabanejewia balcanica*. Both authors did not report *S. balcanica* from the spring itself, but *C. elongatoides*. According to B ă n ă r e s c u (pers. comm.), *S. balcanica* was collected the first time from the pond on 19 September 1980 (ISBB 3870), but was not found on several earlier visits of the site since November 1955.

Material and Methods

C. elongatoides and *S. balcanica* were collected from the pond of the Baile Episcopesti thermal spring on 18 April 1998. Fish were collected at different sites of the spring using a hand net (mesh size 4 mm). A representative subsample was overanaesthetized in MS222, fixed in 5% formaldehyde and stored in 70% ethanol. In *C. elongatoides*, sex was determined by the presence of a *lamina circularis* at the base of the second pectoral fin ray. The *lamina circularis* is a platelike ossificated extension of the ray and a secondary sexual character of males (C a n e s t r i n i 1871). Males of *S. balcanica* are characterised by the presence of lateral swellings (V l a d y k o v 1928). Because this character can be difficult to observe in very small specimens, the sex of all *S. balcanica* was verified by gonad inspection. Specimens with undeveloped gonads were considered juveniles.

Results

Both *C. elongatoides* and *S. balcanica* occurred in high densities in the pond. Other abundant fish species in the pond were *Poecilia reticulata* and *S. racovitzai*. Single juvenile specimens of *R. amarus*, *G.* sp. and *C. carpio* were found. The length-frequency distribution for both sexes of *C. elongatoides* and *S. balcanica* is illustrated in Fig. 1. Most of the specimens had well-developed gonads, but were of comparably small body size. Mature males of *C. elongatoides* measured 35 to 51 mm in total length while the largest female reached 69 mm. Males of *S. balcanica* from Baile Episcopesti measured 40 to 53 mm, the largest female had a total length of 54 mm. Among the 101 specimens of *C. elongatoides,* 51 were males (50%) and 50 were females. Therefore, the sex ratio was balanced. In contrast, among 68 specimens of *S. balcanica,* 11 were juveniles (total length 26–32 mm), 13 were females and 44 (77% of the adult specimens) were males. The sex ratio was 1 : 3.4 in favour of males.

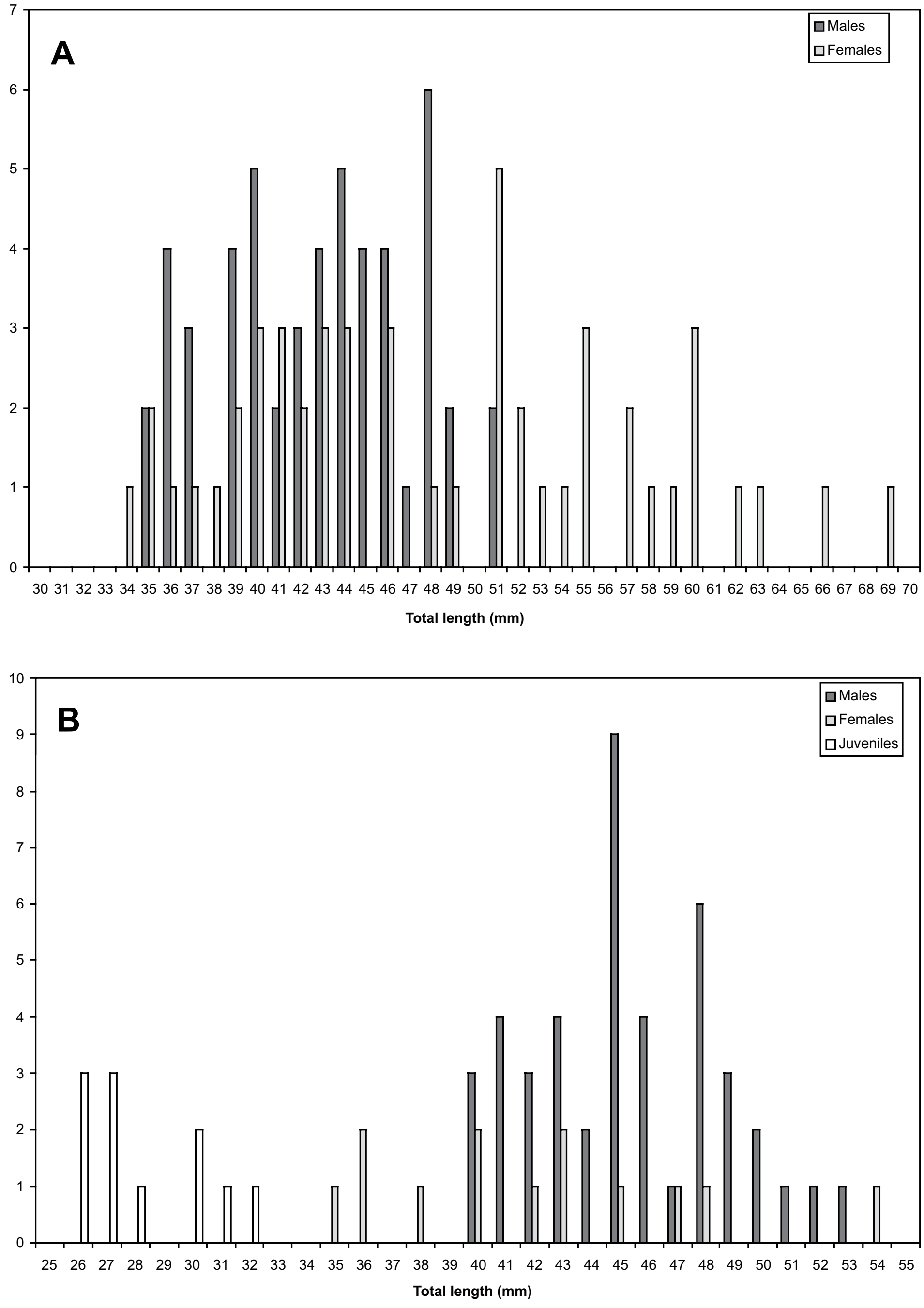

Fig. 1. Length-frequency distribution of *Cobitis elongatoides* (A; n = 101) and *Sabanejewia balcanica* (B; n = 57) collected in the Baile Episcopesti thermal spring in April 1998.

Discussion

The existence of small juveniles and individuals carrying mature gonads in April indicates an earlier reproduction of *S. balcanica* in the spring pool than in other waters in Romania (B ă n ă r e s c u 1964). This underlines the strong impact of the thermal spring habitat on the life history of *Sabanejewia* loaches and may lead to a partial or total reproductive isolation of the spring population.

Both *C. elongatoides* and *S. balcanica* displayed remarkable dwarfism. In comparison, B ă n ă r e s c u (1964) reported a maximum total length of 93 mm for males and 115 mm for females of *C. elongatoides* from Romanian waters. He gave a maximum size of 90 mm for both sexes of *S. balcanica* in Romania. The size at establishment of secondary sexual characters in males usually is about 45–50 mm in *Cobitis* and >50 mm total length in *Sabanejewia* (B o h l e n , unpubl. results). A stout morphology and a parallel trend to develop a relatively small body size is also characteristic for the spring-endemic *S. racovitzai* and suggests that dwarfism may be a general effect of the high temperature environment in Baile Episcopesti.

As can be seen from a survey of literature reports on the sex ratio of cobitine loaches, in these fishes the sex ratio is usually balanced or shifted towards the females (Table 1). The balanced sex ratio of *C. elongatoides* from Baile Episcopesti matches this general tendency.

Table 1. Percentage of males in different species of Cobitinae. Reports are sorted with increasing male proportion. Complex populations that were shown to include gynogenetic female lineages have not been considered in this table.

Species	Percentage of males	Number of specimens	Source
Cobitis elongatoides	50.5	101	present study
Sabanejewia balcanica	77.2	57	present study
Cobitis calderoni	3.3	62	B a c e s c u (1961)
Cobitis bilineata	10.0	?	C a n e s t r i n i (1871)
Cobitis narentana	22.7	211	Z a n e l l a et al. (2003)
Niwaella delicata	23.1	861	S u z u k i (1966)
Cobitis lutheri	27.0	1766	K i m & P a r k (1992)
Iksookimia choii	27.0	85	K i m & S o n (1984)
Cobitis lutheri	32.4	139	K i m & S o n (1984)
Cobitis bilineata	40.1	651	L o d i (1967)
Sabanejewia larvata	37.4	294	R a s o t t o et al. (1990)
Iksookimia longicorpus	35.9	157	K i m & L e e (1990)
Cobitis lutheri	38.7	1789	K i m & J e o n g (1988)
Cobitis sinensis	41.5	125	K i m & L e e (1990)
Cobitis bilineata	43.5	?	M a r c o n a t o & R a s o t t o (1989)
Cobitis taenia	43.5	751	R o b o t h a m (1981)
Sabanejewia kubanica	43.5	645	V a s i ľ e v a & V a s i ľ e v (1988)
Cobitis bilineata	45.4	14,346	G a m b e t t a (1934)
Cobitis „taenia“	45.4	326	S l a v í k & R á b (1996)
Cobitis paludica	50.0	340	L o b o n - C e r v i a & Z a b a l a (1984)
Cobitis bilineata	50.0	997	L o d i (1980)
Sabanejewia balcanica	50.0	45	W i t k o w s k i et al. (1990)
Sabanejewia balcanica	47.6	32	M i š í k (1958)
Sabanejewia caucasica	51.9	77	V a s i ľ e v a & P o z n y a k (1985)

S. balcanica from Baile Episcopesti provides the first record of a strongly male-biased sex ratio in a field population of cobitine fishes. This imbalance can hardly be explained by microhabitat segregation between the sexes or sex-dependent mortality rate, since these mechanisms have to be coupled with differences in size or ecology (Jormalainen & Shuster 1997, Schultz 1996). No such intersexual differences have been reported for *S. balcanica*. Additionally, the small size of the thermal pool and the absence of *S. balcanica* from the effluent creek (pers. observ.) make habitat segregation unlikely. Environmental sex determination by high temperature is the most likely explanation for the male-biased sex ratio of *S. balcanica* in the Baile Episcopesti thermal spring. Similarly, an increase of male proportion with increasing temperature was demonstrated experimentally in Atherinidae (Conover & van Voorhees 1990, Strüssmann et al. 1996), Chichlidae (Desprez & Melard 1998, Wang & Tsai 2000), Poecilidae (Schultz 1993) and others. Nomura et al. (1998) demonstrated the same phenomenon under laboratory conditions in a cobitid fish, *Misgurnus anguillicaudatus*. In this species, the sex ratio was balanced at a rearing temperature of 20°C but significantly male-biased at temperatures of 25 and 30°C.

In opposite to *S. balcanica*, no biased sex ratio was found in *C. elongatoides*. Most likely, this is due to different sensitivity of the species to temperature. Bull (1983) emphasizes the importance of species-specific threshold temperatures for ESD in reptiles. In general, *S. balcanica* occurs in colder waters than *C. elongatoides* (Bănărescu 1964), therefore its threshold temperature could be lower. Another possible explanation could be given by the longer phase of adaptation of *Cobitis* in the thermal regime. It was found in the spring 50 years earlier than *Sabanejewia*, thus having more time to adapt to this environment. This line of reasoning implies that the population of *Sabanejevia* (at the time of sampling) was still in an early phase of the colonisation process. Evolutionary processes such as frequency dependent selection would not have optimised the genetic makeup of that population for the thermal spring habitat.

Conover & van Voorhees (1990) showed that under laboratory conditions, a biased sex ratio might adopt towards a balanced sex ratio within a few generations. However, *S. balcanica* was reported the first time from the thermal spring 18 years before sampling, but still has not adapted its sex ratio. The same is true for *M. menidia*, the experimental fish of Conover & van Voorhees, which in the field also has no stable sex ratio (Conover 1984). Therefore, in the field, Fisher`s principle possibly acts more slowly than under experimental conditions. At the moment, it is not clear which process causes this deceleration, but an extreme and stable environment like a thermal spring provides an interesting object to study this question.

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LITERATURE

Bacescu M. 1961: Contribution a la systematique du genre *Cobitis* description d'une espece nouvelle, *Cobitis calderoni*, provenant de L'Espagne. *Acad. Rep. Pop. Rom. Rev. Biol. 6: 435–448.*

Bănărescu P.M. 1964: Fauna Republicii Populare Romine, Pisces – Osteichthyes. *Academia Republicii Populare Romane, Bucarest (in Romanian).*

Bull J.J. 1983: Evolution of sex determining mechanisms. *Benjamin/Cummings, Menlo Park.*
Canestrini G. 1871: Über das Männchen von *Cobitis taenia* Lin. *Arch. Nat. gesch. 1: 222–224.*
Conover D.O. 1984: Adaptive significance of temperature-dependent sex determination in a fish. *Am. Nat.123: 297–313.*
Conover D.O. & van Voorhees D.A. 1990: Evolution of a balanced sex ratio by frequency-dependent selection in a fish. *Science 250: 1556–1558.*
Desprez D. & Melard C. 1998: Effect of ambient water temperature on sex determinism in the blue tilapia *Oreochromis aureus. Aquaculture 162: 79–84.*
Devlin R.H. & Nagahama Y. 2002: Sex determination and sex differentiation in fish: an overview of genetic, physiological and environmental influences. *Aquaculture 208: 191–364.*
Fisher R.A. 1930: The genetical theory of natural selection. *University Press, Oxford.*
Gambetta L. 1934: Sulla variabilita del cobite fluviale (*Cobitis taenia* L.) e sul rapporto numericodei sessi. *Boll. Mus. zool. Anat. Comp. Univ. Torino 44: 279–325 (in Italian).*
Jormalainen V. & Shuster S.M. 1997: Microhabitat segregation and cannibalism in an endangered freshwater isopod, *Thermosphaeroma thermophilum. Oecologia 111: 271–279.*
Kim I.S. & Jeong M.T. 1988: Seasonal variation of the color pattern in the cobitid fish *Cobitis taenia lutheri* from Korea. *Korean J. Ecol. 11: 77–82.*
Kim I.S. & Lee J.H. 1990: Diploid-triploid hybrid complex of the spined loach *Cobitis sinensis* and *C. longicorpus* (Pisces, Cobitidae). *Korean J. Ichthyol. 2: 203–210.*
Kim I.S. & Park J.Y. 1992: Sex ratios and hermaphroditism of *Cobitis lutheri* (Pisces, Cobitidae) from Korea. *Korean J. Ichthyol. 4: 72–76.*
Kim I.S. & Son YM. 1984: *Cobitis choii*, a new cobitid fish from Korea. *Korean J. Zool. 27: 49–55.*
Lobon-Cervia J. & Zabala J. 1984: Observations on the reproduction of *Cobitis paludicola* DeBuen, 1930 in the Jarama River. *Cybium 8: 63–68.*
Lodi E. 1967: Sex reversal of *Cobitis taenia* L. (Osteichthyes, Fam. Cobitidae). *Experientia 23: 446–447.*
Lodi E. 1980: Hermaphroditic and gonochoric populations of *Cobitis taenia bilineata* Canestrini (Cobitidae, Ostheichthyes). *Mon. Zool. Ital. 14: 235–243.*
Marconato A. & Rasotto M.B. 1989: The biology of a population of spined loach, *Cobitis taenia* L. *Boll. Zool. 56: 73–80.*
Mišík V. 1958: Zum Vorkommen und zur Bionomie der *Cobitis aurata* (Filippi 1865) *balcanica* Karaman 1922 natio montana (?) Vladykov 1925 aus dem Flusse Kysuca. *Biológia, Bratislava 13: 810–832 (in Slovak with German summary).*
Müller G.J. 1958: *Scardinius racovitzai* n. sp. (Pisces, Cyprinidae), eine reliktäre Rotfeder aus Westrumänien. *Senck. Biol. 39: 165–168.*
Nomura T., Arai K., Hayashi T. & Suzuki R. 1998: Effect of temperature on sex ratio of normal and gynogenetic diploid loach. *Fisheries Sci. 64: 753–758.*
Pauca M. & Vasiliu G. 1933: Note ichtyologique sur le lac Petea (Oradea Mare). *Notat. Biol. 1: 58–63.*
Purdom C.E. 1993: Genetics and fish breeding. *Chapman & Hall, London.*
Rasotto M.B., Marconato A., Comuzzi M. & Cardellini P. 1990: Osservationi sulla biologia del Cobite mascherato, *Sabanejewia larvata* DeFillippi (Pisces, Cobitidae). *Riv. Idrobiol. 29: 445–460 (in Italian).*
Robotham P.W.J. 1981: Age, growth and reproduction of a population of spined loach, *Cobitis taenia* (L.). *Hydrobiologia 85: 129–136.*
Schultz H. 1996: Drastic decline of the proportion of males in the roach (*Rutilus rutilus* L.) population of Bautzen Reservoir (Saxony, Germany): Result of direct and indirect effects of biomanipulation. *Limnologica 26: 153–164.*
Schultz R.J. 1993: Genetic regulation of temperature-mediated sex ratios in the livebearing fish *Poeciliopsis lucida. Copeia 1993: 1148–1151.*
Slavík O. & Ráb P. 1996: Life history of spined loach, *Cobitis taenia*, in an isolated site (Pšovka Creek, Bohemia). *Folia Zool. 45: 247–252.*
Strüssmann C.A., Cota J.C.C., Phonlor G., Higuchi H. & Takashima F. 1996: Temperature effects on sex differentiation of two South American atherinids, *Odontesthes argentinensis* and Patagonina hatcheri. *Environ. Biol. Fishes 47: 143–154.*
Suzuki R. 1966: Artificial spawning and early development of the loach, *Cobitis delicata* Niwa. *Bull. Freshw. Fish. Res. Lab. 15: 175–188.*
Vasiľeva E.D. & Poznyak V.G. 1985: Morphological characteristics of the Caucasian spiny loach, *Sabanejewia caucasica* (Cobitidae). *J. Ichthyol. 25: 148–156.*

Vasiľeva E.D. & Vasiľev V.P. 1988: A study of the intraspecific structure of *Sabanejewia aurata* (Cobitidae) with a description of a new subspecies, *S. aurata kubanica* subsp. nov. *J. Ichthyol. 28: 192–212*.
Vladykov V.D. 1928: Über den sekundären Geschlechsdimorphismus bei unseren Cobitiden. *Zool. Jahrb., Abt. Systematik 55: 147–162*.
Wang L.H. & Tsai C.L. 2000: Effects of temperature on the deformity and sex differentiation of tilapia, *Oreochromis mossambicus*. *J. Exp. Zool. 286: 534–537*.
Witkowski A., Blachuta J. & Kusznierz J. 1990: Golden loach, *Sabanejewia aurata* (deFilippi, 1865) in the Widawa River – the second locality in the Odra River basin. *Przeglad zool. 34: 319–323*.
Zanella D., Mrakovčić M., Schneider D., Mustafić P., Ćaleta M. & Radić I. 2003: Growth of *Cobitis narentana* Karaman, 1928 in the Neretva River, Croatia. *Folia Biol. (Kraków) 51 (Suppl.): 155–157*.