

## Some biological characteristics of the endemic Neretvan nase, *Chondrostoma knerii* Heckel, 1843, in the Hutovo Blato wetlands (Bosnia and Herzegovina)

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### Summary

The paper presents for the first time important information regarding population structure, growth rate, length-weight relationships and reproductive aspects of the Neretvan nase, *Chondrostoma knerii*, a fish species endemic to the Neretva River. *Chondrostoma knerii* is mainly distributed in the lower parts and delta of the Neretva River wetlands and its tributaries (Krupa River, Hutovo Blato wetlands) shared between Croatia and Bosnia Herzegovina. Samples were collected between 2000 and 2001 during the reproduction period of the species in the Hutovo Blato wetlands area. Population structure analysis indicates that all ages appear, from 0 to 7+. Longevity was estimated at 7.5 years. Main age classes were 3, 4 and 5 years, totalling 91.00% of all individuals. Males ranged between 2 and 5+ years of age, and females between 1 and 7+ years of age. Maximum length was 28.04 cm and maximum weight, estimated from the length-weight relationship, as 206.8 g. Found scattered in streams of high water velocity, eggs were attached to the substrate (aquatic plants or solid objects). Correlation between egg number and length and weight was extremely low. The gonad-somatic index distribution showed an inverse proportionality with total length. Main length classes for reproduction ranged from 20 to 24 cm, while fecundity ranged between 2000 and 16 000 eggs per female.

### Introduction

Neretvan nase, *Chondrostoma knerii* Heckel, 1843, is an endemic freshwater fish species in the Neretva River catchment area, inhabiting waters in Bosnia-Herzegovina and Croatia (Muus and Dahlstrom, 1968; Kottelat, 1997). Mainly inhabiting the middle and lower Neretva River, it is rare in the upper river areas (Vuković and Ivanović, 1971). *Chondrostoma knerii* has been reported in Spain and Syria (<http://www.fishbase.org>), although there is insufficient data concerning these occurrences. It is also under monitoring status by the IUCN in order to be included in the threatened species red book (Hilton-Taylor, 2000). Data on the biology of this species are very scarce in the scientific literature, although locally *C. knerii* has some commercial value for recreational fisheries, attaining highest prices during the spawning season because of its large and edible ovaries. It is considered endangered in Croatian waters (Mrakovčić et al., 1995); published data for Herzegovian waters are limited to older literature (Vuković and Ivanović,

1971). The main populations of the species occur in water bodies of the low plains having little current, but reproduction occurs in streams with high water velocity. A non-guarder, with reproduction occurring in open waters where the eggs are scattered and attached to substrates, the species is threatened mainly by habitat destruction (Crivelli, 1996).

The aim of this study was for the first-time documentation of data on the population structure, growth, length-weight relationship and reproduction aspects of the Neretvan nase, *Chondrostoma knerii*. Problems and future perspectives for this valuable species in the Hutovo Blato wetlands (Bosnia and Herzegovina) are also discussed.

### Materials and methods

Sampling was carried out from January 2000 to April 2001 during spawning migrations and spawning in the Hutovo Blato wetlands. The fish were sampled with driftnets (20 m length, 1 m width, 20–28 mm mesh size) and by electro fishing in the lakes, streams and springs of Hutovo Blato wetlands complex (Fig. 1). Samples were examined for sex (based on milt or egg appearance after abdominal manual pressure) and frozen for laboratory analysis. All specimens were measured for total length (TL) in centimetres using a digital calliper, and weight (W) in grams using an electronic balance. Temperature and salinity were measured with portable digital instruments (WTW).

Age determination was performed by scale reading under a binocular microscope. Scale readings were validated using marginal increase analyses (Magalhães et al., 2003). Scales were mounted dry on microscope slides and annuli interpreted following Bagenal and Tesch (1978). All scales were read twice and a third reading was made if the first two readings differed. When two of the three measures did not agree, the scale was discarded. The same person interpreted the scales on the second occasion, three months after the initial reading.

Instantaneous growth rate was used to describe the growth trajectory of the caught fish cohort. The von Bertalanffy growth curves were not computed because there was no evidence for asymptotic growth, and therefore  $L_{\infty}$  and  $k$  coefficients would probably have been unrealistic.

Ovaries of 320 sexually mature females were dissected and weighed and later preserved in 5% formalin and after 10 days in 70% alcohol. The oocyte diameter was measured on fresh

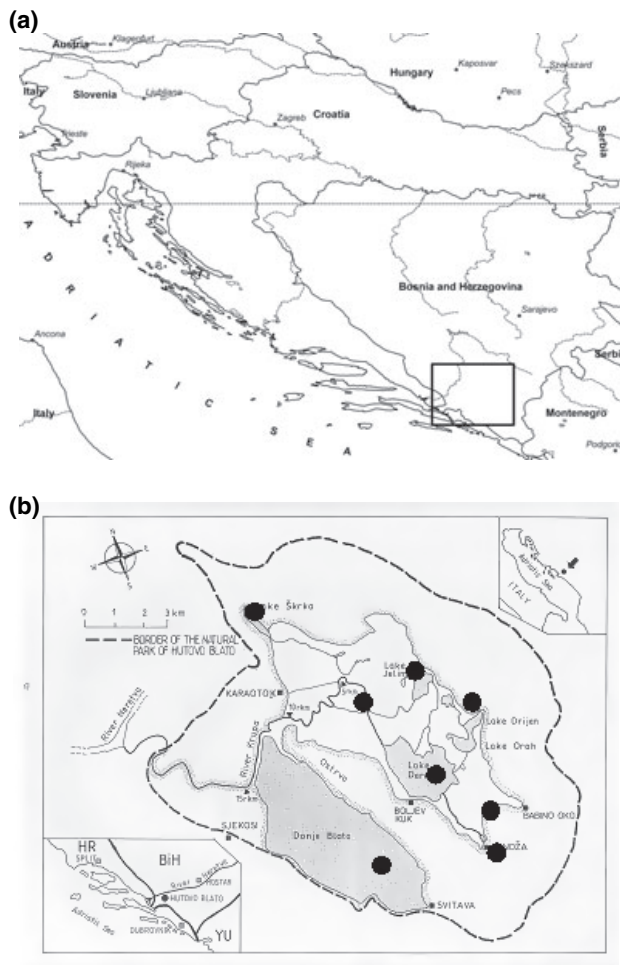


Fig. 1. Map of Hutovo Blato wetlands (Bosnia-Herzegovina) target area, indicating sampling station locations

and thawed materials. The number of oocytes was calculated using the gravimetric method from preserved ovaries. Ovaries of each female were cleaned and dried with tissue paper; the whole ovaries were then weighed and three samples (1 g) were taken from each ovary. All oocytes in these samples were counted under the microscope. Absolute fecundity was calculated using the relation:  $F = nG g^{-1}$  ( $n$  = number of oocytes in gram sample,  $G$  = total weight of ovary,  $g$  = weight of ovarian sample). The gonado-somatic index was used to represent the relative reproductive effort in the population.

Known spawning sites (N. Zovko, unpublished data) and predicted sites, mainly springs, (Vuković and Ivanović, 1971) were investigated in order to find and describe spawned eggs. Chosen sites of spawning (springs of Londža, Posrk, Jamica, Babino oko, Drijen, and Jelimo) were surveyed each week during spawning seasons from January to April in 2001 and 2002. Appearance of eggs was recorded and collected eggs were described and measured and later preserved in 10% alcohol.

The commonly used total length-weight relationship was applied (Ricker, 1975):  $W = aL^b$ , where  $W$  is weight,  $L$  is total length, and  $a$  and  $b$  are constants. The analysis of data was carried out using various software packages for population dynamics. In addition, the empirical models of Froese-Binohlan were used (Froese and Binohlan, 2000).

Table 1

Age-length structure of Neretvan nase, *Chondrostoma knerii*, in Hutovo Blato wetlands ( $n = 724$ )

Age	Length (cm)	No. fish	%
1+	11.0–12.0	3	0.39
2+	13.0–16.0	14	1.93
3+	16.0–19.0	286	39.38
4+	19.0–21.0	229	31.66
5+	21.0–23.0	137	18.92
6+	23.0–24.0	20	2.70
7+	24.0–26.7	35	4.63

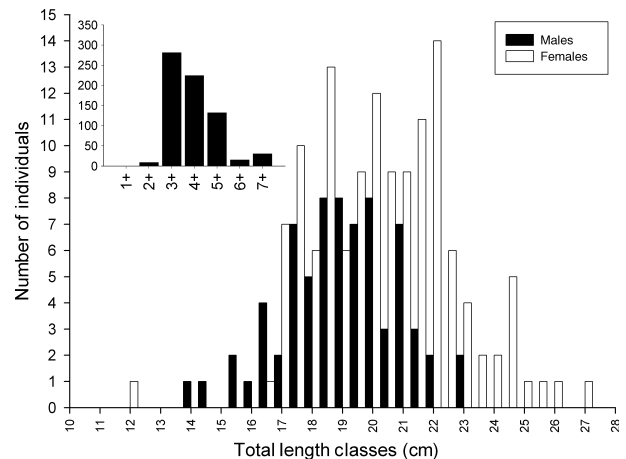


Fig. 2. Population structure of Neretvan nase, *Chondrostoma knerii* in Hutovo Blato per length and per age (smaller graph)

## Results

A total of 724 specimens was collected and measured during the spawning season. The sample was composed of 352 males and 372 females (overall sex ratio 0.95 : 1 in favour of females). TL of males varied from 14.0 to 22.8 cm, and weight from 20.7 to 121.6 g. TL of females varied from 11.7 to 29.4 cm, and weight from 25.4 to 228.6 g. The age-length key is summarized in Table 1 and the length-frequency distribution of the population is illustrated in Fig. 2. Age analyses of the populations showed ages ranging between 0 and 7 years; most abundant were the 3-3+ (40%), 4-4+ (32%) and 5-5+ (19%) classes. There was an age difference between males and females composing the population: males ranged between 2 and 5+ whereas females ranged between 1 and 7+. Based on the growth equation data (Pauly, 1983; Froese and Binohlan, 2000), the average life expectancy is 6.7–7.5 years; this indicates that the population of Hutovo Blato wetland is still intact in terms of expected age classes. The region of Hutovo Blato is a regulated national park and therefore the fishing effort on the population is from moderate to low.

## Growth and growth rates

The species growth and growth rates relationships with age are illustrated in Fig. 3. The equation that shows the relationship between total length and age was estimated based on the logistic and Gompertz models:

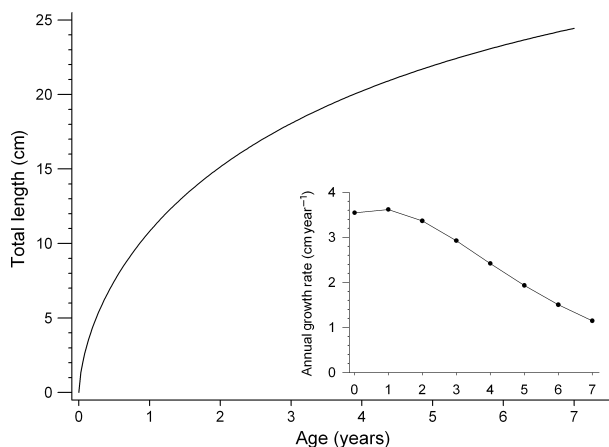


Fig. 3. Growth and annual growth rates (smaller graph) of Neretvan nase, *Chondrostoma knerii*

$$\text{Logistic: TL(cm)} = \frac{26.88}{1 + 2.10e^{-0.45t}},$$

$$r^2 = 0.999, \text{ std.error} = \pm 0.07\text{cm}$$

$$\text{Gompertz: TL(cm)} = 28.40e^{e^{-0.32(0.22 - 0.32t)}},$$

$$r^2 = 0.999, \text{ std.error} = \pm 0.08\text{cm}$$

The maximum length estimated was found to be TL = 28.04 cm (range: 27.7–28.4 cm) and maximum weight W = 206.8 g. Growth rates were estimated as the first derivative of the Gompertz equation:

$$\frac{dL}{dt} = 9.88 \cdot e^{-0.32t+0.22} \cdot e^{-0.32t+0.22}$$

**Aspects of reproduction**

Spawning started in mid-February in the few springs of the Hutovo Blato wetlands at water temperatures of 13° to 13.2°C. Spawned eggs are soft, orange-red and sticky. Eggs can be found attached to various substrates such as water plants *Myriophyllum spicatum* L. and *Potamogeton densus* L., or solid objects such as the metal frames and nets of eel traps. In addition, eggs were found deposited in shallow waters of springs with high water velocities, indicating one of the needs for successful spawning and embryo development. Average egg diameter was 1.78 ± 0.72 mm (range: 1.51–2.16 mm). The eggs in springs with good water velocities developed well and hatched normally. However, in the spring with a low water velocity, the spawned eggs were quickly infected with layers of undetermined fungi (probably *Saprolegnia*) and rotted within a short time, with a bad smell and dead embryos.

The examined ovaries from samples during the two spawning seasons showed that the colour of oocytes changes from brown to orange-red as they mature and the spawning time nears. The egg consistency also changes from hard (brown colour) to soft (orange-red colour).

The correlation between egg number and length and weight is extremely low. The number of eggs is positively correlated with gonad weight (Fig. 4). The ovaries are large and situated on both sides of the body cavity. Average ovary weight was 14.37 g, varying from 5.9 to 35.2 g. Ovaries represented from 4% to 62% of the total body weight, with an average of 21.77%. The histogram of the gonad-somatic index is represented in Fig. 5.

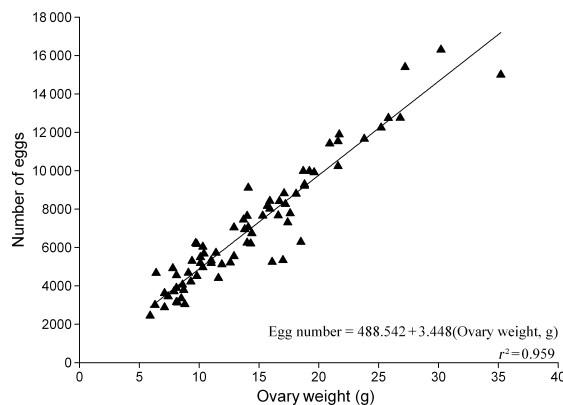


Fig. 4. Relationship between egg numbers and ovary weight of Neretvan nase, *Chondrostoma knerii*, caught in Hutovo Blato wetlands

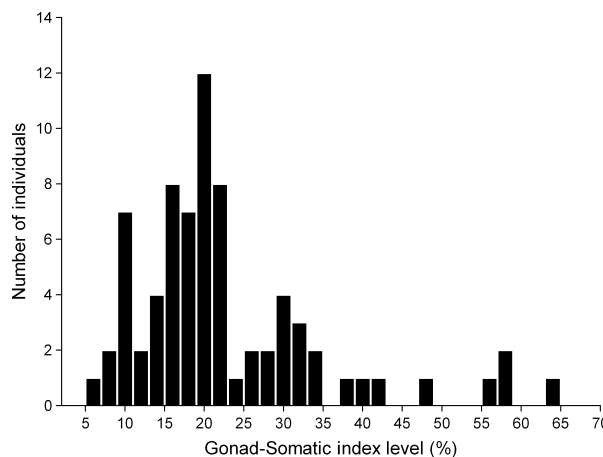


Fig. 5. Variation of female (n = 352) gonad-somatic index (%) in sampled cohort of Neretvan nase, *Chondrostoma knerii*, in Hutovo Blato wetlands

The main length classes for reproduction ranged from 20 and 24 cm. Fecundity ranged between 2000 and 16 000 eggs per female, with an average of 7000 eggs per female.

Using empirical equations, it was estimated that the age-at-first maturity is 1.98 years (16.7 cm TL; range: 12.4–22.3 cm).

**Length–weight relationships**

The length–weight relationships of males and females are presented in Fig. 6. The slopes (b values) differ significantly between sexes (ANCOVA: F = 1.79; F<sub>0.01</sub> = 6.62; P > 0.05). The values of b for males, females and both sexes were significantly different from 3.0 (P > 0.05). TL ranged from 14 to 23 cm TL (for males) and from 11 to 27 cm TL (for females).

Equations that describe the various length–weight relationships are:

$$\text{Males: } W(g) = 0.0029[\text{TL(cm)}]^{3.338},$$

$$r^2 = 0.959, \text{ std.error} = \pm 5.045\text{g}$$

$$\text{Females: } W(g) = 0.0077[\text{TL(cm)}]^{3.063},$$

$$r^2 = 0.928, \text{ std.error} = \pm 11.45\text{g}$$

$$\text{Both sexes: } W(g) = 0.0035[\text{TL(cm)}]^{3.310},$$

$$r^2 = 0.936, \text{ std.error} = \pm 10.55\text{g}$$

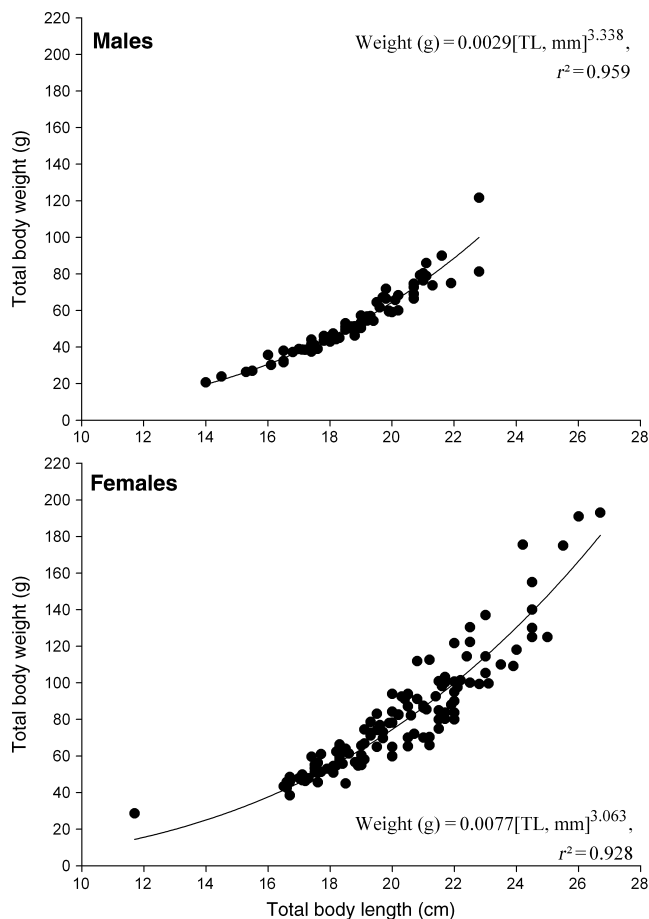


Fig. 6. Length-weight relationships of male ( $n = 352$ ) and female ( $n = 372$ ) Neretvan nase, *Chondrostoma knerii*, from Hutovo Blato wetlands

## Discussion

The population of *C. knerii* in the Hutovo Blato wetlands is composed of individuals ranging from 0 to 7 years of age; the 3-3+ (40%) class was most abundant. Aganović and Kapetanović (1978) found that specimens ( $n = 738$ ) composed nine age classes (from 0+ to 8+) in the middle part of the Neretva River. This is in agreement with the findings of Aganović and Kapetanović (1978), since the biggest number and percentage of units were established in the age class 3+ (26.32%) population of Neretvan nase from the middle part of Neretva River.

The maximum observed total length of Neretvan nase was  $TL = 29.4$  cm, whereas in the Torgal catchment (Portugal) (Magalhães et al., 2003) the *C. lusitanicum* was  $TL = 12.9$  cm (with maximum age of 4 years) Using empirical equations, it was estimated that the age-at-first maturity of Neretvan nase is 1.98 years (16.7 cm TL; range: 12.4–22.3 cm), in agreement with the findings for *C. lusitanicum* (mean maturity reached at age 2 regardless of sex, although males showed 81.5% matures at 70–80 mm, whereas females showed 50% matures only at 80–90 mm) in the Torgal catchment (Magalhães et al., 2003).

The Neretvan nase was mostly caught during its reproductive period in January and April. This period is identical to those of *C. lusitanicum* in Portuguese waters (Magalhães et al., 2003). Later, in the period from April to December, Neretvan nase was only caught sporadically, mainly in colder waters ( $T = 15$ – $17^{\circ}\text{C}$ ) of lakes with underwater springs and with

their streams in the Hutovo Blato wetland complex. The percentage of Neretvan nase in the Upper Hutovo Blato total catch during this period was only 0.23% (Glamuzina and Conides, 2000). The adult specimens migrate to wetlands from the Neretva River for spawning, returning thereafter to the main river flow.

Average egg diameter of Neretvan nase, *C. knerii*, was  $1.78 \pm 0.72$  mm (range: 1.51–2.16 mm), larger than those of *C. lusitanicum*, which was  $1.29 \pm 0.02$  mm (range: 1.12–1.43 mm) (Magalhães et al., 2003).

The exponents of length-weight relationships (males:  $b = 3.338$ , females:  $b = 3.063$ ; both sexes:  $b = 3.310$ ) of the Neretvan nase estimated in the Hutovo Blato wetlands show that growth is allometric. The values of  $b$  from our study could be attributed to the sexually mature specimens and spawning period. Variations in the exponents could also be attributed to different stages in ontogenetic development, as well as to differences in age, maturity and sex. Geographic location and associated environmental conditions, such as seasonality, date and time of capture), stomach fullness, disease and parasite load (Bagenal and Tesch, 1978), can also affect the value of  $b$ . Since there is no literature containing data on this endemic fish species, we have compared our results with those of other species from the genus *Chondrostoma*. Georgiev (2003) reported the value of  $b = 2.944$  (isometric growth) for another endemic fish species *Chondrostoma vardarensis* (River Vardar, Republic of Macedonia).

Spawning seasons during 2000 and 2001 of Neretvan nase in the eastern part of the Hutovo Blato wetlands failed in most of the springs. The main characteristic of water conditions during this period was low capacity for all of the investigated springs. Eggs were found in only two springs of the investigated area in the north-east Hutovo Blato wetlands (two springs of the Londža stream), while some spawners were also caught in two other springs (Posrk and Jamica). In all other areas, neither spawners nor laid eggs were found. It was determined that the main problem was quality deterioration of most streams connecting the springs and lakes of the area. Most were clogged with plant overgrowth, causing local flooding and preventing any fish migration toward the springs, in addition to significantly lowering the water velocity, an important factor for successful reproduction of the species. The adverse effects of the clogged waterways in Hutovo Blato on the reproduction of the species, in addition to lowering the water velocity at the microhabitat level, was the decrease in dissolved oxygen due to decomposition of plant matter. Dissolved oxygen is a crucial factor for the reproduction of the genus (Schiemer et al., 2003).

Main factors recognized as important for the survival of endemic species such as Neretvan nase concern: (i) environmental quality, (ii) presence of pest species, and (iii) presence of related, but competitive, species by intentional introductions or by migration via the river routes of Europe (Povž, 1995). Most of the aforementioned factors are present in the Hutovo Blato wetlands, including the flow of the springs and the lowering of the water capacity (N. Zovko, personal communication), the presence of pests such as the pumpkinseed, *Lepomis gibbosus* (Glamuzina and Conides, 2000), and the introduction of competitive species such as the ruffe, *Gymnocephalus cernuus* (Dulčić et al., 2005).

In order to preserve and enhance the status of Neretvan nase we recommend the cleaning of all streams and springs used by *C. knerii* during their spawning period. The second recommendation based on these studies is to experiment with

different types of egg collectors for collection and relocation of eggs to springs with the most favourable conditions, either in this area or other areas of these wetlands.

### Acknowledgements

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