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Abstract: In this study, the early development of barbel (*Barbus barbus* L.) larvae has been determined by measuring their wet weight, dry weight, whole body mineral content (Ca, Mg, Na and K), skeletal development and gill development. It has been observed that wet weight, whole body mineral contents, the number of lamellae per filament of gill and the number of calcified vertebrae of larvae during early development stage increased by age, whereas dry weight decreased. The increase of whole body magnesium content has been very low compared with that of other minerals. In addition, a possitive relationship between whole body calcium content and the number of calcified vertebrae has been found.

Key Words: *Barbus barbus* L., wet weight, dry weight, whole body mineral content, skeletal development, gill development.

Bıyıklı Balık (*Barbus barbus* L.) larvasının erken gelişimi

Özet: Bu çalışmada, bıyıklı balık (*Barbus barbus* L.) larvalarının yaş ağırlık, kuru ağırlık, tüm vücut mineral içeriği (Ca, Mg, Na ve K), iskelet gelişimi ve solungaç gelişimi ölçülerek larvaların gelişimi saptanmıştır. Erken gelişim safhası süresince larvaların yaş ağırlık, tüm vücut mineral içeriği, solungacın her bir filamentindeki lamella sayısı ve omurga sayısının yaş ile arttığı fakat kuru ağırlığın azaldığı gözlenmiştir. Tüm vücut mağnezyum artışı diğer minerallerin artışı ile karşılaştırıldığında oldukça düşük olmuştur. Ayrıca, tüm vücut kalsiyum içeriği ile kalkerleşmiş omurga sayısı arasında pozitif bir ilişki bulunmuştur.

Anahtar Sözcükler: *Barbus barbus* L., yaş ağırlık, kuru ağırlık, tüm vücut mineral içeriği, iskelet gelişimi, solungaç gelişimi.

Introduction

In order to measure the degree of the larval development and the sublethal stress of aquatic pollutants (e.g. toxicity of metals, low pH etc.), body length, body weight, whole body mineral content and skeletal calcification have been monitored in many studies (1, 2, 3, 4, 5). Since most studies on this subject have been focused on salmonids larvae, there is lack of information on non-salmonid riverine fish larvae such as barbel (*Barbus barbus* L.) etc.

Blaxter (1969) also suggested that only body length gives insuffic ient information about larval development and wet weight in subject to the influence of varying of water content. Thus dry weight, whole body mineral content and skeletal calcification are the most satisfactory measures of gross larval development (1).

So, this study investigates the early development of barbel (*Barbus barbus* L.) by monitoring wet weight, dry weight, whole body mineral content (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}), skeletal development and gill development.

The result will allow comparison about larval development between cyprinids and salmonids and also provide a useful database for cyprinids uncommonly used in experimental work in this particular subject.

Materials and Methods

Experimental animals

Adult male and female barbels (*Barbus barbus* L.) were obtained from Calverton Fish Farm, Nottinghamshire, UK. They were artificially ovulated by injection of pituitary acclimated and incubated in a fibreglass hatchery tank supplying quality running, aerated and filtered tap water (see table 1 for water quality). An artifical lighting regime of 12 h photoperiod was provided for hatchery thank during experiment. Larval fish were fed on *Artemia salina* larvae after yolk-sac absorption (day 8).

Experimental procedure

Temperature, pH and conductivity measurement in experimental medium were made daily. The temperature of experimental room was adjusted to a constant temperature (17±2.0˚C). Conductivity measurements were made by using of a pH mater (Russel pH, model

Table 1. Water quality data for experimental medium and for Calverton Fish Farm hatchery from where experimental animals were supplied. Single value, range or mean±standard error.

Parameters	Experimental medium	Calverton Fish Farm Hatchery
Temperature, °C	17.0±1.0 (n=18)	16.0 (n=1)
Conductivity, µS cm^{-1}	677±9.0 (n=12)	–
pH	8.37±0.02 (n=10)	–
Ca^{2+}, µmol l^{-1}	1312±30 (n=10)	1682 (n=1)
Na^{+}, µmol l^{-1}	510±14 (n=10)	1278 (n=1)

CWL-LCW). Water samples were also taken daily for determination of Ca^{+2} and Na^{+} concentrations. Animals were sampled at intervals. Sampled animals were killed in anaesthetic solution (Sandoz, MS222), rinsed twice in deionised water and separated into three groups for measurement of wet weight, dry weight, whole body mineral, skeletal calcification and gill development.

Wet weight, dry weight and whole body mineral content

Twenty-five of first group animals were transferred into each 4 ml weighed glass vials, to provide 10 replic ates and then they were weighed to determine wet weight of animals. Vials were heated in oven for 24 h at 65±5°C and transferred to a low pressure desiccator for several days until constant weight was obtained. After weighing for dry weight, animals were digested in HNO_3, 16 mol l^{-1} for 24 h at room temperature, followed by dilution with $LaCl_3$ solution in deionised water to give [La]=1% w/v. The diluted digests were filtered through hardened low-ash filter paper (Whatman, grade 540) to remove undigested fats.

Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} concentrations of filtered digests were determined by atomic absorption spectrophotometry (Pye Unicam, model SP90). Blank were prepared for comparison purposes, following the same procedures as above to empty glass vials.

Skeletal development

Second group animals were fixed 70% ethanol, and then bleached and macerated in a mixture of H_2O_2 and dilute KOH solitions for 30 minutes. The specimens were rinsed once in distilled water and stained for 30 minutes in a freshly made solution of alizarin red S (B.D.H., 'Gurr') in 0.2% KOH solution in distilled water and then preserved in glycerol. This procedure was based on that of Taylor (1967) and Nelson (1982) (6, 7). Alizarin red in alkaline solution stains all calcium salts found in bone. For assessment of the degree of skeletal development, numbers of the stained vertebrate were counted under a light microscope.

Gill development

The degree of gill development was determined by measurement of filament length and the number of lamellae per filament. Measurements were made on a single filament from near the middle of the second gill arch, a region considered to represent the longest filaments for each arch (8). The specimens were examined using a scanning electron microscopy (SEM) technique which is given below.

Third group of animals were treated for determination of gill development by using following scanning electron microscopy (SEM). technique. The gills of anaesthetised animals were removed and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 60 minutes. The specimens were then post-fixed in 1% osmium tetroxide (OsO_4) in same buffer for 90 minutes, rinsed in distilled water for 1 minute, and then gill tissues were dried with a critical point dryer (Polaron E3000), using liquid CO_2 as the transitional liquid. After drying, the specimens were stucked on stub and coated with a thin layer of gold in sputter coating unit (Polaron E5100). Coated specimens were observed in a Jeol (JSM-840) scanning electron microscope. The number of lamellae per filament were counted for measurement of the degree of the gill development.

Results

Water quality

Water quality data for experimental medium and for the Calverton Fish Farm hatchery from where experimental animals were supplied are presented in table 1. Water (approx. 200 ml min^{-1}) was supplied to hatchery thank during incubation of eggs. This was increased to approx. 300 ml min^{-1} after hatching. Water temperatures were within the range 17.0±2.0°C throughout the experiment. Na free chlorine was detected in experimental medium.

Wet weiht, dry weight and whole body mineral content

Wet weight and dry weight of barbel are presented in figure 1. Wet weight increased with age. However, dry weight slightly decreased with age.

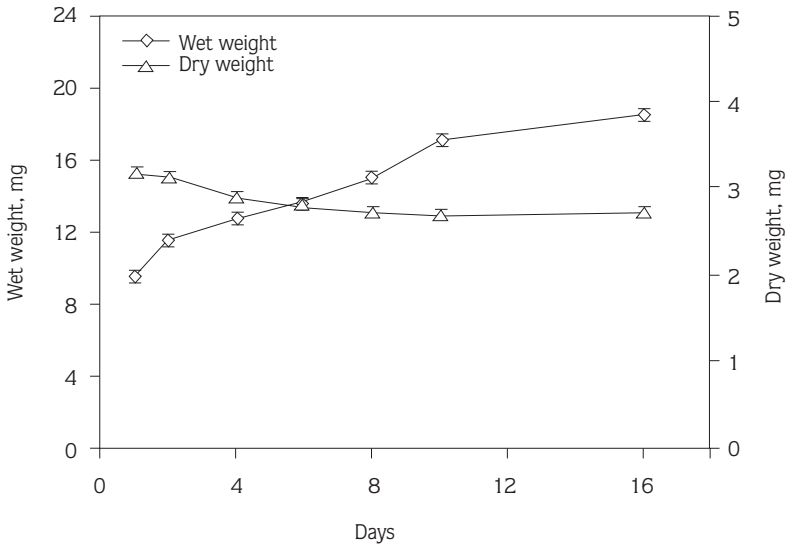

Figure 1. Wet weight and dry weight of barbel larvae (mean±standar error, n=10)

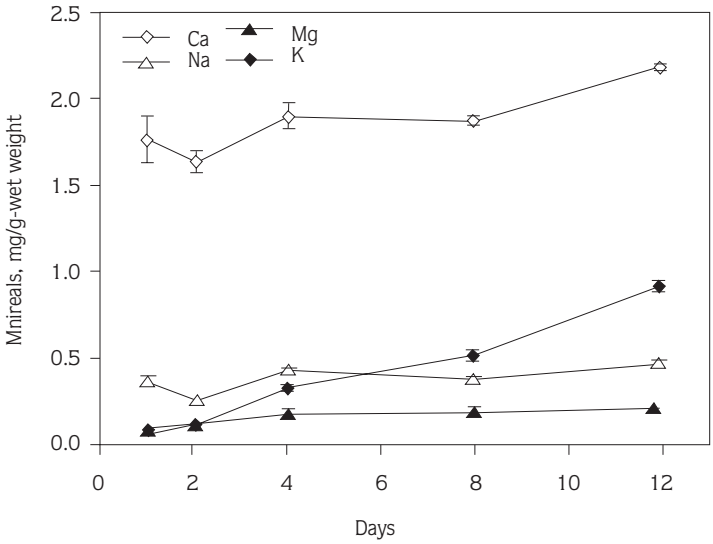

Figure 2. Whole body mineral content in wet weight of barbel larvae (mean±standard eror, n=10).

Whole body mineral contents (Ca, Mg, Na and K) in wet weight and dry weight of barbel during early development are presented in figure 2 and 3. Whole body mineral content of calcium, magnesium, sodium and potassium to wet weight and to dry weight gradually increased with age. This increase was higher for calcium and potassium and lower for sodium and magnesium.

Skeletal development

Skeletons of animals sampled at the beginning of experiment (first day after hatch) took up stain only in certain small area of the skull and in some of caudal fin rays. No calcification of vertebra were observed until day 4 of post-hatch. The onset of calcification in vertebra increased with increasing age. At the end of experiment (day 16 after hatch), it was observed that all vertebrates were stained with calcium specific stain, alizarin red.

Gill development

The lamelallae were first observable in barbel larvae at day 3 and the number of lamellae per filament increased with development of animal. Figure 5 shows that the number of lamellae per filament gradually increased with age of animals.

Disscussion

The results presented in this study provide a useful baseline database for certain riverine cyprinids, previously unavailable, allowing comparisons with more frequently studied species, e.g. salmonids and still-water cyprinids such as the carp, *Cyprinus carpio.*

There is a strong negative correlation between fecundity and egg size at the interspecific level. Kamler

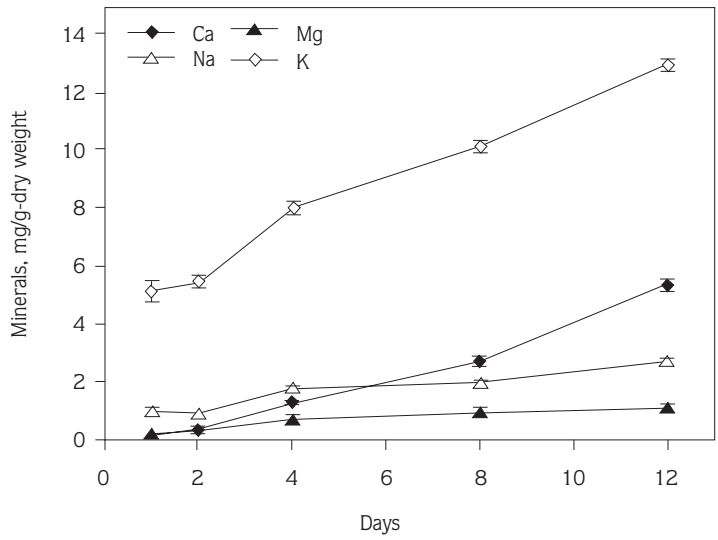

Figure 3. Whole body mineral content in dry weight of barbel larvae (mean±standard error, n=10).

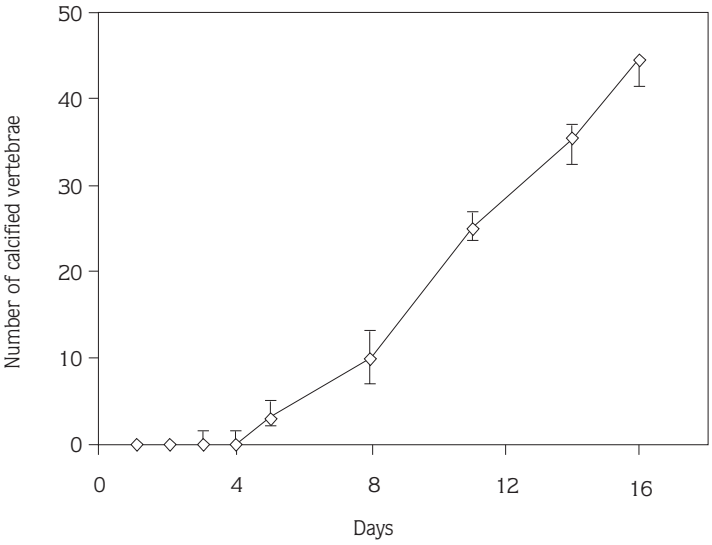

Figure 4. Whole number of calcified vertebrae of barbel during early development stage (mendian±interquartile ranges, n=10).

(1992) summarized this relationship as a function of female temperatures, tend to produce few, large eggs, whereas Cyprinids with short incubation periods in warmer water tend to produce many small eggs. Egg size may vary amongst species of the same family as a function of different ecological niches. The barbel eggs were considerably smaller than the salmonid eggs and larger than the chub eggs (2). This can be explained by their different parental sizes and ecology. The barbel is a large, bottom-dwelling fish and generally prefers the bottom of slower rivers and their deeper parts, whereas the chub is smaller and pelagic. In general, pelagic fishes produce many small eggs compared with non-pelagic fished and also thereare a positive relationship between egg size and larval size (3). The larvae of barbel were considerably larger than those of chub and smaller than those of salmonids, as a result of the difference in egg size (2).

Wet weight of animals were increased by age, whereas dry weight decreased. Wet weight is not useful indicator for assessment of larval development because of the influence of varying of water content (1, 3). Decrease of dry weight of larvae during indigenous feeding period is normal because larvae can not have exogenous food during yolk-sac stage. (3, 9).

The number of calcified vertebrae and the number of lamellae per filament increased by age. Çalta (1996) found that skeletal and gills development were more advanced in the larger larvae with their larger eggs and

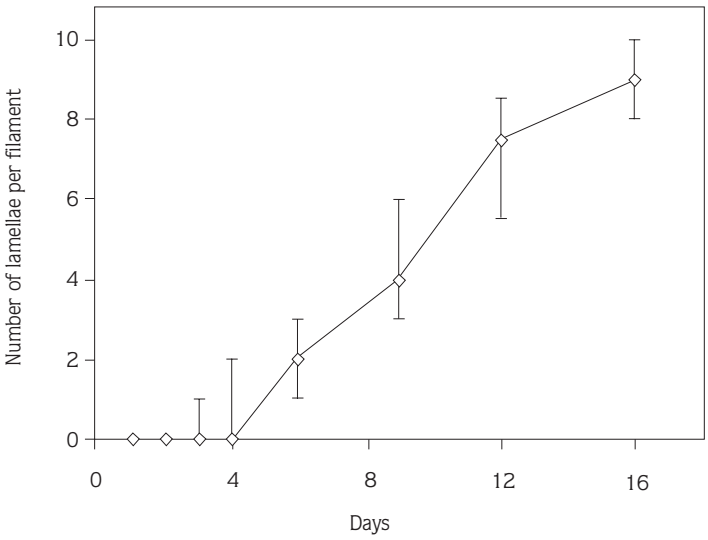

Figure 5. The number of lamellae per filament on gill of barbel larvae (median±interquartile ranges, n=10).

longer periods of embryonic and larval development. (2)

Whole body mineral content of animals increased during early development. This increase accelerated after feeding. Barbel and chub showed very similar pattern of whole body mineral uptake although their larval sizes are considerably different (2).

The experimental data show that there was an increase in the ratio of whole body mineral content to dry weight and wet weight in barbel larvae during the endogenous feeding period. This increase is slightly for magnesium. Similarly , Flik et al. (1993) found that carp (*Cyprinus carpio*) contributed 16% of the magnesium required for growth from the external medium via gills. At least 84% is obtained from food via the intestine. (10) These finding suggests that although a small part of the magnesium uptake occurs normally via the gills, the major site for magnesium uptake is the intestine. However, many recent studies have been showed that salmonids normally do not have any sign of the magnesium uptake from the medium during the endogenous feeding period (2, 4, 11, 12, 13, 14, 15). When fed a low magnesium diet, some fish (guppies, carp) many die even though magnesium in present in the water; other species (rainbow trout, tilapia) survive on such a diet and even continue to grow (10).

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