

## HEADS OR TAILS—FISH HATCHING

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*Department of Animal Physiology, University of Natural Sciences and Humanities in Siedlce, Poland*Ługowska K., Sarnowski P. 2011. Heads or tails—fish hatching. *Acta Ichthyol. Piscat.* 41 (1): 13–17.

**Background.** Hatching is a process in which the embryo emerges from the egg by breaking the protective egg shell. Our preliminary observations indicate that hatching duration and time-distribution may vary among and within the fish species, and that some embryos fail to hatch or hatch incompletely, probably due to the “incorrect” hatching way. So the aim of this study was a detailed description of hatching of three fish species: common carp, barbel, and rainbow trout.

**Materials and methods.** Three species of fish: common carp, *Cyprinus carpio*; barbel, *Barbus barbus*; and rainbow trout, *Oncorhynchus mykiss*; were examined for their hatching modes. The mode and the time of hatching of each larva were noted. Newly hatched larvae were counted and examined. The malformations were classified. Observations of embryos and larvae were done using the stereoscopic microscope Nikon connected to the computer with the MultiScan 8.4 image analysis system; the hatching embryos and larvae were photographed.

**Results.** Three modes of hatching were observed, two of them similar in all three fish species. Some fish started hatching tail first from the egg shell, others head first or—specifically for barbel—yolk sac first. The data obtained in the present study showed that tail hatching was the most successful in all fish species, and shown by most good quality larvae. The majority of tail-hatched larvae developed normally and were viable, and only some of them were deformed and showed slight morphological defects, mainly single vertebral malformations that in most cases were negligible. Head hatching was the precarious in carp, and in all fish species less common and successful comparing to the tail hatching.

**Conclusion.** The hatching mode could be used as another good parameter for estimation of quality of eggs and larvae.

**Keywords:** common carp, barbel, rainbow trout, fish, hatching

## INTRODUCTION

Hatching is a process in which the embryo emerges from the egg by breaking the protective egg shell. Fish hatching includes two mechanisms: the action of the hatching enzyme—chorionase produced by epidermal glandular cells often defined as “the hatching gland cells HGCs” (Hagenmaier 1974, DiMichelle and Taylor 1981, Ostaszewska 1998) that digests the egg membrane, and the vigorous movements of the embryo which propel it from the egg after the chorion has been weakened. Hatching glands differentiate during the later half of the embryonic development. The chemical nature and proteolytic properties of hatching enzyme were described by Bell et al. (1969), Yamagami (1973, 1975), and Shi et al. (2006). The localization of HGCs in the embryo is similar in different fish species. For example, in the goldfish, hatching gland cells appear on the lateral surface of the trunk and on the yolk sac (Ouji 1955). In common carp embryos hatching glands are located on head near the eyes, and on front wall of the yolk sac (Ostaszewska 1998). In rainbow trout embryos the additional glands are located on the front wall of the yolk sac (Hagenmaier 1974). In herring embryos

hatching glands are distributed on the front part of head, a few are present on the lower jaw and on the yolk sac (Rosenthal and Iwai 1979). In Japanese eel embryo HGCs are also located mainly on head (Hiroi et al. 2004).

Despite quite abundant data on the action of the chorionase, very little is known about the process of emerging of the embryo from the egg shell. Our preliminary observations indicate that hatching duration and distribution may vary among and within the fish species, and that some embryos fail to hatch or hatch incompletely, probably due to the “incorrect” hatching way.

The aim of the present study was to perform a detailed observation and comparison of hatching ways and to evaluate their consequences in three species of fish to indicate which of them is the most “correct” and least risky.

## MATERIALS AND METHODS

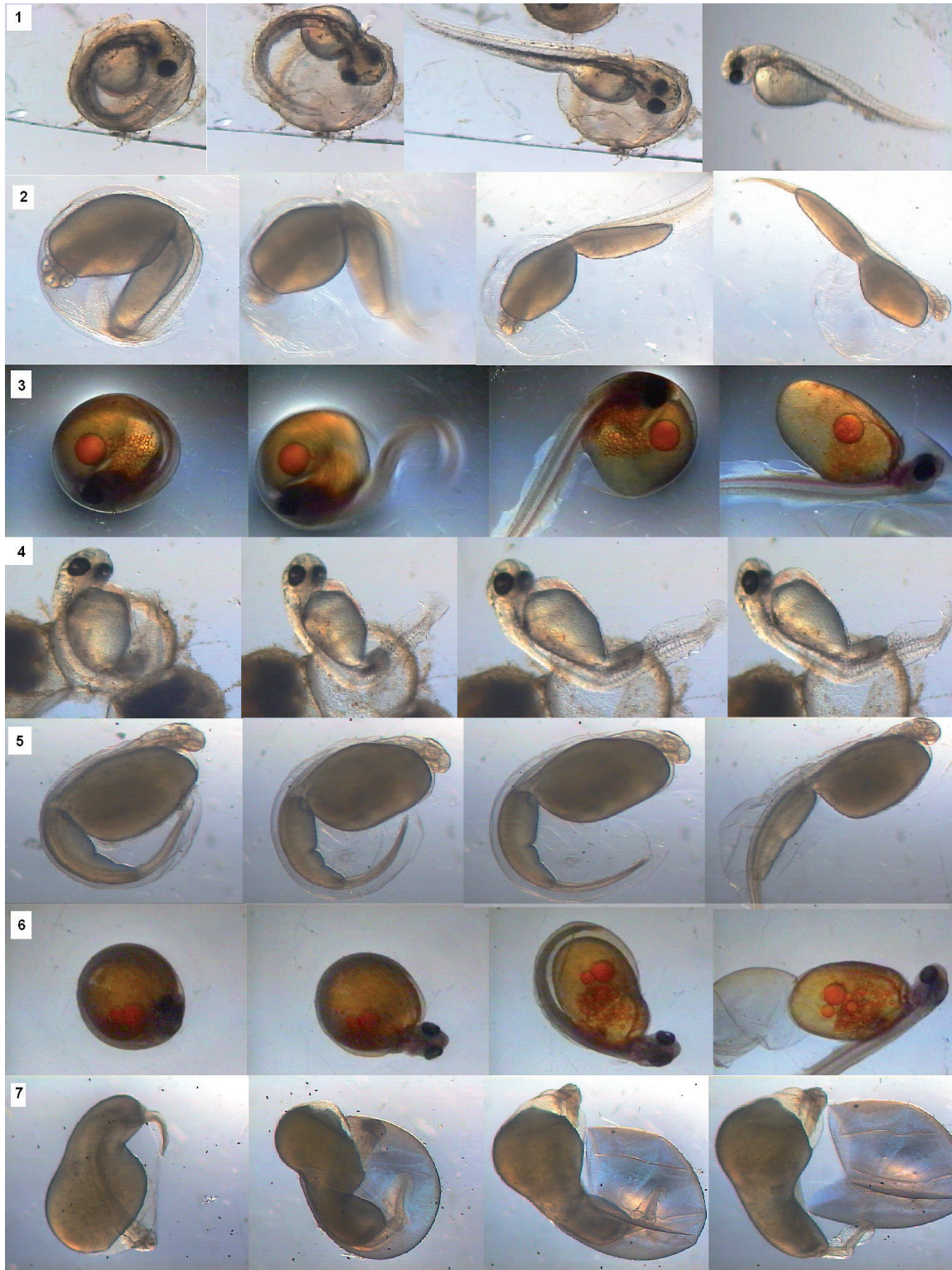
The study was done on embryos and newly hatched larvae of three species of fish, two cyprinids: common carp, *Cyprinus carpio*; barbel, *Barbus barbus*; and one salmonid: rainbow trout, *Oncorhynchus mykiss*. Eggs and sperm of carp and barbel were obtained during stimulated

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reproduction in the Inland Fisheries Institute in Żabieniec, whereas fertilized eggs of rainbow trout were obtained from the Hatchery of Salmonid Fish in Rutki. The eggs were transported in the cold box (5°C) to the laboratory of the Division of Animal Physiology in Siedlce.

Embryonic development of fish took place under controlled conditions in dechlorinated tap water (dissolved

oxygen saturation about 80%, total hardness 167 mg · L<sup>-1</sup> as CaCO<sub>3</sub>, and pH 7.8). Fertilized eggs of common carp (which are sticky) were placed on glass slides (5 replicates of 12 eggs in each), and each slide was incubated in 25 mL glass vessel, at the constant water temperature of 22°C. Barbel eggs were incubated freely dispersed in 2 dm<sup>3</sup> aquaria, at the temperature of 18°C, in 4 replicates



**Figs. 1–7.** Different modes of hatching in: common carp, *Cyprinus carpio*; barbel, *Barbus barbus*; and rainbow trout, *Oncorhynchus mykiss*; **Figs. 1–3.** Tail hatching (1 common carp, 2 barbel, 3 rainbow trout); **Figs. 4–6.** Head hatching (4 common carp, 5 barbel, 6 rainbow trout); **Fig. 7.** Yolk-sac hatching (barbel)



(80 embryos in each). Rainbow trout eggs were incubated in sieves (5 cm diameter) suspended in 12-L aquaria at 8°C, in 3 replicates (25 embryos in each).

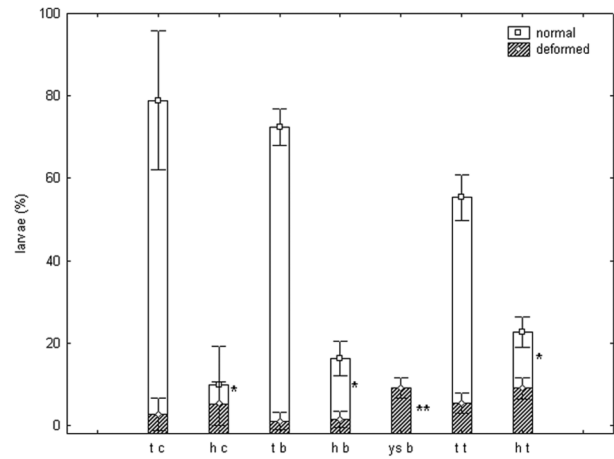
The embryos were observed daily, and when frequency of their movements inside the egg shell considerably increased (2–3 h before hatching for common carp and barbel, and 2 days before hatching for rainbow trout) observations were carried on continuously, until the end of hatching. Way and time of hatching of each larva was noted. Newly hatched larvae were counted and inspected. Body malformations were classified. Observations of embryos and larvae were done using the stereoscopic microscope Nikon connected to the computer with the MultiScan 8.4 image analysis system; the hatching embryos and larvae were photographed. The results were subjected to U Mann–Whitney test to evaluate the significance of differences ( $P < 0.05$ ).

The experiments comply with current Polish law (Certificate of Permission from the III Local Ethical Committee No.25/2007).

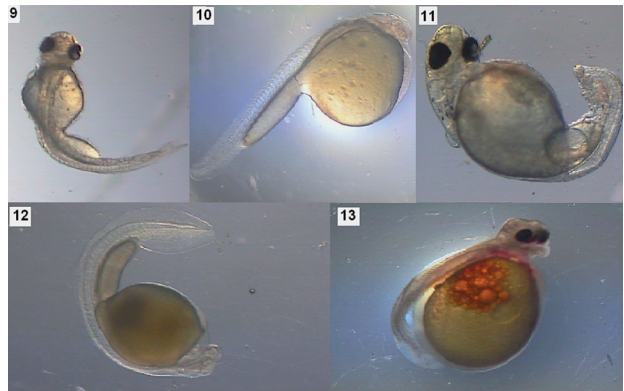
## RESULTS

Three ways of hatching were observed, two of them being similar in all fish species. Most fish started hatching from releasing the tail first from the egg shell (Figs. 1–3). Vigorous movements of tail outside the egg shell led to enlarging a hole and gradual but fast releasing of trunk with the yolk sac and head. Another way of hatching started from emergence of the head (Figs. 4–6). Movements of tail inside of egg caused tearing of egg shell and pushing out of the rest of the body. Third way of hatching—beginning from the yolk sac—was observed only in barbel (Fig. 7). Bending of the body inside the egg led to pulling out head at first, and eventually the rest of the body.

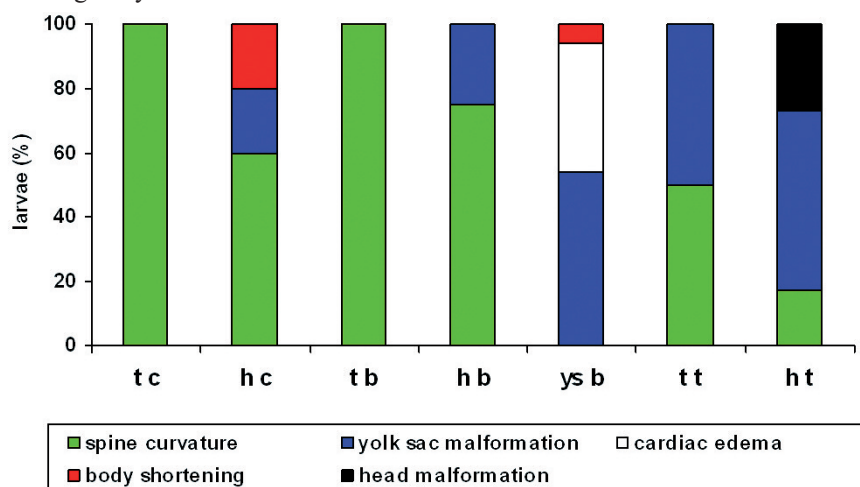
The percentage of larvae hatched each way is shown in Fig. 8. Most individuals of each species (over 70% of carp and barbel, and 57% of trout) hatched with tail first, and only 1%–5% of them were deformed. Among the head hatched larvae significantly higher frequency of body malformations occurred, while no normal larvae were observed among the yolk sac hatched barbels.



**Fig. 8.** The proportion of larvae representing different hatching modes (\*values statistically different from t for each species in normal larvae, \*\*values statistically different from t and h for barbel deformed larvae); Abbreviations: t c = tail hatching common carp, h c = head hatching common carp, t b = tail hatching barbel, h b = head hatching barbel, y s b = yolk sac hatching barbel, t t = tail hatching rainbow trout, h t = head hatching rainbow trout



**Figs. 9–13.** The most common types of larval body malformation (9 spine curvature, 10 yolk sac malformation, 11 body shortening, 12 heart edema, 13 head malformation)



**Fig. 14.** Types of deformations in relation to the way of hatching; Abbreviations: t c = tail hatching common carp, h c = head hatching common carp, t b = tail hatching barbel, h b = head hatching barbel, y s b = yolk sac hatching barbel, t t = tail hatching rainbow trout, h t = head hatching rainbow trout

The detailed examination of the larvae showed 5 main types of anomalies (Figs. 9–13). Single vertebral deformations included axial (lordosis or kyphosis) or lateral (scoliosis: Fig. 9) curvature of the spine. Among the yolk sac anomalies distinct dark spots (in the yolk sac hatched larvae: Fig. 10), and changes in yolk sac shape or/and its oedema were observed. Some larvae showed general body shortening (Fig. 11) accompanied by severe spinal, head and yolk sac malformation. Larvae with heart oedema showed also spine curvature (Fig. 12) and yolk sack deformations. Defects of head included jaw and/or skull malformation (Fig. 13) accompanied by spine curvature.

The data in Fig. 14 show that vertebral deformations were the most common in the tail hatched larvae (100% of all defects recorded for carp and barbel, and 50% for trout). Among the head hatched individuals spine curvature, yolk sac deformations (found in over 50% of trout and up to 25% of barbel and carp), and head malformations (only in trout larvae) were the most common. The yolk sac hatched barbel larvae showed over 50% of yolk sack defects, 40% of heart oedema and 6% of body shortening.

## DISCUSSION

Three ways of hatching: beginning from tail, head and yolk sac were observed in the present study in three fish species. The data on fish hatching process are very scarce. According to Korwin-Kossakowski (1998), most common carp embryos hatch by emerging the tail first, while most tench start hatching head first. According to oral information from fish farmers, also in rainbow trout tail hatching is the most common. However, no data on relationship between hatching way and quality of the larvae were found.

Larval deformations observed in the present study (spine curvature, yolk sac malformation, body shortening, heart oedema, and head malformation) are often observed in fish reared under optimum conditions, and were also reported by Bonnet et al. (2007). These authors noted five types of deformations (cyclopia, torsion, yolk sac resorption defects, prognathia, and “other”, deformations in rainbow trout larvae. According to Krejčí and Palíková (2006), deformities of the vertebral column and yolk sac of common carp were the most frequent in the control groups. Jezierska et al. (2000) also described deformations in common carp under control conditions. Most of them (about 80% of all defective larvae) were single vertebral abnormalities but some larvae showed craniofacial malformations, heart oedema, and yolk sac anomalies. The same types of common carp larvae deformations were also observed by Ługowska and Witeska (2004), and by Ługowska (2007). Moreover, those results show that in some cases single vertebral malformations are not persistent and may even completely reverse.

The data obtained in the present study showed that tail hatching was the most typical and successful in all fish species. Majority of tail-first hatched larvae were correctly developed and viable, while the deformed ones showed slight morphological defects, mainly single vertebral malformations. Tail hatching requires strong and agile body,

and the egg shell is broken by the tail which probably protects more fragile body parts from a possible harm. Head hatching was less common and less successful as compared to the tail hatching. Yolk sac hatching occurred only in barbel, and resulted in 100% of severely deformed larvae. Most of head hatched and all yolk hatched larvae showed body malformations (including body parts oedema and tail shortening) which significantly impeded motility of the embryos. Therefore, the results of present study showed the relation between hatching mode and quality of newly hatched larvae. It is, however, not clear if abnormal hatching is more often undertaken by abnormally developed embryos, or if the injuries result from the incorrect hatching itself.

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Received: 15 September 2009

Accepted: 17 August 2010

Published electronically: 25 March 2011