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Morphological examination of the larval development in Tigris kingfish, *Cyprinion macrostomus* Heckel, 1843

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A B S T R A C T

This study presents a comprehensive morphological examination of the larval development stages and growth rates of *Cyprinion macrostomus*. *C. macrostomus* broodstock have been collected from their natural habitats in Kangal, Sivas, Türkiye, and taken into cultivation under laboratory conditions. The research meticulously observed the early developmental stages, feeding behaviors, and growth rates of the larvae. Findings indicate significant morphological changes during the transformation of larvae into juvenile fish, with morphological metamorphosis completed by day 25. The average length of the larvae reached 14.78 mm by the end of day 25. This study provides fundamental information for the cultivation and conservation of *C. macrostomus*, identifying necessary conditions for optimizing larval growth and development processes. Moreover, this research contributes to the development of strategies for the sustainable management and conservation of *C. macrostomus*, offering a foundation for future studies to build upon for the species' conservation and successful aquaculture cultivation.

INTRODUCTION

Cyprinion macrostomus is a fish species belonging to the Cyprinidae family, commonly found in the basins of the Euphrates and Tigris rivers. This species typically has a grey back color and whitish sides, with some individuals displaying a large orange-reddish spot on the operculum. *C. macrostomus* has been utilized in health tourism in Türkiye, especially by the Kangal community, due to its healing properties discovered by chance (Özçelik & Akyol, 2008).

This species coexists with *Garra rufa* in natural spring waters and is used for therapeutic purposes in health service establishments at spas (Wildgoose, 2012). While *C. macrostomus* nibbles at the skin with its firm mouth movements, *C. macrostomus* consumes the softened skin, removing it from the environment (Wildgoose, 2012). This cooperation plays a significant role in the therapeutic process for both species.

Achieving success in the aquaculture of Tigris kingfish, *C. macrostomus* Heckel, 1843 necessitates a comprehensive morphological analysis of the species' larval development. The morphological progression observed during the larval phase of this species provides critical insights into the optimization of aquaculture conditions and environmental parameters. Specifically, the analysis of larval growth rates, morphological transitions, and developmental stages constitutes a foundational framework for the effective planning and management of aquaculture operations (Alwan et al., 2009).

The detailed examination of larval development from a morphological perspective aids in addressing challenges encountered in the aquaculture of *C. macrostomus*. These

investigations facilitate the identification of optimal nutritional requirements, growth trajectories, and the requisite water quality parameters for healthy larval development (Faris et al., 2014). Armed with this knowledge, the design and management of aquaculture systems can be aligned with the biological requisites of the species, thereby ensuring the robust growth and development of larvae.

Moreover, the morphological examination of *C. macrostomus* larval development holds significant implications for the conservation and sustainable management of the species. Such scientific inquiries enhance our understanding of the species' ecological needs and contribute to the formulation of strategies essential for the support of natural populations (Khalaf, 2008). Consequently, a meticulous morphological analysis of *C. macrostomus* larval development is imperative not only for augmenting aquaculture success but also for bolstering the conservation of the species within its natural habitats.

Therefore, it is imperative that research endeavors related to the aquaculture and conservation of *C. macrostomus* accord special emphasis to the analysis of morphological development during the larval stage. This approach furnishes the requisite scientific foundation for the sustainable management and conservation of the species, thereby facilitating the attainment of positive outcomes from both an economic and ecological perspective.

MATERIALS AND METHODS

Study Area and Fish Collection

Approximately 200 *C. macrostomus* specimens were collected from the natural habitats in Kangal, Sivas, Türkiye. The collection was carried out using nets, and the specimens were immediately transported to the laboratory within 24 hours post-capture. Upon arrival, the fish were placed in specially prepared aquariums for acclimatization and further study.

Aquarium Conditions and Maintenance

The specimens were housed under controlled conditions in the laboratory. Each aquarium was designed to accommodate a maximum of 50 fish, ensuring ample space and optimal living conditions for the specimens. The fish were maintained in these conditions for a period of 12 months. The diet provided was a commercial fish feed composed of 42% protein, 17% fat, 3% fiber, 12% ash, and 8.5% moisture, administered two to three times daily to meet the nutritional requirements of the fish.

Water Quality Monitoring

Water quality parameters, including temperature, pH, and dissolved oxygen (DO) were closely monitored throughout the broodstock culture period. The water temperature was maintained at 28±1°C, pH levels ranged from 8.0 to 8.4, and DO levels were kept between 6.0 to 6.2 mg l^{-1}. These conditions were consistently maintained to ensure an optimal environment for the growth and development of the fish.

Breeding and Incubation

After a period of 12 months, sexually mature males and females were identified based on morphological characteristics and segregated. The breeding ratio was set at 2 females to 2 males per tank. The breeding tanks were equipped with sponge filters and aeration systems to maintain water quality. The tanks were filled with coarse gravel and small stones to mimic natural breeding environments.

Post-spawning, eggs were carefully collected from the breeding tanks, and the total count of eggs and hatchlings was recorded. The eggs were then transferred to 30-liter glass incubation tanks, which contained water from the breeding tanks and were supplemented with aeration through air stones. The incubation tanks were maintained at a constant temperature of 28±1°C.

Larval Rearing

Newly hatched larvae were reared in the incubation tanks from hatching to 30 days after hatching (DAH). From 4 DAH to 30 DAH, 10% of the water in the tanks was changed daily to maintain water quality. The larvae were fed with brine shrimp (Artemia) from 3 DAH to 15 DAH, after which they were gradually transitioned to artificial diets until the end of the experiment.

Sampling and Observation

Random sampling of larvae (n=2) was conducted daily from the day of hatching until the end of the study period. The sampled larvae were observed under a stereo microscope for detailed morphological analysis. Additionally, general observations on morphology were conducted to monitor developmental changes throughout the larval stage.

This methodology provided a comprehensive approach to studying the growth, development, and morphological changes in *C. macrostomus* larvae under controlled laboratory conditions.

RESULTS

This study aims to determine the early developmental stages and larval growth rates of *C. macrostomus*. Throughout the research, the morphological developments, feeding behaviors, and growth rates of the larvae have been meticulously observed.

Early Developmental Stages

1 DAH (Days After Hatching): Newly hatched larvae had an average total length of 4.5 mm, with the yolk sac constituting more than 50% of the total length.

2 DAH: The mouth and anus remained closed, and the yolk sac size reduced.

3 DAH: Larvae exhibited limited swimming activity. The yolk sac continued to diminish, with increased pigmentation over the eyes and body.

4 DAH: The mouth and anus opened, larvae developed a one-chambered swim bladder, and swimming activity increased. The notochord's end was slightly flexed, marking the beginning of exogenous feeding.

5 DAH: Anal and dorsal fins started to develop, and the yolk sac was completely absorbed.

8 DAH: The postflexion stage was observed, with the dorsal and caudal fins beginning to separate from the continuous fin.

9 DAH: The dorsal and pelvic-fin spines started to elongate, with most larvae homogeneously distributed in the water column.

10 DAH: A second inflation of the swim bladder occurred between 9 and 10 DAH. Pigmentation increased on the head and lateral parts of the body.

18 DAH: All fins were well-developed, indicating significant progress in the larvae's morphological development.

25 DAH: The body shape and pigmentation pattern resembled those of adult fish, with the body completely covered in pigment. This marked the completion of morphological metamorphosis.

These findings highlight the rapid growth and significant morphological changes *C. macrostomus* larvae undergo in their early life stages. The transition from yolk sac dependence to active feeding and swimming, followed by the development of key anatomical features, reflects the larvae's adaptation to their aquatic environment. The completion of morphological metamorphosis by 25 DAH demonstrates the species' efficient early development, providing valuable insights for aquaculture practices and conservation strategies aimed at supporting the *C. macrostomus* population.

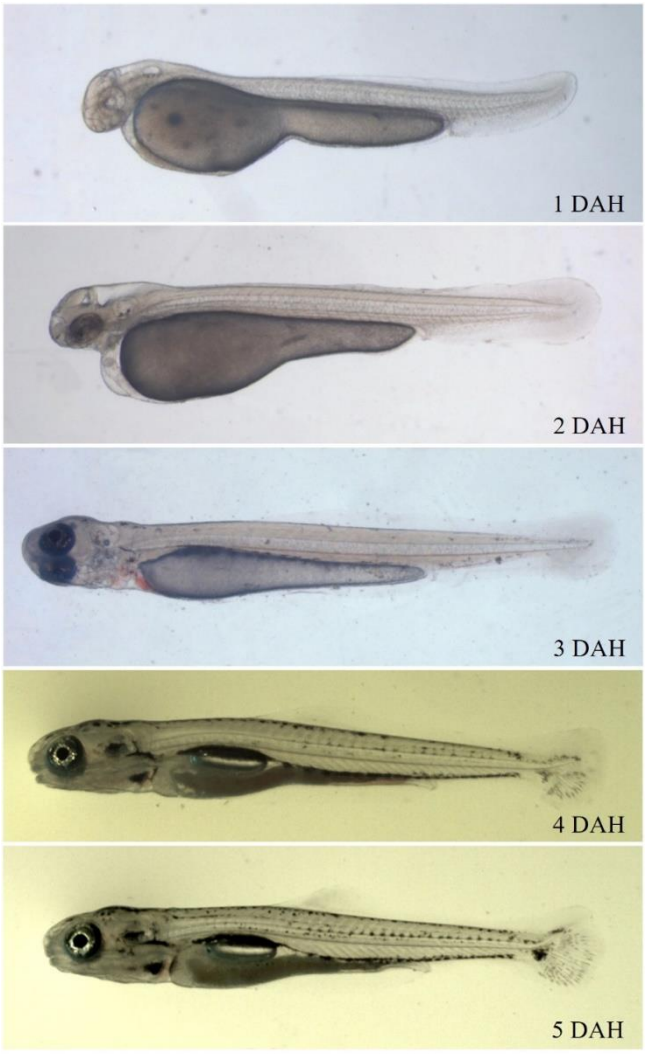

Figure 1. Morphological development of *C. macrostomus* larvae during the first 5 days post-hatching.

Larval Growth Rates

By the sixth day, the average length of the larvae was recorded at 7.42 mm. By the end of the eighth day, the larvae reached a length of 8.00 mm, and by the tenth day, a more pronounced increase in length was observed, averaging 9.48 mm. From the eleventh day onwards, the larvae's length rapidly increased, reaching 10.84 mm by the sixteenth day, 11.54 mm by the eighteenth day, and 14.78 mm by the twenty-fifth day.

These findings indicate that *C. macrostomus* larvae exhibit a rapid growth rate during their early developmental stages. The initiation of active swimming and feeding behaviors following the consumption of the yolk sac plays a significant role in the increase in larval growth rates. Furthermore, the development of pigmentation and the maturation of the

mouth structure during the larval period reflect critical morphological changes in the early life stages of the species.

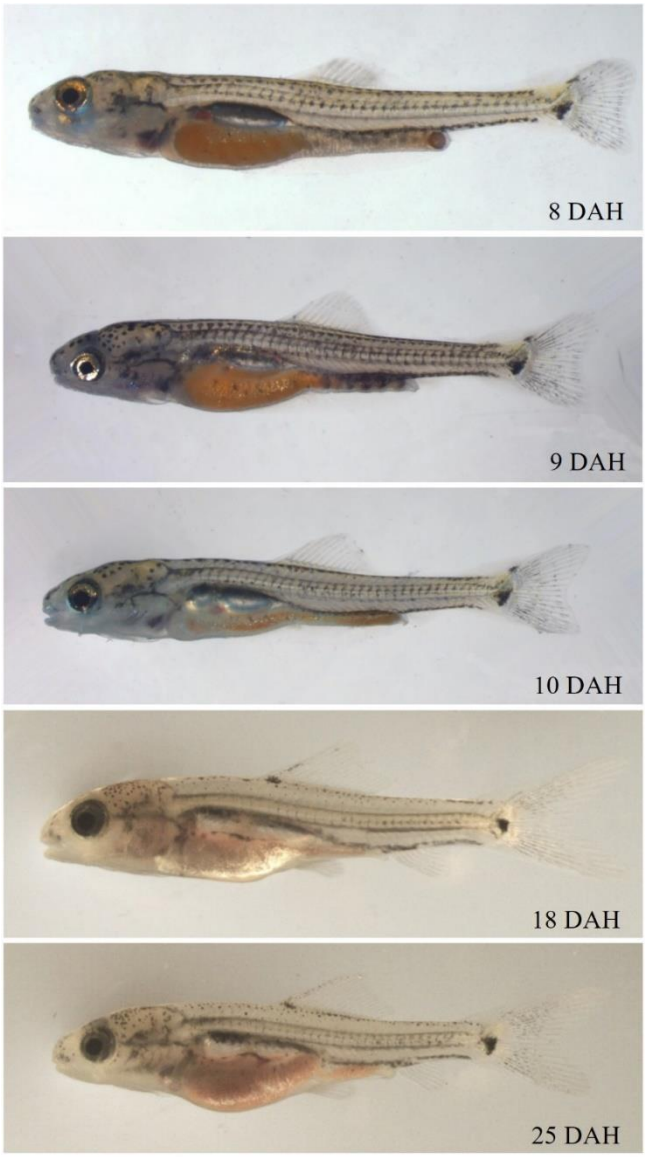

Figure 2. Morphological development of *C. macrostomus* larvae from day 8 to day 25 post-hatching

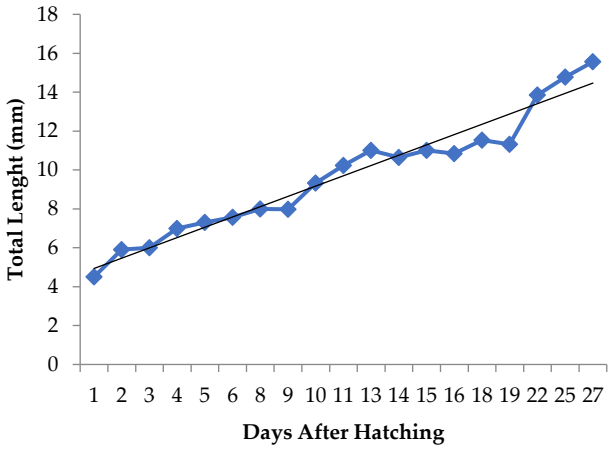

Figure 3. Growth chart of *C. macrostomus* larvae from hatching to the juvenile stage during the first month (mm)

This study provides essential insights into the larval development and growth dynamics of *C. macrostomus*, contributing to the development of strategies for the species' cultivation and conservation. This study has also meticulously examined the larval development stages and growth rates of *C. macrostomus*, shedding light on the morphological changes and growth dynamics of the species during its early life stages.

DISCUSSION

The study has demonstrated that *C. macrostomus* larvae undergo rapid morphological transformations within the first 25 days, developing critical anatomical structures such as the swim bladder, anal and dorsal fins. These findings underscore the importance of rapid growth and development during the early stages for effective aquaculture management and enhancement of larval survival rates.

This study presents a comprehensive morphological examination focused on the larval development stages and growth rates of *C. macrostomus*, aiming to fill gaps in this area. The research documents significant morphological changes during the transformation process from larvae to juvenile fish, based on detailed observations of the early development stages, feeding behaviors, and growth rates of this species. It has been determined that morphological metamorphosis is completed by the twenty-fifth day, with the larvae reaching an average length of 14.78 mm by the end of this period. This study provides critical information for the cultivation and conservation of *C. macrostomus*, identifying the necessary conditions to optimize larval growth and development processes. Additionally, the research contributes to developing strategies for the sustainable management of this species while establishing a solid foundation for future studies.

The results of the research highlight the rapid growth and significant morphological changes observed in the early development stages of *C. macrostomus* larvae. The transition from dependence on the yolk sac to active feeding and swimming behaviors, followed by the development of main anatomical features, indicates the successful adaptation of the larvae to the aquatic environment. The completion of morphological metamorphosis on the twenty-fifth day demonstrates the efficiency of the species' early development and offers valuable insights for aquaculture practices and conservation strategies aimed at supporting *C. macrostomus* population.

This study provides significant insights into the larval development and growth dynamics of *C. macrostomus*, contributing to the development of strategies for its cultivation and conservation. The research enriches the

existing literature in this field by offering detailed information on the morphological changes and growth dynamics during the early life stages of the species. In the discussion and conclusion sections, the main findings of the study are interpreted, comparisons are made with the existing literature, the limitations of the research are addressed, and suggestions for future studies are provided.

Previous studies by Alwan et al. (2009) have documented the morphological variations of *C. macrostomus*, but this study contributes significantly to the literature by detailing the larval development stages and growth rates. Research by Faris et al. (2014) explored the effects of water temperature on the growth performance and survival rate of *C. macrostomus* larvae. The findings of this study support the critical role of water quality parameters and feeding regimes on larval growth.

Scientific studies on the cultivation and production of *C. macrostomus* have evaluated the growth performance of this species under different water temperatures. One such study found that the best growth performance of *C. macrostomus* juveniles was achieved between the temperatures of 28-30°C when reared in temperatures ranging from 24°C to 34°C (Çelik & Güzel, 2017). These findings represent an essential step in determining the optimal temperature range for the cultivation of *C. macrostomus* juveniles.

The larval development process of *C. macrostomus* is characterized by a morphological metamorphosis that is completed by the twenty-fifth day. During this period, it has been observed that the larvae develop critical anatomical structures such as the swim bladder, anal, and dorsal fins, facilitating their adaptation to the aquatic environment (Çelik & Güzel, 2017). Rapid growth and development in the early stages are of critical importance for effective aquaculture management and increasing larval survival rates to support the *C. macrostomus* population (Özçelik & Akyol, 2008). Research contributes to the development of strategies for the sustainable management of *C. macrostomus* by identifying the necessary conditions for its cultivation and conservation (Wildgoose, 2012).

One limitation of this study is its confinement to laboratory conditions. Future research could explore the development of *C. macrostomus* larvae under different environmental conditions. Additionally, the effects of genetic factors on larval development could be investigated in more detail.

In addition to marine fish species, the larval development of freshwater fish also exhibits interesting differences and similarities. For instance, *Cyprinus carpio* (common carp) larvae hatch with a larger yolk sac and can survive longer on this endogenous nutrition compared to *C. macrostomus* (El-Sayed, 2006). In contrast, *Oncorhynchus mykiss* (rainbow trout) larvae begin exogenous feeding early in their development, similar to marine fish (Kendall et al., 1984).

Morphological development of freshwater fish larvae also varies among species. *Oreochromis niloticus* (Nile tilapia) larvae develop certain anatomical features more rapidly compared to *C. macrostomus* (Fujimura & Okada, 2007). On the other hand, the morphological development of *Salmo salar* (Atlantic salmon) larvae follows a more similar trajectory to that of marine fish (Gorodilov, 1996).

Larvae of different freshwater fish species may exhibit variable adaptations to environmental factors and threats. For example, *Clarias gariepinus* (African sharptooth catfish) larvae are tolerant to low oxygen levels and high temperatures (Hecht & Appelbaum, 1988). Similarly, *Ictalurus punctatus* (channel catfish) larvae show good development in turbid and nutrient-rich waters (Tucker & Robinson, 1990).

CONCLUSION

In conclusion, this study reveals that *C. macrostomus* larvae undergo rapid morphological transformations within the first twenty-five days, including the development of critical anatomical structures such as the swim bladder, anal, and dorsal fins. These findings highlight the critical importance of rapid growth and development in the early stages for effective aquaculture management and increasing larval survival rates. This research serves as a valuable reference source for the conservation and culture of *C. macrostomus* while laying a solid foundation for future studies.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

İÇ: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing

PÇ: Investigation, Visualization, Writing – original draft, Writing – review & editing

All authors read and approved the final version of the article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Both authors have a Certificate for the Use of Experimental Animals.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

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