Hindawi
Journal of Applied Ichthyology
Volume 2023, Article ID 2560365, 6 pages
https://doi.org/10.1155/2023/2560365

Research Article

Differences in Otolith Microstructure between Reared and Wild *Hedinichthys yarkandensis*

Liwei Xia, Chengxin Wang, Xinyue Wang, Linghui Hu, Fangze Zi, Liting Yang, Gulden Serekbol, Yong Song, and Shengao Chen

College of Life Science and Technology, Tarim University, Tarim Research Center of Rare Fishes, Alar 843300, Xinjiang, China

Correspondence should be addressed to Shengao Chen; shengao@taru.edu.cn

Received 15 July 2023; Revised 25 November 2023; Accepted 27 November 2023; Published 8 December 2023

Academic Editor: Ömerhan Dürrani

In this study, a significant difference was demonstrated between the otolith increments of wild and reared *Hedinichthys yarkandensis* (day, 1877) ($P < 0.05$). By comparing the width of the first 40 daily increments of otoliths, it was found that the otolith width of the wild *H. yarkandensis* was significantly wider than that of the reared *H. yarkandensis*. The otolith daily increment width of wild *H. yarkandensis* tends to increase significantly with age and then decreases slowly, while the otolith daily increment width of reared *H. yarkandensis* grows more steadily. This may be related to the different growing conditions of the two groups. This study investigates the relationship between the early life history of *H. yarkandensis* and the daily otolith increment and provides a basis for identifying fish groups and fish life histories through differences in otolith microstructure.

1. Introduction

Hedinichthys yarkandensis (Day, 1877), belonging to the order Cypriniformes, family Cyprinidae, subfamily Nemachilinae, genus Triplophysa, and subgenus Hedinichthys, is endemic to the Tarim River system in Xinjiang [1]. *Hedinichthys yarkandensis* (*H. yarkandensis*) is a dominant economic fish, mainly found in slow-moving shallow water habitats [2] and active physiological functions at 6°-7°C, indicating that this fish is a cold-water fish. The Tarim River is the longest endorheic river in China and the fifth largest inland river in the world, with a total length of 1,321 km, and is a typical seasonal river in arid conditions [3]. It is located in the northern part of the Tarim Basin in the Xinjiang Uygur Autonomous Region. It originates in the Tianshan Mountains and the Karakoram Mountains, runs along the northern edge of the Taklamakan Desert, through the southern part of the counties (cities) of Aksu, Shaya, Kucha, Luntai, Korla, and Yuli, and flows into Lake Taitma along the end [4]. In recent years, overfishing of this species and climate change have led to a decline in river flows in the Tarim River basin, resulting in a dramatic decline in *H. yarkandensis* population. Researchers have begun to study *H. yarkandensis* and measured the full mitochondrial DNA sequence of this species [5, 6]. To understand the genetic status of *H. yarkandensis* and to protect the normal reproductive life of the species, the researchers studied the genetic diversity and population differentiation of the species [2].

In recent years, researchers have carried out a series of studies on the otoliths of fish. Some studies have confirmed that estimating age through otoliths is accurate through the use of oxygen isotopes [7]. The otoliths have been used to classify and identify *H. yarkandensis* by their morphological characteristics [8]. Due to different growth environments, the morphology of otoliths in the same species of fish varies. A study analyzed the differences in otolith morphology of five *Scorpaena* species in different sea areas [9]. Research has analyzed abnormal otolith morphology information of blind eye sagittal otoliths in different marine *Flatfish* species through otolith morphology and characteristics [10]. In addition, morphological, morphometric, and contour shape analysis methods were used to compare the sagittal otoliths of the same sea area and genus of *Lepidorhombus* *whiffiagonis* and *Lepidorhombus boscii*, and the differences between the two were also found [11]. This indicates that otoliths can exhibit significant specificity under the influence of fish genetics and living environments. Researchers find daily increments of otoliths can assess early growth rates in fish [12]. Microstructural analysis of the otoliths of *Stigmatopora argus* and *Stigmatopora nigra* verifies the initial occurrence and periodicity of incremental otolith deposition [13]. The researchers assessed the growth of the larval stage in detail by daily incremental analysis of otoliths [14–17]. Some studies show that otolith microstructure data can be used to reveal the impact of the environment on the growth of young fish, the transfer process between life stages, and the performance of young survival in specific habitats [18–20]. No relevant studies have been reported to discern the early life history of *H. yarkandensis* by otolith daily increments.

Otolith microstructure analysis provides important biological and ecological information on the early life history of fish. This information is particularly important for interpreting and predicting the population dynamics of socioeconomically important fishery species [19]. In this study, the differences between wild and reared *H. yarkandensis* were investigated by observing the width of daily otolith increments in *H. yarkandensis* caught in the Tarim River and reared at the Tarim University Experimental Station. The relationship between the early life history and daily otolith increments of *H. yarkandensis* was investigated to provide a basis for inferring the living environment of the fish through otolith microstructure.

2. Materials and Methods

2.1. Material Sources. Fifty-six wild *H. yarkandensis* were collected in July 2021 in the Tarim River (N 40°30′50.62″ E 80°59′6.11″) (see Figure 1) using custom-made gillnets (mesh 4–8 cm) and ground cages (mesh 1 cm). Fifty reared *H. yarkandensis* were collected randomly in October 2021 at the Tarim University Experiment Station. Samples were collected up to 1 year of age. A study comparing scales, bones, and otoliths of *H. yarkandensis* found otoliths to be the most reliable structure for measuring their age [21]. Among the three types of otoliths in *H. yarkandensis*, the lapilli otoliths are the most structurally stable and more suitable for age estimation. Samples were collected, and otoliths were removed and then washed and dried with anhydrous ethanol.

2.2. Preparation of Otolith Samples and Daily Increment Counts. A randomly selected subset of left and right otolith samples ($n = 50$) was used for comparison. There was no significant difference between the left and right otoliths ($P > 0.05$); therefore, the right lapillus otolith was uniformly used for analysis. Lapillus otoliths are small in size, and to prevent damage to their internal microstructure, it is simpler and clearer to polish them by hand [21–23]. The lapilli were removed from each specimen and fixed on slides using a liquid plastic soldering tool (Bondic®). Otoliths were polished repeatedly by gently pressing them on 1000 and 1200 grit sandpaper in a circular motion until the origin and daily whorl of the otolith were clearly visible (see Figure 2). Otoliths with unclear or unrecognizable otolith origins and daily whorl outlines due to excessive grinding were not used. An Olympus CKX41 camera system was used to photograph each otolith and save them, and then, the first 40 daily otolith whorls were counted using ImageJ v1.8.0. The otoliths were measured along the short axis starting at the first increment of the otolith. The same sample was read twice in duplicate with an error of no more than 10%, at least 1 month between readings and a random sample.

2.3. Data Analysis. Data were checked for conformity to a normal distribution using the Shapiro–Wilk test. Homoscedasticity was checked using Levene's test. Then, analysis of variance (ANOVA) was used to test whether there were differences in the width of daily increments between wild and reared *H. yarkandensis*. The daily round data were processed using SPSS 16.00 software, and plots were made using Origin 2020 and Adobe Photoshop CS6.

3. Results

There was a significant difference in the width of daily otolith increments between the reared and wild *H. yarkandensis* ($P < 0.05$). The width of the daily increment in reared *H. yarkandensis* ranged from 2.1 to 9.5 μm, while that of wild *H. yarkandensis* ranged from 2.2 to 22.8 μm. The width of daily increments of wild *H. yarkandensis* was wider than that of the reared group. The width of the daily increments in the first 40 days of the age estimations of wild *H. yarkandensis* is more variable; however, overall, the width of the daily increments gradually widens with increasing age, peaking at 34 days of age and then gradually narrowing. The width of the daily increments in the first 40 days of the age of the reared *H. yarkandensis* is relatively stable (see Figure 3).

4. Discussion

The results of this study show that the daily increment width of the wild of *H. yarkandensis* is wider than that of the reared group, verifying that the difference in the early living environment of individuals between the two groups can cause differences in otolith microstructure. The difference in the living environment is an important reason for this difference [24, 25]. The temperature of water is an important factor [26], and research has shown a positive correlation between otolith growth and fish growth [27]. A warm water environment will increase the abundance of bait, which is conducive to the rapid feeding and growth of fish. At the same time, the palatability of bait also affects the deposition of mineral elements on otoliths [28]. The growth and development of individual fish can also lead to rapid growth of otolith microstructures, leading to greater daily widening of wild populations. This study found that the daily incremental width of otoliths in the wild population showed a trend of increasing and then decreasing growth. It was found that a daily growth cycle occurs during the rapid

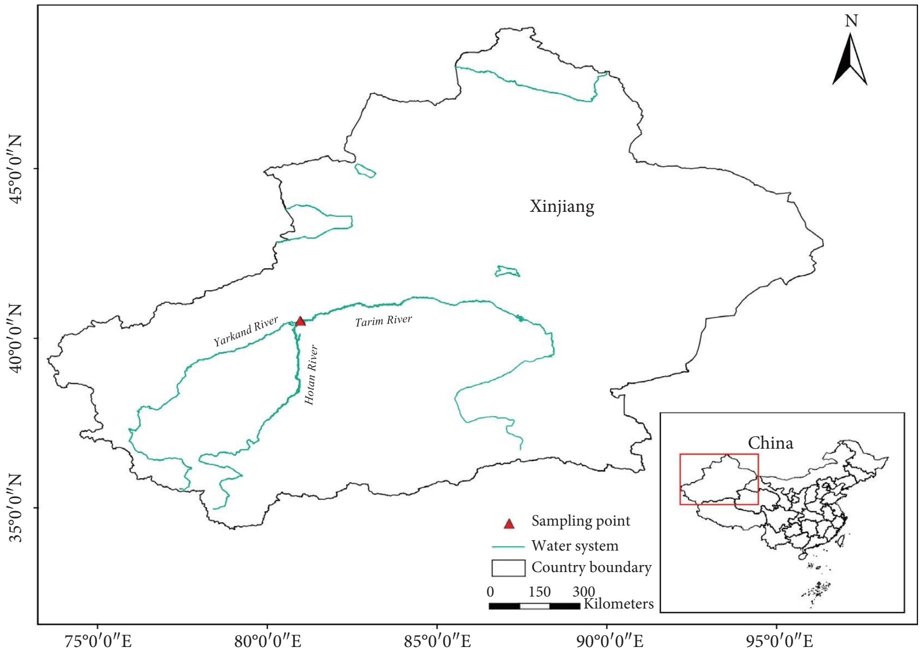

Figure 1: Wild *Hedinichthys yarkandensis* sampling point.

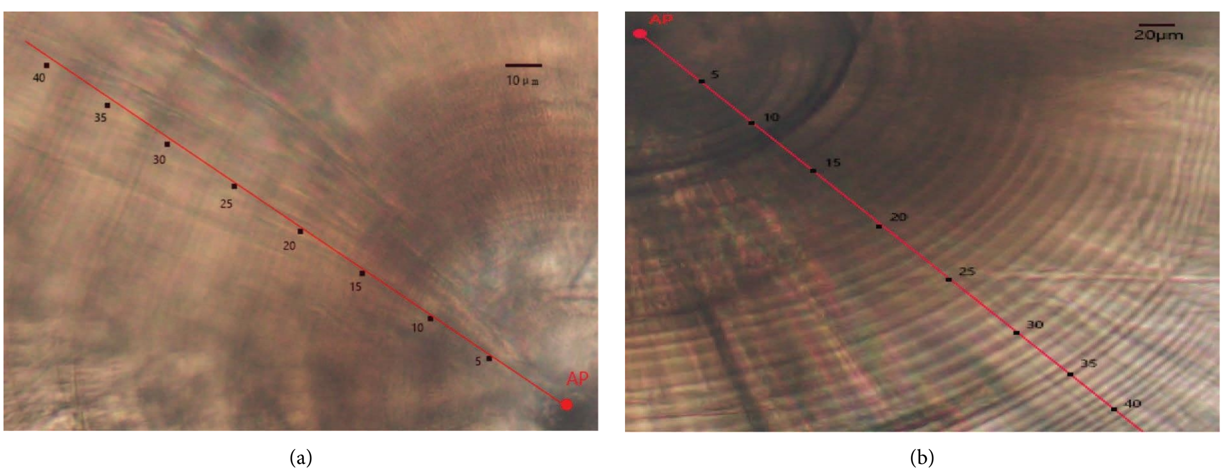

Figure 2: Otolith microstructure of the (a) reared and (b) wild *Hedinichthys yarkandensis* (AP: red dot is otolith origins; black dots represent the daily increase in otoliths).

growth period of fish around 7-8 months [29]. The water temperature in the Tarim River rises gradually during 7-8 months after a long period of a high temperature and light. The breeding period of *H. yarkandensis* is from the end of May to late June [30], so the juvenile stage of *H. yarkandensis* is during the period of rising water temperature in the Tarim River, promoting the growth of *H. yarkandensis* juveniles. The growth of fish varies greatly with the seasons, with high feeding and rapid growth of fish, and subsequently otoliths, at high temperatures, and vice versa [31]. Therefore, the width of the daily increments in the wild population widened significantly with the significant increase in temperature, with the width peaking at 34 days of age estimation. It is presumed that the water temperature on day 34 of age and the abundance of bait in the water reached the optimum growth value for *H. yarkandensis* within 40 days of age. It is generally accepted that the otolith incremental width decreases rapidly towards the edge after reaching a maximum [32–35]. Perhaps due to temperature and oxygen transport capacity, the daily increment width in this study began to decrease after approximately 34 days. Fish have been attributed to an increase in the aerobic standard metabolic rate and a decrease in appetite at temperatures above optimal. Therefore, with the increasing temperature in the wild environment, it may limit the growth of fish [36].

The study illustrated that the daily incremental rate of otolith deposition may be influenced by fish activity patterns [37, 38]. The wild *H. yarkandensis* has the biology of a benthic organism with a life pattern of periodic migration, selective spawning, and intense predation [39]. Daily

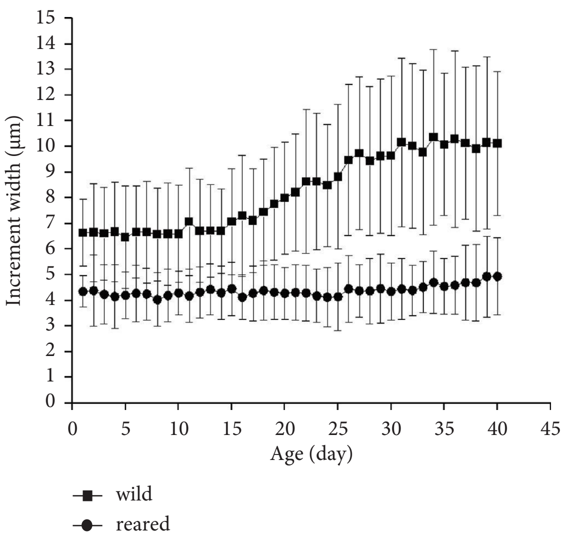

FIGURE 3: Overall trend chart of daily incremental width of otoliths in the first 40 days of reared and wild *Hedinichthys yarkandensis* ("ᴛ" the error bars on both sides represent each otolith of the same age).

incremental growth of otoliths in wild H. yarkandensis may also vary with seasonal factors. Researchers have observed seasonal changes in the composition of otoliths, which are usually related to and lagging behind the environmental temperature cycle [40]. During the growing season with high temperatures, the amount of otolith elements added is the highest [41]. Environmental instability in the wild can also lead to unstable growth rates in the wild *H. yarkandensis*, which in turn leads to uneven daily incremental widths. The reared *H. yarkandensis* is well-fed and reared in a stable environment, and as a result, the daily increment width is narrower and more regular in the farmed population than in the wild fish [42].

This study demonstrates the difference in daily increment widths between wild and reared *H. yarkandensis* populations. This practical and straightforward method can be used to identify wild and reared fish and provide a database for the study of otolith microstructure in fish. It enriches the basic biological data of *H. yarkandensis* and promotes the conservation of germplasm resources of *H. yarkandensis* [19].

Data Availability

The data that support the findings of this study are openly available in 4TU. ResearchData at https://doi.org/10.4121/f9250f19-182b-477f-bb09-94b7c8d84048.

Disclosure

Liwei Xia and Chengxin Wang are the co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Liwei Xia and Chengxin Wang contributed equally to this study.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31360635), the Special Agriculture and Rural Finance Project (Investigation on Fishery Resources and Environment in Key Waters of Northwest China), and the Corps Science and Technology Bureau Project (2017DB003 and 2022DB019).

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