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A Study on Morphological Discrimination between *Tribolodon brandti* and *T. hakonensis* at the Early Developmental Stages

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Summary

In ecological studies on fish in their early life history, it is necessary to distinguish the objective species from taxonomically close species. In the genus *Tribolodon*, species are very similar morphologically, so it is considerably difficult to identify them. Moreover, reliable morphological discrimination of their larvae and juveniles has not been established yet. In this study, the authors reviewed available literature on morphological discrimination between two *Tribolodon* species, *T. brandti* and *T. hakonensis*, at the early developmental stages. Basing the morphological comparison between the two species at larval and juvenile stages with special reference to developmental processes of melanophores, we indicated that availability of following characters as the keys for discrimination. 1) At yolk sac stage and early post-larval stage, melanophores on lateral midlines are located posterior to the 7-8th myomeres in *T. brandti* but the 11-13th myomeres in *T. hakonensis*. 2) At post-larval stage, *T. brandti* has some remarkable melanophores on both pectoral fins and anal fins, but *T. hakonensis* has few melanophores on both fins. 3) At juveniles stage, *T. brandti* has numerous spotty melanophores on pectoral fins and anal fin, but *T. hakonensis* has elongated melanophores along the fin rays of both fins. The renults obtained in this study suggest that more accurate morphological discrimination between the two species is possible by the use of these view points in combination with those described by other researchers.

Tribolodon brandti is a unique fish in the family Cyprinidae because of its anadromous mode of life, mainly inhabiting in the lower parts of rivers, embayments and coastal waters around the mouths of rivers, and it is known that *T. brandti* is one of the most dominant fish in the brackish waters in the northern part of Honshu Island and Hokkaido. In spring, a great number of adult fish migrate to the middle parts of rivers and spawn in the shallow rapids, so it is considered that *T. brandti* plays an important role in the biological production systems of rivers.

The authors have been studying the biological production of *T. brandti* in its

early life history. This study has been conducted in the River Natori where numerouns information on the structure of aquatic community were obtained by some ecological studies.

In ecological studies on fish in their early life history, it is necessary to sort out the objective species from taxonomically close species at each developmental stage. In the River Natori, two species of the genus *Tribolodon*, *T. brandti* and *T. hakonensis*, inhabit. Therefore, it is important for us to find out a connvenient method of discrimination between the two species at early developmental stages.

In the genus *Tribolodon*, species are very similar morphologically, so it is difficult to distinguish them. Accordingly, their classification has been confused for a long time (1-6). Currently, *Tribolodon* fishes are classified into four species, *T. hakonensis*, *T. brandti*, *T. ezoe* and *Tribolodon* sp., due to some morphological characters such as nuptial color, cephalic lateral-line system and gas-bladder (7-9). Nevertheless, identification based on these characters is not applicable to larvae and juveniles which are not developed enough to exhibit the characters.

Nakamura and Mochizuki (6) compared two *Tribolodon* species, *T. hakonensis* and *T. brandti*, morphologically, and reported on differences between two species as follows. 1) At the post-larval stage, a series of melanophores on the ventral tips of myomeres connect with melanophores on the abdomen in *T. brandti*, but they don't connect in *T. hakonensis*. 2) At the juvenile stage, there is a black longitudinal band on the lateral midline in *T. hakonensis*, but there is a slight black line in *T. brandti*. Nevertheless, in our experiment to distinguish the two species on the basis of these view points, we found that it is difficult to make clear-cut identification due to some intraspecific variation.

On the other hand, in the recent studies, electrophoretically distinct isozymes were utilized to distinguish *Tribolodon* species (10, 11). These biochemical methods are very reliable, however, it is very difficult to apply the electrophoretic analysis to individual identification of numerous specimens in the ecological field-work.

In this study, the authors tried to find an effective and convenient method of discrimination between two *Tribolodon* species at the early developmental stages. The results obtained in this study demonstrated specific characteristics which are available for morphological discrimination revealed out by detailed comparison between developmental processes of melanophores in both species.

Materials and Methods

In the River Natori, *Tribolodon* fishes spawn en masse on rapids with gravel bottoms in fresh water areas of the river during April to June. Parental fish of *T. brandti* and *T. hakonensis* aggregating for spawning were caught by a cast net in a spawning ground, about 9 km above the mouth of the river (Fig. 1), on 30 April 1993. Two ripe females, *T. brandti* : 417 and 488 mm SL, *T. hakonensis* :

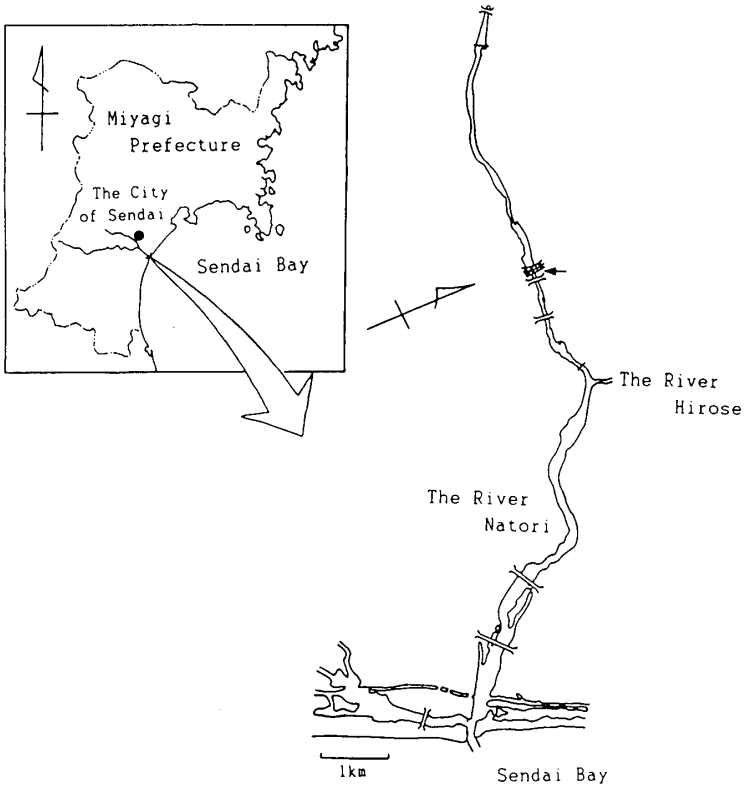

FIG 1. The River Natori in Miyagi Prefecture. The arrow mark indicates the site where parental fish of *Tribolodon brandti* and *T. hakonensis* for artificial fertilization were sampled.

216 and 270 mm SL, and three males, *T. brandti* : 378-422 mm SL, *T. hakonensis* : 208-277 mm SL, of each species were used for artificial fertilization. Eggs fertilized by the wet method were placed in aquariums and incubated at 20°C.

Hatching occurred three days after fertilization, and larvae were reared for ninety days until they reached the juvenile stage at 25°C. Larvae after absorption of yolk were fed on nauplii of *Artemia*.

Three to ten larvae and juveniles of each species were sampled from reared fish at one to three days intervals after hatching. They were preserved in 10% formalin. The outward appearance of specimens was observed under 10 to 40× magnification with special reference to meristic characters and location of melanophores on the bodies in order to distinguish the differences between the two species.

Results

Yolk Sac Stage

Newly hatched larvae of *T. brandti* are 5.2-6.7 mm (mean 6.1 mm) SL (Fig. 2A). Number of myomeres is 43-46. Yalk-sac is spherical in the anterior half of the trunk but slender in the posterior half. Head of the larvae bend ventrally

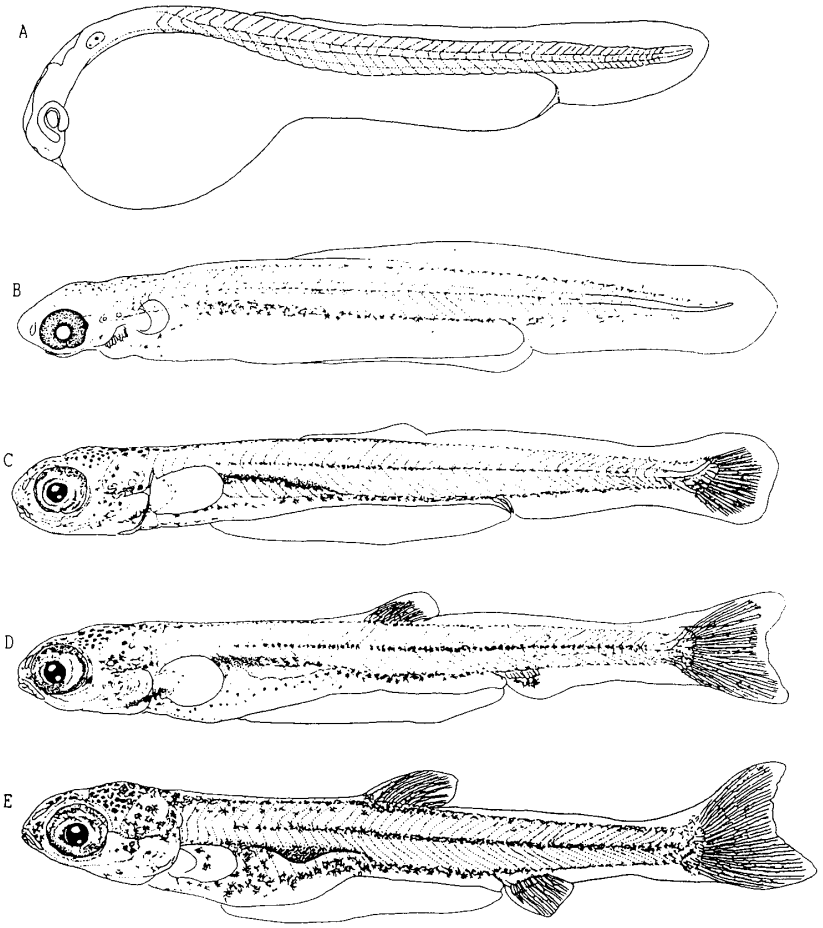

FIG 2. Morphology of larval *T. brandti*.
A, immediately after hatching, 6.1 mm SL; B, 5 days, 9.4 mm; C, 13 days, 10.4 mm; D, 28 days, 10.7 mm; E, 52 days, 12.4 mm.

along the yolk-sac. Melanophores are not observed at all. On the other hand, newly hatched larvae of *T. hakonensis* are 5.9-6.9 mm (mean 6.3 mm) SL (Fig. 3A). They show no obvious differences in the outward appearance and meristic characters from *T. brandti*.

Four days after hatching, tiny melanophores appear on the head, dorsal and ventral tips of myomeres, and lateral mid-line in both species, and they appeared clearly five days after hatching. This is the first stage where morphological difference can be observed between the two species; namely, melanophores on lateral mid-line are located posterior to the 7-8th myomeres in *T. brandti* (Fig. 2B) but 11-13th myomeres in *T. hakonensis* (Fig. 3B).

Post-larval Stage

Thirteen days after hatching, larvae of both species finish absorption of the yolk completely and attain to the post-larval stage. Although interspecific

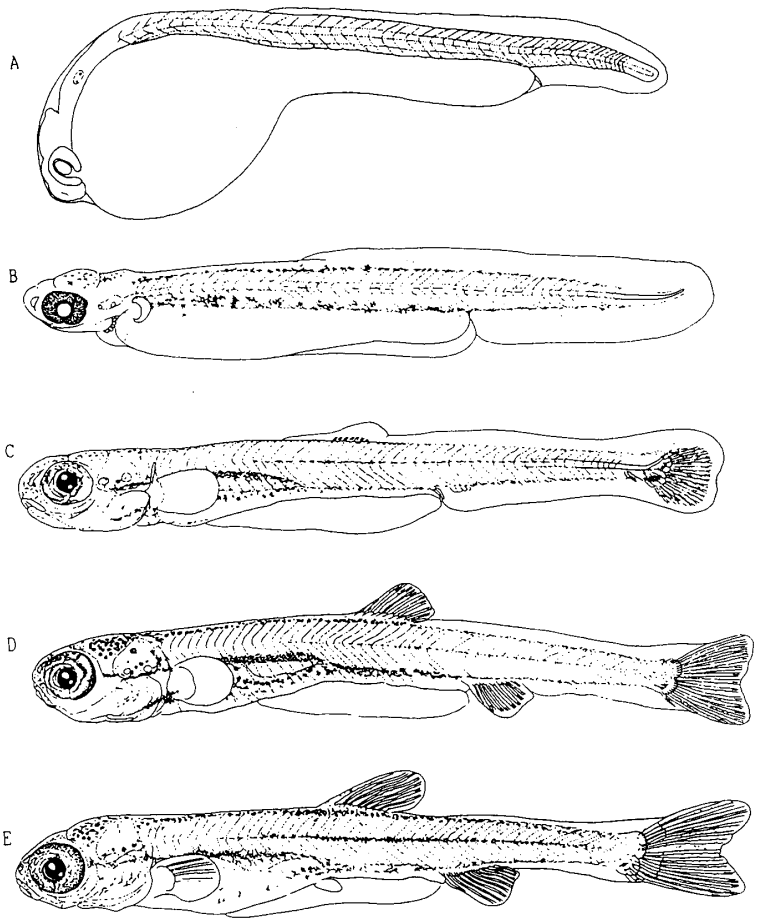

FIG. 3. Morphology of larval *T. hakonensis*.
A, immediately after hatching, 6.3 mm SL; B, 5 days, 9.8 mm; C, 13 days, 11.3 mm; D, 28 days, 11.5 mm; E, 52 days, 14.1 mm.

difference in location of melanophores in lateral midline still remains at the early post-larval stage, it becomes indistinct in specimens larger than 12 mm SL. In addition to this difference, presence or absence of melanophores on pectoral fins and anal fins are available as criteria for morphological discrimination between the two species at this stage. In the case of post-larvae of *T. brandti*, some melanophores are observed on the pectoral fins (Fig. 2C), and melanophores also appear on the anal fins according to growth (Fig. 2D, E). On the other hand, there are few remarkable melanophores on both the pectoral and anal fins in post-larvae of *T. hakonensis* (Fig. 3C-E).

Juvenile Stage

All fin rays fix in number to adult complement in specimens of both species larger than 20 mm SL, and larvae attain to the juvenile stage. At this stage, *T. hakonensis* also begins to have some melanophores on the pectoral fins and anal fin,

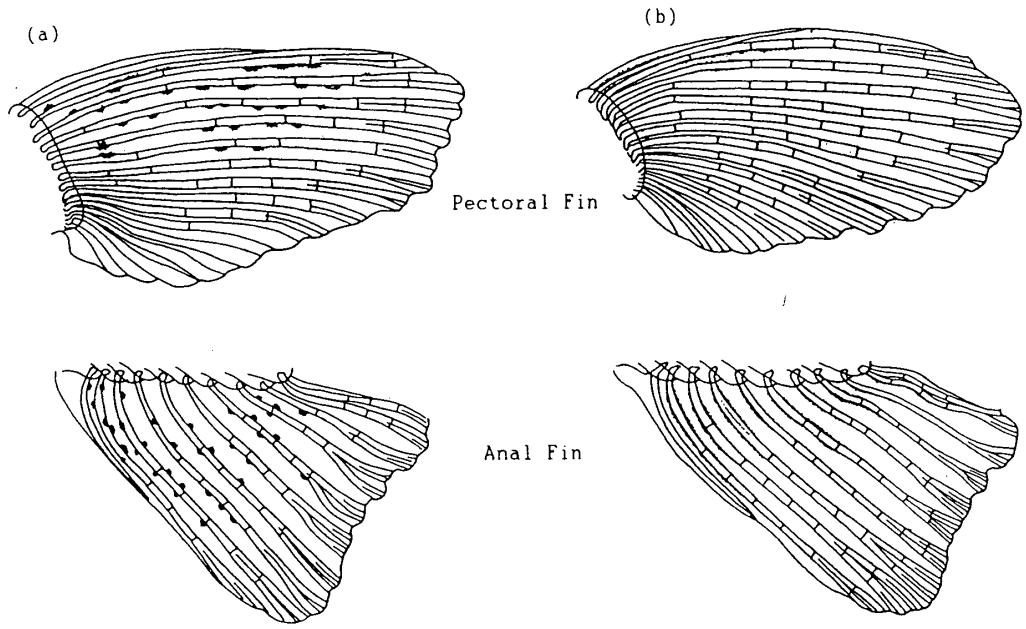

FIG. 4. Melanophores on the pectoral fin and anal fin of *T. brandti* (a) and *T. hakonensis* (b) at juvenile stage.

but the melanophores are not so marked as those of *T. brandti*. Namely, while *T. brandti* has many spotty melanophores on both pectoral fins and anal fin (Fig. 4a), *T. hakonensis* has elongated melanophores along fin rays on these fins (Fig. 4b).

Discussion

The results presented in this study indicate that there are differences in distribution of melanophores between *T. brandti* and *T. hakonensis* at the larval and juvenile stages. Identification based on these interspecific morphological differences in combination with those described by Nakamura and Mochizuki (6) will be advantageous for discrimination of both species at early developmental stages. Nevertheless, we don't examine whether these specific characteristics of reared fish are generally observed in wild fish, so it is necessary to determine availability of the characteristics as the keys for morphological discrimination of wild *Tribolodon* species.

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