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Bluefin tuna larval rearing and development – State of the art

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SUMMARY – In Japan, trials for mass seed production of Pacific bluefin tuna, *Thunnus thynnus,* have been conducted vigorously in recent years. There are three major problems concerning seed production: (i) early mortality during the first 10 days after hatching; (ii) cannibalism around 20 days after hatching; and (iii) collisions against the tank wall observed from juvenile period. Studies on the developmental biology of bluefin tuna larvae have been published intensively over the past years, using laboratory-reared specimens. Developmental characteristics of bluefin tuna are summarized in the second part of the present paper.

Key words: Digestive system, RNA/DNA, larvae, juvenile, growth, enzyme.

RESUME – *"Elevage larvaire et développement du thon rouge : Etat des connaissances". Au Japon, des essais pour la production en masse de semence de thon rouge du Pacifique* Thunnus thynnus *ont été menés activement pendant ces dernières années. Il y a trois grands problèmes en ce qui concerne la production de semence : (i) la mortalité précoce pendant les 10 premiers jours après éclosion ; (ii) le cannibalisme environ 20 jours après éclosion ; et (iii) les collisions contre les murs des bassins observées pendant la période juvénile. Des études sur la biologie du développement des larves de thon rouge ont été publiées à profusion pendant ces dernières années, en utilisant des spécimens élevés en laboratoire. Les caractéristiques développementales du thon rouge sont résumées dans la seconde partie du présent article.*

Mots-clés : *Système digestif, ARN/ADN, larves, juvénile, croissance, enzyme.*

Introduction

Since the Pacific bluefin tuna *Thunnus thynnus* (BFT) is one of the most expensive fish in Japan, the fish has received considerable attention as an important candidate for future aquaculture and stock enhancement. Spontaneous spawning of BFT in captivity was first recorded in 1979 at Kinki University, Wakayama, Japan (Kumai, 1997). Since then, the seed production of BFT has been attempted vigorously in some hatcheries. Although there remain many challenges before a rearing technique can be established, efforts are beginning to yield increased survival rates of BFT larvae and early juveniles.

To establish suitable seed production techniques and facilities for finfish, it is indispensable to accumulate fundamental knowledge about species-specific early life history traits. In particular, results of seed production trials of BFT strongly suggest that they have unique eco-physiological characteristics during their early life history (Miyashita, 2001). The basic developmental biology of hatchery-reared BFT, therefore, has been studied and published intensively over the last several years, by taking advantage of the ability to use laboratory-reared BFT larvae (Kaji *et al*., 1996; Takii *et al*., 1997; Miyashita *et al*., 1998; Miyashita *et al*., 1999; Ito *et al*., 2000; Kaji, 2000; Miyashita *et al*., 2000a,b; Sawada *et al*., 2000; Hattori *et al*., 2001; Masuma *et al*., 2001; Miyashita, 2001; Miyashita *et al*., 2001; Shimizu and Takeuchi, 2002).

The first part of this paper briefly introduces the present state of larval rearing of BFT. Development of BFT larvae is summarized in the second part in terms of morphological, physiological and biochemical data (Kaji, 2000).

Rearing

The present state of mass seed production for BFT larvae in Japan was well described by Kumai (1998), Miyashita (2001) and Tezuka (2001). They noted that there are three major mortality stages

during the seed production. The first stage is the first ten days after hatching (DAH). The second one is from 10 DAH to the juvenile, caused by active cannibalistic behaviour. The third one is from the juvenile to the young stage, caused by collisions against the tank and the net-pen wall.

The mechanism of these collisions have been investigated by several researchers, because it is unique and the most serious problem for BFT culture (Miyashita *et al*., 2000; Miyashita, 2001; Masuma *et al*., 2001). Miyashita *et al.* (2000) and Miyashita (2001) investigated the development of the external morphology concerning swimming performance and found that BFT attain remarkable propulsion power at the juvenile period to young stage, but their control system for swimming develops later, resulting in inability to avoid the tank wall. Masuma *et al*. (2001) carried out retinomotor response experiments using cultured bluefin tuna juveniles and concluded that visual disorientation due to the incompatibility of the retinal adaptation with the change in the ambient light intensity at dawn is one of the causes for colliding with the net-pen wall.

Development

All the fish described in this part were reared at the Amami and Yaeyama Stations, Japan Sea Farming Association. Results were mainly based on Kaji (2000), and are summarized in Fig. 1. To describe the early ontogeny of BFT, the developmental phases proposed by Kendall *et al*. (1984) were used in the present study. Briefly, the larval period was divided into 3 phases based on the state of notochord tip flexion (preflexion phase: first feeding to onset of notochord flexion, flexion phase: to completion of notochord flexion, postflexion phase: to completion of fin formation).

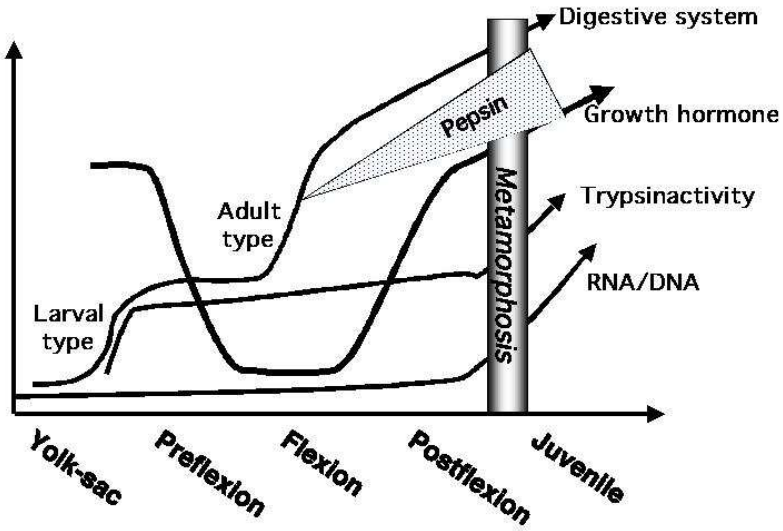

Fig. 1. Schematic drawing of the early development of bluefin tuna *Thunnus thynnus* reared in the laboratory. Data are based on Kaji (2000). Developmental phases proposed by Kendall *et al*. (1984) were used in the present figure.

Digestive system

The BFT larvae initially fed on rotifers from 3 DAH, concurrent with the establishment of a primitive larval type digestive system. The gastric glands and pyloric caeca first appeared during the flexion phase, indicating early formation of the juvenile-type digestive system. The number of gastric glands and pyloric caeca and volume of the gastric blind sac increased markedly as larvae developed toward the juvenile period. When compared to the other marine fish larvae hatched from pelagic eggs (Tanaka, 1973), the development of the digestive system of BFT larvae appeared to be precocious, supporting early shift to piscivory and resultant rapid somatic growth.

Digestive enzymes

Specific activities of trypsin of BFT larvae were determined individually by the methods reported by Ueberschär (1988). The specific activity exhibited three peaks, 3, 14, and 25 DAH, which coincided with first feeding, flexion phase, and transformation phase to juvenile, respectively. Pepsinogen synthesis in the stomach, detected by immunohistochemistry, started just before the flexion phase, and activated as larval development proceeded. These results suggest that the digestive capability increase markedly from the flexion phase.

Growth hormone

Growth hormone (GH) immunoreactive cells in the pituitary were first detected 3 DAH at first feeding. Percent GH, defined as the ratio of GH cell-mass volume to pituitary volume, was very high during a few days after initiation of feeding. Then it rapidly decreased, remained at the lowest level throughout the flexion phase, and began to increase from the postflexion phase to the juvenile period. Such a V-shaped ontogenetic pattern of %GH was also observed in yellowfin tuna *T. albacares* larvae (Kaji *et al*., 1999). The %GH of tuna larvae was considerably higher than those of the other marine fish species previously examined throughout the larval period.

RNA/DNA ratio

RNA/DNA ratio is used as an index of protein synthesis and the nutritional condition of marine fish larvae. Thus, the RNA/DNA ratios were determined individually using an ethidium bromide technique (Clemmesen, 1993; Sato *et al*., 1995). The ratios remained at a constant low level during most of the larval period with a temporal decrease around first feeding. Then the ratios increased steeply from 20 DAH at metamorphosis, suggesting drastic increase of protein synthesis capacity.

Developmental characteristics of bluefin tuna

Altricial yolk-sac and preflexion larval phases

Histological, physiological, and biochemical data suggest that BFT undergo an altricial and immature early larval period from hatching until the end of the preflexion phase. Overall developmental features during this period, e.g. primitive body structure with normal-size mouth and larval-type digestive system structure and function, are similar to those of common coastal marine teleosts hatched from small pelagic eggs.

The existence of a "critical period" around first feeding, represented by slow growth and high mortalities under rearing conditions, was confirmed consistently by histological, physiological, and biochemical data. Because of tuna's high fecundity and batch spawning mode offshore, they could be a representative group with a life history pattern characterized by very high annual production of larvae and subsequent high mortality at sea. Vulnerability of tuna larvae revealed in the laboratory-reared fish could be common to in the open ocean. This mode of spawning may also force larvae to undergo an altricial larval period offshore in which the densities of available food organisms are relatively low. Primitive body structure of tuna at an early larval period would be adaptive to utilize small but abundant invertebrate zooplankton, such as copepod nauplii (Uotani *et al*., 1990).

Flexion phase as a turning point of development

The flexion phase should be noted as a "turning point" in tuna larvae. External morphology common to most fish species transformed into a specialized form of scombrid larvae characterized by a disproportionately large head with large mouth and eyes. Although the other body structures are still of the less-advanced larval type, only the digestive system attained the adult type; differentiation of gastric glands in the stomach followed by differentiation of pyloric caeca. Compared to other marine fish larvae, apparently precocious development of the digestive system coupled with an enlarged head and mouth

allow the larvae to shift the survival strategy from "altricial larval life with planktivory" to "large prey-fast growth" (Hunter, 1981) with a potential piscivorous food habit.

Large prey-fast growth during postflexion phase to juvenile

Histological, immunohistochemical and physiological data, as well as such morphological features as posterior shift of the anus position, demonstrated that the structure and function of the digestive system developed in an extremely quantitative way during the period from postflexion phase to the juvenile period. At the transition to juvenile, fins completed and vertebral column ossified, which are generally observed in the metamorphosis of many marine teleosts, indicating marked increase in swimming ability which enable the tuna juvenile to actively exploit prey fish larvae at relatively low densities in their offshore habitat. Therefore, the developmental feature of BFT at postflexion phase and juvenile period can be described as "large prey-fast growth", as demonstrated by the steep increase of %GH and RNA/DNA ratio. In fact, Miyashita *et al*. (2001) noted that the growth of BFT was markedly accelerated from about 2 weeks after hatching, while the growth during first 10 days was low and similar to those of common marine fish larvae previously examined.

In summary, the early development of BFT divides into two contrasting periods; altricial larval period and rapidly developing postflexion phase to juvenile. The flexion phase intervenes between these two periods as a turning point.

Further studies on ecological traits of wild tuna larvae, as well as a comparative study among other scombrid species, are required to understand the early survival strategy of tuna.

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