Estimation of Early Growth History in the Japanese Sandfish with Otolith Microstructure

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Early growth of the hatchery-reared Japanese sandfish *Arctoscopus japonicus* was deduced by analyzing the otolith microstructure.

A positive linear relationship was observed between the radius of lapillus and the total length in the 19 juveniles, 34.2 mm in TL and 92 d in age, suggesting that the otolith growth could represent the body growth of fish in length. The relationship between the age and the radius of each corresponding daily increment in the lapilli revealed that otolith radius increased rapidly at the mean rate of 3.9 $\mu\text{m}/\text{d}$ between 31 d and 52 d, and thus early growth pattern divided into three phases (Ph-1; 0–30 d, Ph-2; 31–52 d, Ph-3; 53–92 d). The rapid growing phase, Ph-2, seemed to have some relation with an abrupt increase in rearing temperature from 10 to 14°C.

The otolith growth rate of a phase was positively correlated to an increment radius at the end of the phase. Furthermore, total length had a positive correlation with otolith growth rate of the latest phase, Ph-3, showing that body size might be determined by the latest growth rate of the individual.

The Japanese sandfish *Arctoscopus japonicus* (Steindachner) Trichodontidae, is an important commercial fish in Japan. In recent years, the population decreases rapidly,¹⁾ and intensive survey is now required on their life history and population. In the previous study,²⁾ we validated the daily increments in the lapillus of the Japanese sandfish, and developed ageing technique of this species in daily precision. The present study analyzed the otolith microstructure of the hatchery-reared juveniles to deduce their early growth pattern as the first step for a future intensive field survey of this species.

Materials and Methods

Newly hatched larvae, 0–3 days after hatching and 13.7 mm in mean total length (TL), were immersed in 400 mg/l tetracycline hydrochloride (TC) solution in 100 l plastic tank for 24 h to make a time mark in the otoliths.³⁾ They were reared for 95 days after that according to the standard procedure of Japan Sea-Farming Association (JASFA),⁴⁾ fed twice a day on rotifer and *Artemia* nauplii. They were kept under natural photoperiod in an indoor tank with running sea water supply. Water temperature ranged from 9 to

20°C. At the end of rearing experiment, the juveniles of 34.2 ± 2.5 mm in TL (mean $\pm$ SD) were fixed in 90% ethanol (Table 1).

After measurement of TL, lapilli of 19 juveniles were extracted under dissecting microscope and mounted in epoxy resin on a glass microscope slide. They were ground with sandpaper to the center of the otolith. The increments between the nucleus and the otolith edge was traced along the longest 'radius' with a camera lucida (Fig. 1). The position of TC mark in the otolith was also confirmed under ultraviolet light and was conventionally defined as 0 d. Every increment visible outside the TC mark was numbered from the inside (N_0) toward the otolith edge (N_3). Radii of the every second increment were measured, and the relationship between the radii of each increment and their increment number, i.e. the growth pattern of the otolith, was obtained (Fig. 2). As described below, the otolith growth pattern was divided into 3 phases (Ph-1, Ph-2 and Ph-3) based on the slope of the linear regressions (S_1 , S_2 and S_3) for each phase (Fig. 2). They were characterized by the otolith parameters shown in Fig. 2: increment numbers (N_0 , N_1 , N_2 and N_3) and their corresponding radii (R_0 , R_1 , R_2 and R_3). The otolith parameters were obtained for each 19 specimens,

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Table 1. Mean, maximum and minimum values of total length (TL: mm) of the juvenile sandfish together with otolith parameters: radius of TC mark (R_0 : μm), slopes of Ph-1, 2 and 3 (S_1 , S_2 , and S_3 : $\mu\text{m/d}$), increment numbers (N_1 , N_2 , and N_3) and their corresponding radii (R_1 , R_2 , and R_3 : μm). See terminology in Fig. 2. Otolith growth (R_2-R_1) during a rapid growing phase (Ph-2) and its duration (N_2-N_1) are also shown

Parameter	Mean	SD	CV	Max	Min
TL	34.2	2.5	7.4	38.5	30.0
R_0	34.4	4.5	13.0	42	26
R_1	102.2	12.1	11.9	126	87
R_2	185.2	17.5	9.5	221	157
R_3	268.4	23.1	8.6	300	222
N_1	30.1	5.9	19.7	50	24
N_2	51.6	3.9	7.6	62	46
N_3	91.6	4.0	4.4	96	78
S_1	2.3	0.2	9.7	2.7	1.8
S_2	3.9	0.5	11.7	4.9	3.2
S_3	2.0	0.4	21.2	3.2	1.1
R_2-R_1	83.1	19.0	22.9	130	59
N_2-N_1	21.5	4.7	21.7	32	12

Fig. 1. Photograph of a lapillus of the juvenile sandfish (34 mm in TL, 92 days old) which was immersed in TC solution of 400 mg/l for 24 h 0–3 d after hatching. Check corresponding to TC mark (TC) and otolith radius (OR) are shown.

and their correlation matrix was obtained (Table 2).

Results

Otolith Parameters

Under UV light, a distinct yellow fluorescent ring (TC mark) of about 34.4 μm radius was confirmed around the nucleus of lapilli in every fish examined (R_0 in Table 1). There was observed

a deep check at the same position with the TC mark (Fig. 1). The mean radius of lapilli (R_3) was 268.4 μm (Table 1). The radius was positively correlated with TL ($p < 0.05$, Table 2), suggesting that the otolith growth could represent the length growth of fish body.

Total number of increments outside the TC mark (N_3) was 91.6 (Table 1), showing a little smaller value than expected number, corresponding to the rearing period of 95 days after TC treat-

ment. This was 3.6% underestimation in ageing which seemed negligible for a further analysis on early growth history. The number of increments (N_3) had no significant relationship with TL (Table 2), suggesting that the increment deposition was independent to fish growth.

Rapid Growing Phase

The relationship between the increment number (age) and the radius of each daily increment

Fig. 2. Terminology for otolith growth expressed by relationship between increment number (e.g. N_0 - N_3) and radius of each increment (e.g. R_0 - R_3) in the lapillus of the sandfish. Each increment was numbered from the clear check (N_0) of TC treatment till the otolith edge (N_3). Otolith growth was divided into 3 phases (Ph-1, Ph-2, Ph-3) based on the slope of the regressions (S_1 , S_2 , S_3).

showed a common pattern in every fish examined: i.e. the growth rate of otolith increased rapidly between about 30 d and 52 d comparing to the other periods before and after (Fig. 3), although the period shifted forward or backward according to the individuals (N_1 and N_2 in Table 1). Based on the plotting of individual fish, otolith growth pattern was divided into 3 phases (Fig. 2): the first slow growing phase (Ph-1), the second rapid growing phase (Ph-2) and the third slow growing phase (Ph-3). The slope of linear regression fitted to each phase (S_1 , S_2 and S_3) was 2.3, 3.9 and 2.0 $\mu\text{m}/\text{d}$ in mean for Ph-1, Ph-2 and Ph-3, respectively (Table 1). The duration (N_2 - N_1) of the rapid growing phase (Ph-2) was 21.5 d in mean (Table 1). These rapid growing phases of about 3 weeks roughly coincided with an abrupt increase in water temperature from 10 to 14°C (Fig. 3).

Otolith Growth and Body Growth

Otolith growth rate (slope) of each phase (S_1 , S_2 and S_3) correlated positively ($p < 0.05$, $p < 0.01$ and $p < 0.05$) to an increment radius at the end of the phase (R_1 , R_2 and R_3 , respectively; Table 2). Furthermore, the slope (S_3) of Ph-3 showed a positive correlation ($p < 0.025$) with TL (Table 2). These results suggested that the otolith size at a timing was dependent to the latest otolith growth rate, and further that body size might be determined by the latest growth rate of the individual.

Both otolith growth (R_2 - R_1) during rapid growing phase and its duration (N_2 - N_1) had no significant relationship with TL and otolith growth rates of three phases, S_1 , S_2 and S_3 (Table 2).

Table 2. Matrix of correlations among TL and otolith parameters: radius of TC mark (R_0), slopes of Ph-1, Ph-2 and Ph-3 (S_1 , S_2 , and S_3), increment numbers (N_1 , N_2 , and N_3) and their corresponding radii (R_1 , R_2 , and R_3), otolith growth (R_2 - R_1) during a rapid growing phase (Ph-2) and its duration (N_2 - N_1). See terminology in Fig. 2

Parameter	TL	S_1	S_2	S_3
TL	—	NS	NS	$P < 0.025$
R_0	NS	NS	NS	NS
R_1	NS	$P < 0.05$	$P < 0.025$	NS
R_2	NS	NS	$P < 0.01$	NS
R_3	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$
N_1	NS	NS	$P < 0.025$	NS
N_2	NS	$P < 0.025$	$P < 0.05$	NS
N_3	NS	NS	NS	NS
S_1	NS	—	NS	NS
S_2	NS	NS	—	NS
S_3	$P < 0.025$	NS	NS	—
R_2 - R_1	NS	NS	NS	NS
N_2 - N_1	NS	NS	NS	NS

Fig. 3. Relationships between increment number and radius of each increment in the lapilli of 3 sandfish (top), and the corresponding change in water temperature during the experimental period (bottom). Three phases (Ph-1, Ph-2, Ph-3) are also shown based on the mean values of 19 individuals examined.

Discussion

The present study showed that growth histories of the juvenile sandfish could be estimated by the examination of otolith microstructure. Volk *et al.*⁴⁾ suggested the possibility that otolith microstructure recapitulated juvenile chum salmon growth histories during estuarine residence. Penny and Evans⁵⁾ demonstrated a clear estimation of individual growth histories of larval redfish by the otolith microstructure. Such estimates were grounded on the positive relation between otolith size and fish length, which was confirmed

in various species such as green sunfish,⁶⁾ chinook salmon,⁷⁾ redbfish⁸⁾ and ayu.⁹⁾ Volk *et al.*⁴⁾ observed a direct relationship between mean otolith increment width and fish growth rate in weight.

Otolith increment formation including both increment number and width is known to be influenced by environmental factors such as temperature,⁷⁾ photoperiod^{7,9)} and feeding frequency.⁷⁾ In the present study, abrupt increase in water temperature seemed to facilitate the otolith growth in the sandfish (Fig. 3), since no drastic change occurred in other environmental factors such as salinity, light condition, pery taxa and daily ration levels near 30 d and 52 d during the experiment.* However, because of a large variation in the timings of beginning and ending of the rapid growing phase (Table 1), it is also probable that the phase represents a species specific growth pattern and that an increase in water temperature might facilitate a rapid growth of a fish. This consideration well explains the discordance of timing of the phase in otolith. The timing of ending of the phase might be related to an upper limit of optimum temperature of this species, although no exact experimental datum is available at present. The sandfish can be reared throughout a year when water temperature is kept at 8~13°C¹⁰⁾ and spawning could be observed in a aquarium at 8~9°C.¹¹⁾ These observation suggest that fish could not maintain a rapid growth at temperatures higher than 14°C near the end of Ph-2.

If it is assumed that the larval growth of sandfish was linear, then the growth rate of fish used here could be calculated as 0.22 mm/d. Other rearing data showed similar values from 0.17¹²⁾ to 0.27 mm/d.* Sugiyama¹³⁾ estimated the growth rate of juvenile sandfish as 0.46 mm/d for wild fish obtained from various water depths following their offshore migration.

Estimates of growth rates based on regressions of TL on date of sampling from rearing pond or the sea are indirect, since size-dependent mass mortality and immigration into or emigration from the sampling area cause a bias. In fact, mass mortality of smaller individuals was observed two weeks after hatching when yolk was completely absorbed, and about 6 weeks when effect of overfeeding of *Artemia* nauplii began to appear.¹²⁾ Furthermore, juveniles move from the shallow inshore (5 m in April) to the deeper offshore (38 m in May, and 83 m in June) according to the growth.¹³⁾ JASFA* observed that apparent growth curve

* Notojima Branch of JASFA, unpublished.

of population in a rearing pond showed no special rapid growing phase unlike the individual growth histories shown in Fig. 3. Since the timing of rapid growing phase varied in each individual, clear rapid growing phases could not be detected by the mean growth curve of pond population. Most accurate estimate of growth rate could be given by the mean value of individual rates obtained from the analysis of growth history of a fish. Furthermore, analysis of otolith microstructure seemed one of the most reliable techniques to deduce a growth history of an individual fish since identification of individual larvae was difficult in both field and laboratory.

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