水産海洋研究

Preliminary Information on Internal Structures of Otoliths and Growth of Ling, *Genypterus blacodes* (Ophidiidae), Larvae and Juveniles Collected off Argentine

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The internal structure and growth increments of sagittae and lapilli in *Genypterus blacodes* larvae and juveniles collected off Argentine were investigated. Distinctive checks, referred to as compressed increments fusion and accessory growth center were observed in the sagittae. The formation period of these checks corresponded to the appearance of caudal fin structure and metamorphosis phases. Such checks were not observed in the lapilli. Due to subdaily increments in the sagittae, the lapilli were employed for subsequent analyses on age determination, hatch date estimation and growth trajectory. Reproductive period demonstrated by the estimated hatch date was primarily from December to May. Growth trajectory estimation by back-calculation using lapillus daily increments was applied, and means of back-calculated total length (TL) at ages showed a close pattern to actual age-TL relationship. A remarkable decline in growth was observed from pre-metamorphosis. This decline suggests the ecological and/or physiological changes that are influencing the growth of this species around metamorphosis phase.

Key words: *Genypterus blacodes*, ling, larvae, juveniles, otolith growth increments, sagittae, lapilli

Introduction

Ling, *Genypterus blacodes* (local name ABADEJO in Argentine) is a commercially important fish in Argentine and widely distributed over off southern Brazil and Argentine (Inada, 1986). This species grows to over 1 m total length with the size of sexual maturation estimated at more than 70 cm total length (Machinandiarena, 1999). The life span was estimated to be more than 30 years (Horn, 1993). The fisheries production of ling in Argentine increased steadily to over 20,000 t in 1980s, but catches leveled off during mid 1990s due to decline in the resource (Cordo, 1999). This situation in the fishery of ling initiated the trawl surveys with the purpose of estimating the biomass of the species, the standardization of commercial catch and effort data analyses. Early growth of ling, which may influence recruitment success, is scarcely known. Additionally, the fundamental information on growth and stock structure is not obtained at present for Argentine ling stocks, although research on the age-growth analysis and stock identification of adult ling have been made in New Zealand (Horn, 1993), Australia (Withell and Wankowski, 1989) and Chile (Chong and Aguayo, 1990). Research on growth in larval and juvenile stages is important for understanding growth history before recruitment. For conducting researches on biological and ecological aspects of fish early stages, the analysis using otolith daily growth increments has been broadly applied and developed since Pannella (1971). Morioka and Machinandiarena (2001) have attempted to validate the daily increments formation in juvenile ling and resulted that the lapilli were the better character for daily increments analysis than the sagittae. This can be attributed to the good agreement in days—increment counts and scarcity of subdaily increments occurrences in the lapilli, while the sagittae contained numerous subdaily increments in juvenile stage, making it difficult for accurate daily increment analysis. However, the growth analysis with otolith daily growth increments in ling juveniles has not yet been carried out.

This study was made in order to obtain fundamental information of *G. blacodes* on the otolith (sagittae and lapilli) microstructure, the age in days of larvae and juveniles, and to provide an estimation of the reproductive period off Argentine. The lapilli were used for the latter two analyses. Furthermore, the early life of this species was discussed on the basis of the back-calculated growth trajectory using daily increment counts of the lapilli. The assumption used for age analysis was that the increments were formed on daily basis after hatching since the validation of the period of first increment formation has not been made.

Materials and Methods

Collection of Samples

Fifty-two larvae and juveniles (3.04–320.00 mm total

Table 1. Number and size (TL in mm) of fish collected with each gear from different areas and months.

Month of collection	Number of fish collected with each gear				
	BONGO	NACKTHAI	PILOT	IKMT	Other
Northern area: 35.05–39.29S, 52.46–58.13W					
May		2		2	
Jun.		3	2		
Sep.					1
Southern area: 41.51–46.53S, 62.15–66.58W					
Dec.	1				
Jan.			2		
Feb.	1				
May	2		10		
Jul.			26		
TL range	4.60–9.17	15.00–23.30	40.07–320.00	21.00–40.00	71.00 (SL)*

* This fish was collected from stomach of *Psammobatis* sp. and TL was not observable because caudal fin rays were digested.

length (TL)) of ling, collected intermittently from December to September of 1995–1999 off Argentine (Table 1, Fig. 1), were used in this study. Demersal mini trawl (referred to as PILOT, $2.4\,m^2$ of net mouth, 25 mm mesh at wing, 10 mm mesh at codend), mid water trawl (modified IKMT, $10\,m^2$ of net mouth, 50 mm mesh at wing, 1.5 mm mesh at codend) were used for juveniles collection. High speed plankton sampler (NACKTHAI, 20 cm diameter of net mouth, 0.4 mm mesh, 1.80 m length) and BONGO (60 cm diameter of net mouth, 0.3–0.5 mm mesh, 2.75 m length) operated obliquely from the bottom to surface were used for larvae and juveniles collection. Samples were collected during cruises of R/V OCA BALDA, DR. HORNBERG of INIDEP (National Institute for Fisheries Research and Development), Mar del Plata, Argentine. The water depth was ranged from 30 to 100 m.

Otolith treatment

Sagittae of all specimens (3.0–320.0 mm TL, n=52) and lapilli of 26 specimens (4.7–234.0 mm TL) were used. Otoliths were embedded in epoxy resin on glass slide. Otolith of fish smaller than 20 mm TL could be observed through to nucleus without grinding. Sagittae of fish larger than 20 mm TL were ground from both ventral and dorsal sides as thin frontal sections and lapilli of those fish were ground from both distal and proximal sides as thin sagittal sections using sand paper (#600, 800, 1200) and lapping film (12 µm, 3 µm mesh). Otolith daily increments were observed under an optical microscope (×200–1000), then, the number, width and radius of each increment were recorded.

Growth trajectory by means of back-calculation of total length at age

Campana (1990) recommended the use of a biologically
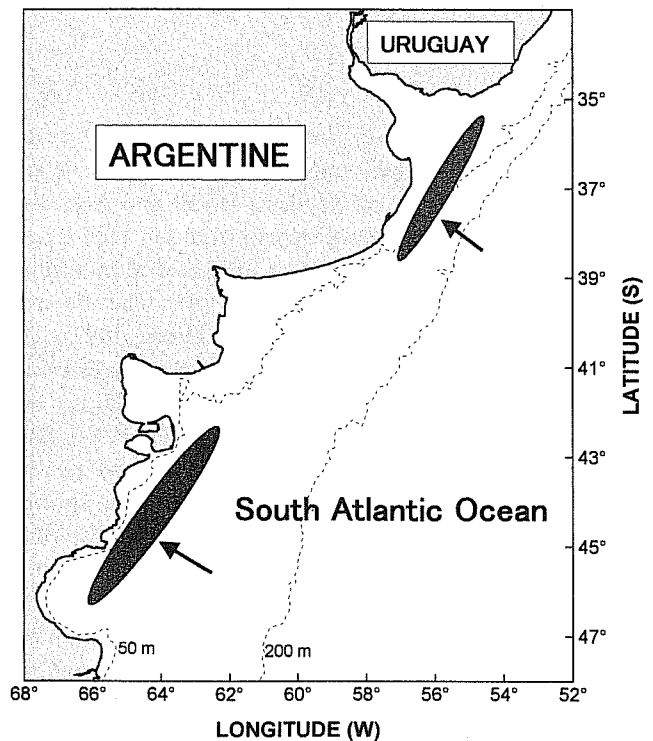

Figure 1. Site of fish collection. Upper and lower arrows indicate the northern and southern areas for collection, respectively.

based intercept instead of a statistically estimated one for describing otolith radius–fish size relationships. He defined the biological intercept as the fish size and otolith radius corresponding to the initiation of proportionality between

fish and otolith growth. The size at hatching of ling is not well known, although the notochord length of the congener *G. capensis* immediately after hatching was reported as 3.9 mm when fresh and 3.6 mm when formalin-fixed (Brownell, 1979). However, a yolk-sac absorbed *G. blacodes* larva of 2.2 mm TL (formalin-fixed) has been found off Argentine (Machinandiarena, pers. comm.). Therefore, we assumed the larval size at 1 day after hatching to be 2.0 mm TL and the first daily increment to be formed on 1 day after hatching, which were used as the biological intercept for growth back-calculation (Watanabe and Kuroki, 1997).

Lapilli were used for growth back-calculation in this study based on Morioka and Machinandiarena (2001). Relationship between maximum lapilli radii (R, μm) and total length (L, mm) were resembled with two allometric regressions intersecting at (X, Y). Hence, we assumed that the relationship between *i*-th lapillus increment radius (R_i) and L on the day of *i*-th increment formation (L_i) could also be expressed by two allometric regressions intersecting at (X, Y) for individual fish. In order to estimate the fish size at age, we applied the following procedure instead of directly using allometric regressions between R and L.
- For fishes smaller than the intersecting point (X, Y).

Allometric parameters a_1 and b_1 were calculated for each fish by solving the following two equations; $L_1 = a_1 \cdot R_1^{b1}$ and $L_c = a_1 \cdot R_c^{b1}$ where L_1 is total length at 1-day after hatching (given as biological intercept, 2.0 mm TL), R_1 is otolith radius at 1-day after hatching, L_c is total length at capture and R_c is otolith radius at capture.
- For fishes larger than the intersecting point (X, Y).

Before the estimated fish size at ages reaching the intersecting point, the allometric parameters a_1 and b_1 were obtained by solving the following two equations; $L_1 = a_1 \cdot R_1^{b1}$ and $L_p = a_1 \cdot R_p^{b1}$ where L_1 is total length at 1-day after hatching, R_1 is otolith radius at 1-day after hatching, L_p and R_p are Y and X each of the intersecting point. Posterior to the intersecting point, the other allometric parameters a_2 and b_2 were obtained by solving the regressions $L_p = a_2 \cdot R_p^{b2}$ and $L_c = a_2 \cdot R_c^{b2}$ where L_c is total length at capture and R_c is otolith radius at capture.

Additionally, the instantaneous growth rate (%) of fish was obtained using a result of back-calculated total length in individual fish. IGR was calculated as follows; $\mathrm{IGR}_i = (L_{i+1} - L_i) / L_i \times 100$ where IGR_i and L_i are the instantaneous growth rate at *i*-th day and fish total length at the age of *i*-th day-old, respectively.

Results
Otolith internal structure
The sagittae and lapilli of early larvae (<10 mm TL) were round and disc shaped (Fig. 2). As fish grew, the com-
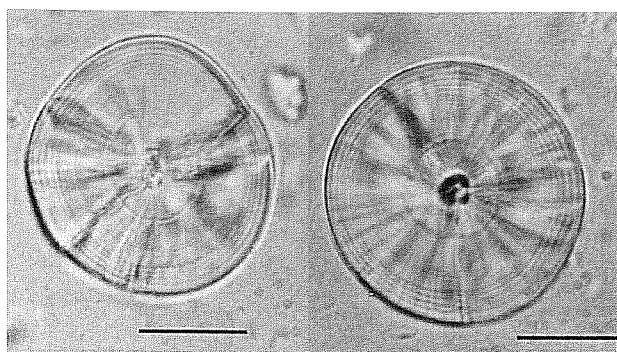
Figure 2. Sagitta (left) and lapillus (right) of ling larvae (4.7 mm TL). Bars indicate 20 μm.

pressed increments fusion (CIF) and accessory growth center (AGC) were observed in the sagittae (Fig. 3), which were characterized as follows;
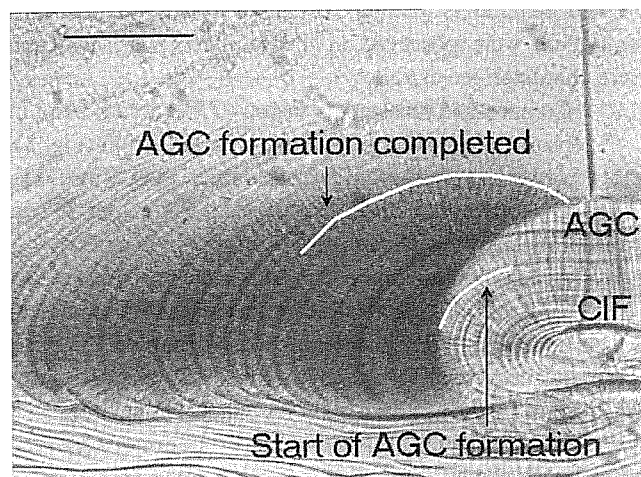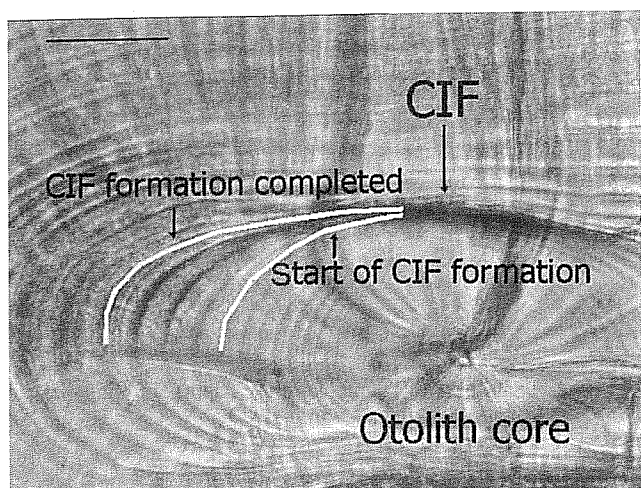- Compressed increments fusion (CIF): a dark band in proximal ward of the sagittae frontal section (Fig. 3), which was observed in fish larger than 8 mm TL, formed mostly from 24th (mean 24.36, n=31) to 29th increments deposition (mean 28.95, n=31),
- Accessory growth center (AGC): a fan shaped structure well distinguishable in the sagittae frontal section observed in fish larger than 15 mm TL (Fig. 3), formed mostly from 37th (mean 36.68, n=31) to 43th (mean 43.00, n=31) increments deposition.

In contrast, such distinctive marks were not observed in the lapilli.

Growth increments around the otolith core were narrow (1 μm or less), and their width increased as fish grew, both in sagittae and lapilli (Figs. 3, 4). During AGC formation in the sagittae (Fig. 3), the width of increments was observed to be wider and the sagittae grew rapidly anterior- and posterior-ward along body axis with the rostrums development. The maximum width of increments was observed for over 30 μm at the time (Fig. 3). The increments were clearly identified in the sagittae up to c. 50th increment deposition. It was observed that beyond the 50th increments deposition, numerous subdaily increments occurred in the sagittae, and the identification of daily increments became difficult. In contrast, measurement of the maximum increment width in the lapilli (3 μm) was observed between the 30th and 40th increments deposition. After approximately the 40th increments deposition, the width became narrower. The daily increments in the lapilli were deposited clearly and subdaily increments were rarely observed (Fig. 4).

Hatch date estimation
Hatching date estimated by the increment counts of the lapilli was from December to April (summer–autumn) in

Figure 3. Compressed increments fusion (CIF, top) and accessory growth center (AGC, bottom) in ling juvenile (175.0 mm TL). Each image shows the start and completion of CIF/AGC formation. Bars in the top and bottom images indicate 20 μm and 100 μm, respectively.
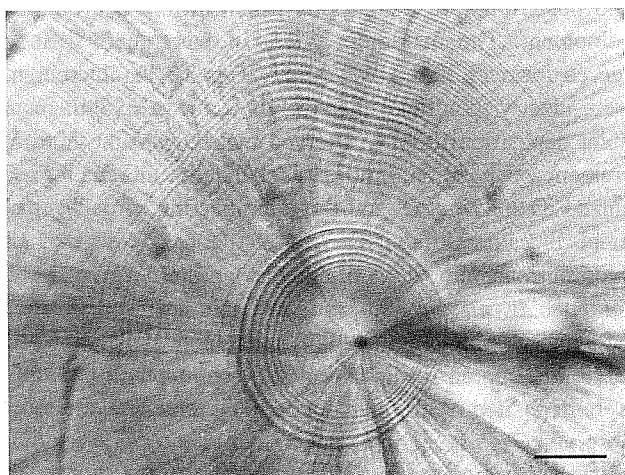
Figure 4. Daily growth increments in lapillus of ling juvenile (175.0 mm TL). Bar indicates 20 μm.
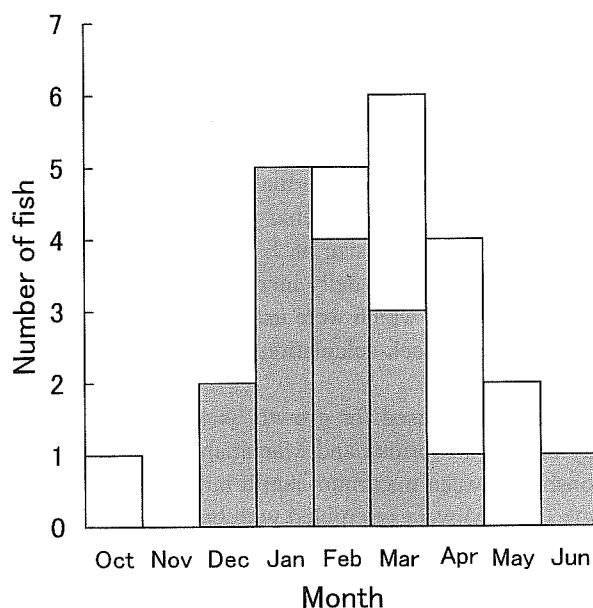
Figure 5. Hatching month of ling estimated by growth increments count in lapilli. Dark bar: fish collected from the southern area, white bar: fish from the northern area.
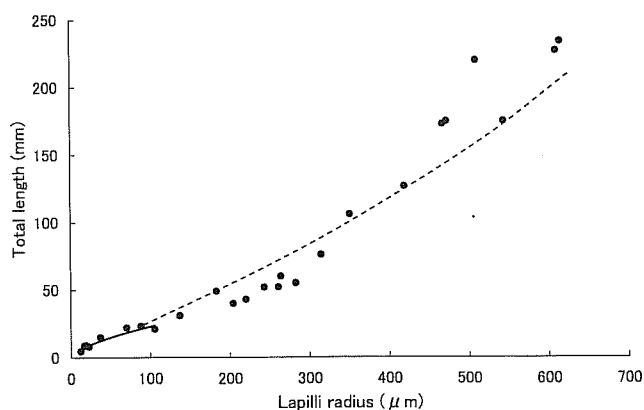

Figure 6. Relationship between maximum radii of lapilli and total length in ling larvae and juveniles (n=26).

fish from the southern area and February to May (late summer–late autumn) in fish from the northern area (Figs. 1, 5).

Growth trajectory by back-calculated total length

Since TL-age relationship or age-increment width relationship between fishes from northern and southern areas demonstrated a close relationship, they were treated as one group for growth trajectory analysis. The relationship between lapilli radii (R, μm) and total length (L, mm) were expressed by two allometric regressions intersecting at (110.82, 24.47) (Fig. 6) as follows; $L=1.268 \cdot R^{0.629}$ ($r=0.931$), $L=0.075 \cdot R^{1.229}$ ($r=0.962$). Mean back-calculated total length at ages showed a good agreement with the
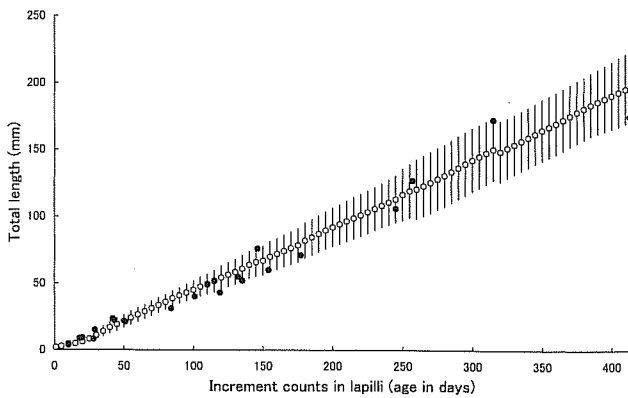
Figure 7. Relationship between growth increments count in lapilli (age in days) and total length. Open circles: back-calculated total length at ages averaged at every 5 days, vertical bars: standard deviations, solid circles: actual age–TL plots.
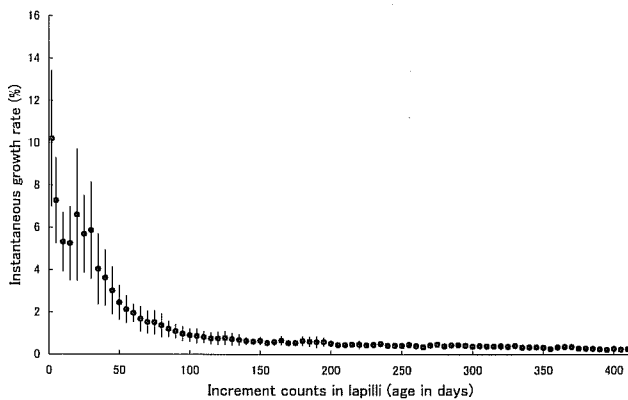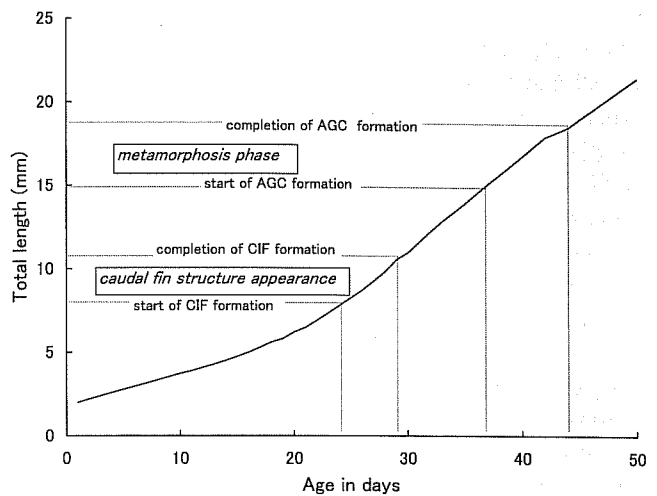

Figure 8. Relationship between growth increments count (age in days) and instantaneous growth rate averaged at every 5 days. Vertical bars: standard deviations.

Figure 9. Back-calculated total length, growth increments count in lapilli (age in days) and developmental stage at CIF and AGC formation. Solid line shows the mean back-calculated total length at ages. Periods of CIF and AGC formation, which are 24~29 day-old and 37~43 day-old (see Results), correspond to 8~11 mm TL and 15~19 mm TL in back-calculated total length, respectively.

actual age–TL relationship (Fig. 7).

Instantaneous growth rate (IGR, %) was individually calculated on the basis of back-calculated TL. Mean IGR was observed to be as high as more than 5% per day until reaching 30 day-old, and remarkably decline thereafter until less than 1% per day at c. 100 day-old (Fig. 8). The IGR, then, gradually decreased as fish grew (Fig. 8). We obtained the mean back-calculated TL and mean IGR up to 410 day-old with at least five fishes for each data.

Discussion

As observed in Morioka and Machinandiarena (2001) as well as in this study, the lapillus was a better material for daily increment analysis in juvenile stage than the sagitta because subdaily increments rarely occurred in the former. By this reason, we chose the lapilli as materials for hatch date estimation and growth back-calculation. Hatch date based on growth increment counts in lapilli in this study

showed that the reproductive period was estimated to be primarily during December–May off Argentine (summer–late autumn in the Southern hemisphere) and seemed to move successively from south to north as season elapsed from summer to autumn (Fig. 5). This result agreed with the reproductive period estimated by larval occurrence and sexual maturity analyses of adult ling (Machinandiarena, 1999). This agreement also suggests that the increment counts in lapilli correspond to the age in days of this species, although the validation of daily increments in larval stage of this species has not been carried out so far both in the sagittae and lapilli. The reproductive period estimated in this study was different from *Genypterus blacodes* in New Zealand (Horn, 1993) and the congener *G. capensis* of South African waters (Olivar and Sabates, 1989), where the reproduction occurred from June to December and August to December, respectively.

The morphological features of sagittae found in this study, such as the compressed increments fusion (CIF) and accessory growth center (AGC), were often observed in other demersal species, e.g. *Theragra chalcogramma* (Nishimura, 1993), *Micromesistius australis*. (Cassia and Morioka, 1999) and *Macruronus magellanicus* (S. Morioka: unpublished data). In *Platichthys stellatus* (Campana and Neilsen, 1985) and *Pleuronectes americanus* (Jearld *et al.*, 1993), AGCs were also observed. Changes in spacing of increments and internal structures were considered to demonstrate the record of ecological events in the early life history (Brothers and McFarland, 1981; Radtke

and Dean, 1982). In Theragra chalcogramma (Nishimura, 1993), CIF and AGC are considered to reflect the morphological and ecological changes in the period of caudal fin structure appearance and metamorphosis, that is defined as an appearance of all fin rays structure, respectively. Periods of CIF and AGC formations found in this study, the former being estimated as 24~29 day-old and the latter as 37~43 day-old, corresponded to 8~11 mm TL and 15~19 mm TL each in mean back-calculated TL (Fig. 9). These size ranges in this species mostly fell under the periods of caudal fin structure appearance and beginning of metamorphosis, respectively (Machinandiarena, unpubl. data). These observations suggest that such checks in the sagittae would commonly take place in demersal species during the caudal fin development and metamorphosis that drastically change their swimming ability. These phases are probably related with the eco-physiological shifts (Tanaka et al., 1989), such as changes in habitat or feeding habit.

In this study, growth back-calculation was made on the basis of the assumption that the radius–TL relationship at metamorphosis was mostly constant among individuals. This is because the metamorphosis is expected to be more size-dependent than age-dependent as reported in Chambers and Leggett (1987). As a result, means of back-calculated TL demonstrated a close pattern to an actual age–TL relationship (Fig. 7). So, it is considered that the method employed in this study for growth trajectory would be more or less acceptable for generalizing the growth pattern of this species, although fish used in this study was obtained from different areas and times. The expanded reproductive period covering at least 6 months as well as the regional difference of reproductive period estimated in this study (Fig. 5) suggest the existence of more than one population of this species off Argentine. Future research should focus on obtaining more specimens from various areas and time order to examine the stock structure.

The instantaneous growth rate showed a drastic decline from some 30 to 100 day-old (Fig. 7). Low water temperature could explain the low growth rate at this phase, because water temperature generally declines after the hatching period in both southern and northern areas (Figs. 1, 5) toward autumn and winter (Brunetti, 1999). Size of ling at 30~100 day-old was estimated to correspond with pre- to post-metamorphosis phase (Fig. 9). The metamorphosis phase of demersal species, in general, is coincided with the transitional stage from pelagic to benthic life, and fish undergoes various physiological changes during this phase (Forstner et al., 1983; Oikawa et al., 1991). As known in Pleuronectiformes, the difficulties for adapting to changes in behavior, feeding habit and habitat during and after metamorphosis could potentially cause a mass-mortality (Van der Veer et al., 1987; Tanaka et al., 1989; Seikai et al.,

1993). There may be similar difficulties causing the growth decline in ling during this period.

Acknowledgment

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アルゼンチン産アシロ科底魚 *Genypterus blacodes*
仔稚魚の耳石内部構造と成長

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アルゼンチン産アシロ科魚類 *Genypterus blacodes* 仔稚魚の扁平石および礫石の日輪と耳石内部構造を調査した．扁平石内部には複数の輪紋の融合からなる暗帯 (CIF) と2次核 (AGC) が形成され，これらの構造の形成時期は，それぞれ尾鰭骨格出現期および変態期に該当すると考えられた．このような構造は礫石には見られなかった．扁平石には多くの偽日輪が出現し，正確な日輪の特定が非常に困難であるが，礫石では偽日輪の出現が稀で日輪は明瞭に観察されることから，孵化日の推定および成長の逆算に礫石を用いた．その結果，本種の産卵期は主に12月から翌年5月頃にわたるものと推定された．また，耳石日輪数と各輪紋形成時の耳石半径から個体別に成長を逆算したところ，その平均値は実際の日齢–全長関係に近い傾向を示したことから，本研究で適用した逆算手法は概ね実際の成長を反映するものと考えられた．逆算成長をもとに得られた平均瞬間比成長率は変態期開始前から減少する傾向が見られ，これは変態期前後に生じる生息域の変化（浮遊性から底生性）およびそれに伴う生態的・生理的変化による現象と推定された．

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