Reproduction and Early Life History of Gunnel, *Pholis fangi* in the Yellow Sea off Korea

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Larvae of the gunnel *Pholis fangi* were collected in coastal waters off Daecheon with a bag net from March to June, 1988, and with a ring larva net in February 1989. Maturity and spawning period were analyzed by examination of the gonads of adult fish collected with a bag net from May 1998 through November 1999.

In February, the larvae were widely distributed in the outer and inner Cheonsu Bay. From March to April the larvae were present mainly the inner bay; they were absent there in May and found mainly in the outer bay. After June, few gunnel larvae were collected in the study area. This suggests a seaward movement of gunnel from the nursery grounds of the bay to offshore feeding grounds.

The otolith of larvae smaller than 10 mm in total length did not show a distinct growth stop. The growth stop is believed to be formed in the early larval stage when the total length is about 10 mm. This period coincides with the time of shoreward migration, suggesting a metabolic change during this period. At a total length of 30 to 40 mm, the shape of the otolith changes from spherical to elongate. Daily growth rate in length was estimated by the Gompertz equation, which is represented as follows:

 $TL = 6.702 \exp\{2.925[1 - \exp(-0.008 t)]\}$ (r² = 0.94, N = 92)

Assuming daily deposition of growth increments in the otolith, the time of first growth increment formation was shown to be from December to January. Gonad observations show that *Pholis fangi* spawns from November to December. So, the hatching time is thought to be about one month.

Key words : Pholis fangi, larvae, otolith daily increment, reproduction

Introduction

Gunnel, *Pholis fangi* Wang and Wang (formerly *Enedrias fangi*) is one of species among the Pholididae with *P. nebulosus*, and distributes in the Yellow Sea, the Po-hai Bay and the northern part of the East China Sea (Chyung, 1977; Yatsu, 1981; Hur and Yoo, 1983; Masuda *et al.*, 1984).

The adult *P. fangi* is not edible owing to the strong spine of dorsal fin and the thin flesh although larva named 'silchi' as local dialect is used to make dried slices of meat seasoned and swallow up without chewing. Also, this fish is fed at the fish farm. Its production links secondary plankton production to fisheries output and is very important to the energetic processes of the ecosystem. As widespread and abundant, *P. fangi* larvae may play a major role as prey species in the trophic structure of the Yellow Sea with *Engraulis japonicus* and *Ammodyres personatus* (Ryu and Lee, 1984; Tang, 1993; Hwang, 1998).

Because of one of the useful fishery resources in the Yellow Sea of Korea, *P. fangi* was studied in taxonomy (Yatsu, 1981; Hur and Yoo, 1983), morphological characteristics with development of larvae (Yoo, 1985) and geographical distribution (Huh *et al.*, 1981; Hur *et al.*, 1984; Yoo *et al.*, 1995). However, the informations on most aspects of its biology are lacking despite its importance, especially its reproductive biology. Its early life history with age using otolith has not been known.

Since Pannella (1971, 1974) first demonstrated the presence of daily growth increments in the otolith of marine fishes, many fishery biologists have substantiated their suggestion from a wide application to the reared fish of known age (reviewed by Campana and Neilson, 1985; Jones, 1986). The onset of the daily growth increments in an otolith is known as being formed at hatching, at yolk-sac absorption or at the start of external feeding after yolk-sac absorption (Brothers et al., 1976; Marshall and Parker, 1982; Miller and Storck, 1982; Tsuji and Aoyama, 1982; Radtke and Scherer, 1982; Nishimura and Yamada, 1984; Tabeta et al., 1987; Alemany and Alvarez, 1994; Hare and Cowen, 1994; Regner and Dulčić, 1994; Quiñonez-Velázquez, 1999). Therefore, the total number of growth increments is regarded as the days from at hatching or external feeding to before sampling date, so this ageing technique by the daily growth increments has been useful to the ecological examination in estimating the birth date of field-caught larvae and understanding the growth pattern of each individual.

Objectives of this study are to describe the spatio-temporal appearance patterns of larval *P. fangi* in the coastal waters off Daecheon, Korea from 1988 to 1989, and to analyze the relationship between larval morphologic changes and otolith growths observing the microstructures in otoliths. Also, the spawning time and the patterns of early growth are estimated.

Materials and Methods

P. fangi larvae and adults were collected in the coastal waters off Daecheon, Korea with a bag net (mesh size: $0.33 \sim 1$ mm) from March to June 1988 (st. 7), a 1 m ring net with 0.33 mm mesh in February 1989 (st. $1 \sim 8$) and a bag net from May 1998 to November 1999 (open circles), respectively (Fig. 1). In addition, the surface water temperature was measured daily at a fixed station off Daecheon during the sampling.

Ethanol was used as a preservative to prevent dissolution of otolith (Methot and Kramer, 1979),



Fig. 1. Map showing sampling sites in the coastal waters off Daecheon in the Yellow Sea, Korea.

and to reduce variations in shrinkage of larvae (Theilacker, 1980). These samples were fixed in 70% alcohol, and were identified as the species according to Yatsu (1981) and Hur and Yoo (1983).

More than 50 individuals among the larval specimens subsampled in 1988 and all sampled in 1989 were measured to the nearest 0.05 millimeter in total length, and their meristic and morphometric characters were observed.

Body length (BL) of 1,156 adult *P. fangi* was measured to the nearest 0.1 millimeter, and body weight and gonads were weighted to the nearest 0.1 g, respectively. Macroscopic stages of gonad development for both sexes were identified: immature, maturing, ripe, spawning and spent. The gonadosomatic index (GSI) was calculated by dividing gonad weight by total fish weight and multiplying by 100. The ovaries of 12 fish with mature oocytes were fixed in Gilson solution for $2 \sim 3$ months. Thereafter, the oocytes were separated using a stirrer. Of a total 100 ml with water, eggs of 1 ml pipetted five times repeatedly were counted and averaged (ň). Fecundity (F) was calculated using equation as follows.

$$F = \frac{\dot{n}}{1 \text{ (ml)}} \times 100 \text{ (ml)}$$

The otoliths of larvae were extracted, brushed free of tissue and rinsed with distilled water under a dissecting microscope. The otoliths were prepared for observation by embedding them in the unsaturated polyester resin, by hand grinding them to the proximal surface of sagittal plane passing through the otolith center with a series of graded silicon carbide papers (400, 800 and 1,200 grits) and by polishing with 0.3 µm alumina powders. Prepared otoliths were examined and photographed at $200 \times$, $400 \times$, $500 \times$ magnification under a light microscope (Leitz diaplan) using transmitted and/or reflected dark -field illuminations. This method of examination permitted observation of not only growth increments but also the optical density that is determined by organic contents of the otolith. A dark field metallurgical microscope was also particularly helpful to monitor the grinding surface of otoliths during preparation.

The maximum distance (i.e., the longest radius along the long axis) in otoliths was measured with a ocular micrometer. For viewing under the scanning electron microscope (SEM; AKASHI SSX-40), the prepared otolith was etched in 0.1 N HCl solution for $1 \sim 2$ sec and coated with gold –palladium (vacuum: $0.1 \sim 0.2$ Torr, ion current: $4 \sim 5$ mA, coating time: $4 \sim 5$ min). Photographs at $1000 \times$, $1500 \times$ and $2000 \times$ were taken for each otolith. Measurements and counts of the otolith microstructure were made on SEM photographs, and they were compared with the light microscopical observations.

The relationship between total length of larvae, radius of the otoliths and number of otolith increments was established by the least square method with the SPSS/PC⁺ (Norusis, 1986) program. The total length was estimated by back– calculation (Fraser–Lee method in Bagenal and Tesch, 1978) from otolith radii. The growth formula was calculated following Gompertz growth function using Basic Fishery Science Programs (Saila *et al.*, 1988).

Results

Appearance pattern

In February, 1989 (4~5°C) P. fangi of early lar-



Total length (mm)





Fig. 3. Thin sectioned sagitta of *P. fangi*.

A. Otolith of a larva (30.7 mm TL) under transmitted light (left) and reflected dark field. Bar : 50 μ m B. Sagittal plane of the otolith of *P. fangi* (68.2 mm TL). Bar : 100 μ m val stage at $7 \sim 28$ mm TL were widely collected at the all sampling sites (Fig. 1). The larvae appeared mainly in the inner Cheonsu Bay from March to April. In this period, the range of the averaged total length of the larvae was $25 \sim 33$ mm and the variation of total length was not distinguished districtly. Larvae did not occur in the bay but occur mainly in the outer bay from May at the water temperature of about 16°C. The averaged total length of postlarval stage was about 52 mm at that time. The lengths in May increased remarkably contrast to those of previous months (Fig. 2). After June, few *P. fangi* larvae were collected in the study area.

Growth of otoliths in larvae

The sagitta of early larvae was transparent and concentric (Fig. 3–A). It had an opaque core in the center (about 50 μ m in diameter), which was surrounded by a narrow transluscent zone and the opaque zone of outer portion under dark



rig. 4. Microstructures of otofich of the farvae measured 10.4 mm (A) and 29.0 mm (B) in total length under the scanning electron microscope. Bar : 5 μm





Fig. 5. Incremental growth sequence in a postlarval (60.8 mm TL) otolith under the scanning electron microcope.



Fig. 6. Plots of the mean total length (top), the mean otolith radius (middle), the mean number on otolith growth increments (bottom) and its standard error (vertical bar) of larval *P. fangi* collected off Daecheon from March to June, 1988 and in February 23, 1989 (*).

field. The outer opaque zone became gradually transparent approaching the margin. The core region of the disk shape with a diameter of about 20 μ m was observed on the ground and etched otolith of larvae (Fig. 4–A). On the core region "kernel" (Pannella, 1980), there were 3~4 holes which were thought to be as the trace of some particles playing the role of nucleus. From the outer of kernel, increments were unclearly shown with about 13 μ m in width and limited by the innermost etched groove. The increment widths from the core to the first prominent check

were relatively narrow, and the number of increments were about 30 (Fig. 4-B). Thereafter, sagittae became laterally compressed with the highest growth rate along with the anteriorposterior axis and intermediate growth rate in the dorso-ventral direction (Fig. 3-B). The increments were obviously recognizable after the check, and the width of each increment was still narrow to the 110th increment and gradually increased after this (Fig. 5). Growth increments were mostly recognizable at the sagittal anterior portion. Lapillus and asteriscus were not used for the age determination because of their small size, the narrow incremental width and the poor visibility. Each increment was composed of an alternation of two different structural layers. One was a wide, crystallized layer (incremental zone) and the other was a thin layer (discontinuous zone) that does not appear to be as heavily crystallized as the incremental zone.

The longest radius of otoliths of February 23, March 14, March 29, April 9, April 23, May 19 and June 11 averaged 36.5 (\pm 1.9 standard error, SE) µm, 64.7 (\pm 4.7) µm, 70.7 (\pm 5.1) µm, 77.9 (\pm 4.7) µm, 95.5 (\pm 9.6) µm, 202.9 (\pm 5.5) µm and 277.9 (\pm 8.1) µm, respectively (Fig. 6). The average of the longest radius in larval otoliths showed a pattern of remarkable increase from April through May. This was resulted from being wider at the widths of each increment and being elongated in the shape of otoliths.

The mean number of growth increments of the specimens of February 23, March 14, March 29, April 9, April 23, May 19 and June 11 were 53.9 $(\pm 3.3 \text{ SE})$, 77.7 (± 4.1) , 82.6 (± 4.4) , 97.3 (± 4.8) , 113.7 (± 7.8) , 159.8 (± 4.0) and 183.3 (± 4.9) , respectively (Fig. 6). The time lags of collection dates did not exactly agree to differences in the mean number of increments among the samples, suggesting the multiple spawning and/or an extended spawning season.

Age structure in larvae

The number of the otolith growth increments was from the minimum 23 on February 23 to the maximum 215 on June 11 (Fig. 7). Its ranges of 23 February, 14 March, 29 March, 9 April, 23 April, 19 May and 11 June were $23 \sim 76$, $58 \sim 104$, $54 \sim 110$, $68 \sim 123$, $79 \sim 159$, $128 \sim 178$ and $150 \sim 215$, respectively. When we assumed daily deposition of growth increments in otolith of *P. fangi*, the time for the first growth increment formation was from November to February (Fig.

Table 1. The period of first growth increment formation estimated by back-calculation from the number of daily growth increments in otolith

Formation period	Number of specimen	Frequency (%)	Cumulative frequency (%)
1987, 1988			
Nov. $1 \sim \text{Nov.} 10$	1	1.09	
Nov. 11~Nov. 20	1	1.09	
Nov. $21 \sim$ Nov. 30	5	5.43	7.61
Dec. 1~Dec. 10	12	13.04	
Dec. 11 ~ Dec. 20	18	19.57	
Dec. 21~Dec. 31	16	17.39	57.61
1988, 1989			
Jan. 1~Jan. 10	14	15.22	
Jan. 11~Jan. 20	14	15.22	
Jan. 21 ~ Jan. 31	7	7.61	95.65
Feb. $1 \sim$ Feb. 10	4	4.35	100.00

7), mainly from December to January (Table 1).

Growth in length of larvae

The relationship between total length and otolith radius calculated by the least square method was divided into two groups (Fig. 8), from February to April ($r^2 = 0.85$, N = 68, P < 0.01) and from May to June ($r^2 = 0.76$, N = 24, P < 0.01), based on the change of otolith growth rate at $30 \sim 40$ mm TL.

The individual growth pattern from total length that was back-calculated by the width of the increments averaged for every five increments showed a changing point at the 110th increment of otolith and $30 \sim 40$ mm TL of larvae (i.e., after April, timely) (Fig. 9).

The growth curve of larval *P. fangi* off Daecheon, based on the data of the back-calculation of otolith increments of wild larvae, is showed in Fig. 10. The estimated growth formula applicable for a size range $10.35 \sim 59.00$ mm in total length was calculated as

TL = 6.702 exp
$$\{2.925[1 - \exp(-0.008 t)]\}$$

(r² = 0.94, N = 92)

where TL is the total length in millimeter and t is the age in days after the formation of the otolith increment (= number of increments in the otolith). This result was likely to the variation plot of individual growth patterns by back-calculation, indicating that the back-calculation method is reliable for growth estimation in the early life stage.



Fig. 7. Frequency distributions of the number of growth increments counted in otolith (left) and the time at first increment formation estimated by back-calculation from the number of growth increments (right) of larval *P. fangi* collected off Daecheon from March to June, 1988 and in February 23, 1989.



Fig. 8. Relationship between total length and otolith radius of larval *P. fangi* collected off Daecheon from March to June, 1988 and in February, 1989. The symbols, □, +, ◊, △, × are the data collected in February, March, April, May and June, respectively.



Fig. 9. Variation in width of growth increment with sequential number of growth increment from a larva in 29.0 mm TL (top) and other larva (60.8 mm TL) sampled in May, 1988 (middle), and growth in length by back-calculation from the otolith of the individual in 60.8 mm TL (bottom).



Fig. 10. Scattered diagram between the number of growth increments and total length of larval *P. fangi* collected off Daecheon from March to June, 1988 and in February, 1989. The curve represents a least square fit of the Laired–Gompertz model. The symbols, □, +, ◊, △, × are the data collected in February, March, April, May and June, respectively.

Monthly variation of length in adults

Length frequencies of 1,156 adult P. fangi collected from May, 1998 to November, 1999 ranged from 49.2 mm to 159.5 mm in body length (Fig. 11). Mean and standard error of length on 28 May, 1998 was 110.2 (±11.2) mm. Two modal length of 78.3~86.4 mm and 95.2~147.7 mm occurred on 22 June, and the pattern of two modal length distribution continued until July. We could not collected any fish from August through September in this study area with same sampling gears. Mean length on 17 October was high as 124.0 (\pm 17.5) mm, but they decreased 107.3 (\pm 14.5) mm on 19 November instead. After this, mean lengths tended to increase slightly up until May, 1999. Two modal length of $43.2 \sim 70.7$ mm and 101.5~134.9 mm occurred on 26 May, and were $70.5 \sim 90.4 \ mm$ and $107.8 \sim 152.1 \ mm$ on 27June, showing abrupt increase of adolescent fish. A few fish were collected during summer although 112.2~151.9 mm and 129.1~135.8 mm occurred on July and September, respectively. Mean lengths were 128.0 (\pm 12.8) mm and 118.9 (\pm 11.9) mm, on 28 October and 25 November, 1999, showing lower than that of September.

Ovarian maturation

We examined monthly variation of gonadosomatic indices (GSI) of *P. fangi* collected from May, 1998 to November, 1999 except adolescents



Fig. 11. Monthly variation of length (mean±standard error) and gonadosomatic index (GSI) of adult *P. fangi* collected from May 1998 to October 1999.

(Fig. 11). Mean and standard error of GSI in May, 1998 was 0.02 ($\pm < 0.01$). These low GSI value continued to July. GSI began to increase to 0.28 (± 0.04) in October, were 0.40 (± 0.05) in November and peaked 0.43 (± 0.06) in December. GSI abruptly decreased 0.02 (± 0.00) in January. After this, Mean GSI lowed 0.02 ~ 0.11, were high as 0.23 (± 0.02) in October and peaked 0.67 (± 0.09) in November.

The female ovaries of *P. fangi* collected from May to September, 1998 were immature stage (Fig. 12). We could observed that most of them were maturing or ripe in October and all of them were ripe or spawning in November. In December, immature, maturing, ripe, spawning and spent occupied 34%, 6%, 12%, 39% and 9% of total, respectively, suggesting that many of them were already spawned. All were spent in January and immature in February, 1999. The development pattern of gonads in 1999 was similar to that of 1998. Therefore, *P. fangi* of the Yellow Sea matured from October and spawned from November to December. Spawning finished in January. Males of *P. fangi* were showed similar



Fig. 12. Monthly variation in percentage composition of gonadal development for female (top) and male (bottom) *P. fangi* collected from May 1998 to November 1999.

patterns, though the data was more limited.

Fecundity

Fecundity of females was estimated by counts of oocytes in the largest size mode. 12 spawning females collected in December 1998 had 257 to 1,076 eggs with averaged 560.

Discussion

It has not been yet known when the first increment deposition of *P. fangi* otolith formed. Also, periodity and interruption of increment formation have not been studied. However, the otolith growth increment was proved to be formed daily in many other fishes (Brothers et al., 1976; Tanaka et al., 1981; Marshall and Parker, 1982; Tsuji and Aoyama, 1982; Radtke and Scherer, 1982; Miller and Storck, 1982; Gartner, 1991a; Sogard, 1991; Warlen, 1992; Parrish et al., 1994; Oxenford et al., 1994; Hoff et al., 1997; Peters and Schmidt, 1997; Quiñonez-Velázquez, 1999; Massutí et al., 1999). Each growth increment was composed of two alternative layers; the incremental zone contains many crystals and the discontinuous zone is poor in crystals and is etched by HCl into a groove. Watabe et al. (1982) found that the discontinuous zone contained more organic matters and fewer crystals, and suggested that the increment pattern may be dependent on periodical changes in deposition of organic and mineral substances. Evidence is available that a diurnal change in calcium deposition under photoperiod control is responsible for increment formation (Mugiya et al., 1981; Tanaka et al., 1981). It is possible that diurnal changes in physiological activities, directly or indirectly, could cause daily growth increment formation in the otolith. The number of increments tended to increase linearly with time and the days counted from the number of increments agreed to the number of days after spawning dates. Therefore, the growth increment may be inferred to be a daily growth increment.

In this experiment, supposedly, the ring in otolith of *P. fangi* larvae was a daily growth increment, and the time of the initial incremental deposition of *P. fangi* appeared in the coastal waters off Daecheon was estimated as the period from early November through next Februray, mainly December ~ January at the water temperature $3 \sim 8^{\circ}$ C. However, the birth dates must be taken into consideration by adding the time

from spawning to the first increment formation to the period of the initial increment deposition above-mentioned. Different species begin to deposit the first growth increment at different ages: i.e., at hatching, at yolk-sac absorption or first feeding (Jones, 1986). Russell (1976) demonstrated that the incubation time was different depending on the water temperature, the egg size and the yolk-sac amount. We can interpretate that *P. fangi* began to be matured from October, spawned mainly from November through December and finished the spawn in January as the result of the fact that ripe and spawning were peak from October through December and spent was present in January, and the gonadosomatic index increased in October, peaked in December and decreased in January. As compared with the results of previous studies (Matarese et al., 1983) that hatching duration of some species of Family Pholidae spawning in cold months is about two months, P. fangi of the Yellow Sea is considered to have the duration time of about one month.

In February, 1989, the larvae of mean total length (13.5 mm) were widely distributed both in the outer and inner Cheonsu Bay. At that time, *P. fangi* has been in the yolk-sac absopted stage already and early larvae have the otoliths which are almost round in the shape. The otolith of the larvae being smaller than the 10 mm in total length sampled at the outer did not show a district growth stop. The growth stop was believed to be formed in early larval stage when the total length was about 10 mm on the way onshore from offshore. This period coincided with the time of shoreward migration, suggesting a metabolic change during this time. Changes in spacing of increments and internal structures has been suggested as a record of ecological events in the early life history of fishes (Nishimura and Yamada, 1984; Victor, 1986; Tabeta et al., 1987; Sogard, 1991; Hare and Cowen, 1994; Regner and Dulčić, 1994; Deegan and Tompson, 1987; Nishimura and Yamada, 1988; Watanabe and Kuroki, 1997; Quiñonez-Velázquez, 1999; Massutí et al., 1999). From March to April, the larvae occurred mainly in the inner bay, and the range of the mean total length of larvae was about $20 \sim 30$ mm and the variation of length was not distinguished districtly. It is estimated that larvae continued to influx from the outer due to the extended spawning duration. Another reason is considered as low growth rate at the low water temperature. At this time, *P. fangi* is larval stage and the shape

of otoliths is almost round yet. When the total length ranged from 30 to 40 mm on April~May, larvae were at the postlarval stage and the shape of the otolith was changed from spherical form to elongated one. An abrupt increase of the length of larvae after April was resulted from cease of influx of larvae and the rapid growth at risen water temperature (about 10°C). After this time, the body length increased rapidly and the width of each increment became wider, showing the high growth rate of *P. fangi* on the water temperature over 20°C. After June, few P. fangi were collected in the study area, suggesting a seaward movement from the nursery ground of the bay to the offshore feeding ground. This result agrees to the previous researches (Huh et al., 1981; Hur et al., 1984; Cha, 1986; Yoo et al., 1995; Hwang, 1998). P. fangi seemed to be shared the ecological niche with the warm water species which migrated into the bay in spring after overwintering in the offshore, though the reason was not able to be fully explained.

The validation of daily growth increments and the information about migration route would be a future subject of research.

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한국 서해 흰베도라치(*Pholis fangi*)의 생식과 초기 생활사 황 선 도·이 태 원*

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흰베도라치, Pholis fangi의 초기 생활사를 밝히기 위해 한국 서해의 대천 연안에서 1988년 3 월에서 6월 사이와 1989년 2월에 흰베도라치 치어를 채집하였다. 성장에 따라 외부형태 변화와 이석을 관찰, 측정함으로써 출현양상과 산란시기 및 초기성장 등을 조사하였다. 또한, 1998년 5월 부터 1999년 11월 사이에 매월 대천 해역에서 채집된 흰베도라치의 생식소 관찰로 산란기를 추 정하였다.

2월에는 흰베도라치 치어가 천수만 안팎에서 넓게 분포하였다. 3월에서 4월 사이에 만 안쪽에 서 주로 출현하였고, 5월에는 만밖에서 주로 나타났으며, 6월 이후에는 본 연구 해역에서는 채집 이 되지 않았다.

이석은 초기에 둥근 모양에서 체장이 30~40 mm 정도 성장하면 타원형으로 변하며, 일륜으로 볼 수 있는 미세 성장선이 나타났다. 또한, 핵으로부터 30개의 미세성장선 이후에 깊게 파인 불 연속선이 나타나는데, 체장 10 mm 이하의 자어 형태에서는 성장 정지선이 나타나지 않았다. 이 불연속선은 연안측으로 회유하는 동안에 신진대사의 변화에 따라 형성된 것으로 판단된다. 흰베 도라치의 초기성장은 다음과 같은 Gomertz식

 $TL = 6.702 \exp \{2.925 [1 - \exp (-0.0076 t)]\}$ (r² = 0.94, N = 92)

에 의해 추산되었다.

흰베도라치 이석에 처음으로 미세 성장선이 형성된 시기는 12월에서 1월 사이로 보여지며, 흰 베도라치 성어의 생식소는 10월부터 성숙하기 시작하며 11월에서 12월 사이에 주로 산란하고 1 월에는 산란을 마치는 것으로 나타남으로써 흰베도라치의 부화시간은 한 달 정도인 것으로 판단 된다.