Zoological Studies **37**(4): 322-329 (1998)

Use of Otolith Microchemistry to Investigate the Life History Pattern of Gobies in a Taiwanese Stream

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(Accepted July 3, 1998)

Kang-Ning Shen, Ying-Chou Lee and Wann-Nian Tzeng (1998) Use of otolith microchemistry to investigate the life history pattern of gobies in a Taiwanese stream. *Zoological Studies* **37**(4): 322-329. To understand the migratory history of stream gobies in Taiwan, otolith microchemistry of 11 species of gobies collected in Longlong Brook, northeastern Taiwan from 26 Sept. 1995 through 4 Nov. 1996 was examined by use of an electron probe microanalyzer. According to the time-series changes in strontium (Sr) to calcium (Ca) ratios from the primordium to the edge of the otolith, the 11 species are divisible into 3 life history patterns. *Rhinogobius brunneus* is a freshwater species, its otolith Sr/Ca ratios being less than 10‰. *Glossogobius biocellatus* and *Favonigobius reichei* are estuarine species, with Sr/Ca ratios of around 10‰. *Awaous melanocephalus, Eleotris acanthopoma, Glossogobius celebius, Oligolepis acutipennis, Redigobius bikolanus, Rhinogobius nagoyae formosanus, Sicyopterus japonicus*, and *Stenogobius genivittatus* are amphidromous species migrating between seawater and fresh water, Sr/Ca ratios being higher in inner layers (approximately 10‰-17‰) than outer layers of the otolith (less than 10‰). This indicates that the 8 amphidromous species migrate to a marine environment during early life and back to fresh water at a later stage. The marine phase of post larval duration in the amphidromous fishes lasts from approximately 1 mo in *O. acutipennis* to 6 mo in *Sicyopterus japonicus*. This study reveals that Sr/Ca ratios in otoliths can be used as indicators of a fish's past life history.

Key words: Gobioidei, Amphidromy, Otolith, Microchemistry, Larval duration.

Life history patterns of gobies are the most diversified among freshwater fishes (Dotu and Mito 1953, Dotu 1955 1961, Ryan 1991, Katoh and Nishida 1994). According to their migratory patterns, gobies are classified as marine, freshwater, estuarine, or amphidromous species (Myers 1949, McDowall 1988 1993). Amphidromous gobies deposit adhesive eggs in streams, and after hatching the tiny larvae are swept down to the sea. The larvae spend a period of time as marine icthyoplankton, metamorphose, return to the estuary as juveniles, and then migrate upstream to the habitat of the adult (Iguchi and Mizuno 1991, Bell et al. 1995). Past studies of fishes in Taiwan mainly focus on species inventory. Their life histories are incompletely known (Tzeng 1986, Shao et al. 1992).

Otoliths, located in the membranous labyrinth of the inner ear of teleost fishes, are composed of aragonitic calcium carbonate and an organic matrix (Carlstrom 1963, Degens et al. 1969, Lowenstein 1971). The otolith contains much information about the environment which the fish has experienced previously, and this can be retrieved through otolith microchemistry (Radtke and Shafer 1992). There are a number of trace elements that may contaminate the aragonitic calcium carbonate during otolith deposition. Due to its close proximity to calcium (Ca) in ionic radius size, strontium (Sr) can interchange with calcium in otolith aragonite (Amiel et al. 1973). Sr/ Ca ratios in otoliths are higher for those deposited in marine than in freshwater environments (Casselman 1982, Kalish 1990, Secor 1992, Tzeng and Tsai 1994, Tzeng 1996, Tzeng et al. 1997). Thus, the ratios can be used as indicators in the study of environmental history of am-

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phidromous fishes migrating between seawater and fresh water (Radtke and Kinzie 1996, Radtke et al. 1996). Moreover, Pannella (1971) discovered daily growth increments in otoliths of fish; their age can be determined from the increments. Thus, the timing of the ontogenetic shift from seawater to fresh water can be determined (Campana and Neilson 1985, Jones 1992, Cheng and Tzeng 1996). These findings provide new clues to study the migratory history and ecological behavior of such gobies.

This research aims to clarify the migratory environmental history of amphidromous gobies in a stream of Taiwan and to estimate the duration of their marine larval phase.

MATERIALS AND METHODS

Fish were captured monthly by electro-fishing in the Longlong Brook, northeastern Taiwan during the period 26 Sept. 1995 through 4 Nov. 1996 (Fig. 1). The sampling site was free from tidal influence, and salinity was close to zero. After collection, specimens were fixed immediately with ethanol or frozen fresh.

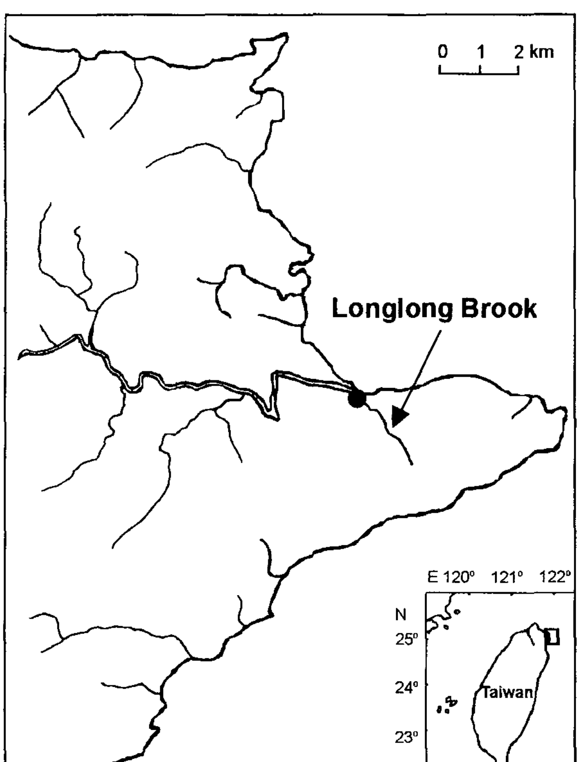

Fig. 1. Sampling site of gobies in Longlong Brook, northeastern Taiwan.

Sagittae, the largest of the 3 pairs of otoliths of the fish, were removed, embedded in epoxy resin, and then polished until the nucleus was revealed. Otolith surfaces must be polished to extreme smoothness to prevent large diffractions of x-rays when Sr and Ca are measured by an electron probe microanalyzer (JET 1800 x-ray electron microprobe). The polished otoliths were coated with carbon to further dampen x-ray diffraction and to increase electron conductance. Apatite and strontium fluoride were utilized as standards to calibrate the concentrations of Sr and Ca in the otoliths of the fish. Samples were analyzed with an electron beam focused on an area approximately 5 µm in diameter. Each area was measured at a distance of 5-µm intervals across the maximum axis of the sagittal plane of the otolith. Sr/Ca ratios in otoliths are expressed by weight ratio in parts per thousand (‰). Detailed procedures and criterion of analyses followed a previous study (Shen 1997).

Counts of growth increments in otoliths were made by both light and electron microscopy to estimate the age of the fish in days. The duration of marine larval life of amphidromous gobies was determined according to counts of daily growth increments in the otolith from the primordium to the position where the Sr/Ca ratio decreased and the increment width increased dramatically (Shen 1997).

RESULTS

Species composition

A total of 1009 individuals representing 9 families and 24 species was collected in Longlong Brook. Gobies were the most abundant, with 14 species being found. *Eleotris acanthopoma* and *Sicyopterus japonicus* were the 2 most abundant species and constituted approximately 28.25% and 26.07% of the total fish caught, respectively, followed by *Rhinogobius* spp. (including 3 species: *R. brunneus*, *R. flumineus*, and *R. nagoyae formosanus*, 11.79%), *Awaous melanocephalus* (8.03%), *Anguilla japonica* (5.35%), and *Anguilla marmorata* (4.56%). These 8 species formed approximately 85% of the total catch (Table 1).

Sr/Ca ratios in otoliths

Sr/Ca ratios from the primordium to the otolith edge in 11 species of gobies were examined (Fig. 2). *Rhinogobius brunneus* is a freshwater species

Table 1. Species composition of fish collected in Longlong Brook from Sept. 1995 to Nov. 1996

Family	Species	No. of fish															
		Sep	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total	%
Anguillidae	*Anguilla marmorata*	5	6	8	6	3	2	2	2	3	1	1	2		5	46	4.56
	Anguilla japonicus	3	2	1						7		1	1	1	38	54	5.35
Cichlidae	*Tilapia zillii*	2								15	1					18	1.78
Cyprinidae	*Acrossocheilus paradoxus*								1							1	0.01
	Zacco platypus				4	7	11	3	1	4					1	31	3.07
Eleotrididae	*Eleotris acanthopoma*	38	81	22	13	5	3		7	25	41	26	18	6		285	28.25
Gobiidae	*Awaous melanocephalus*	24	17	1	7	3	5	5	2	10	2	1	1		3	81	8.03
	Favonigobius reichei										2	3	8	1	1	15	1.49
	Glossogobius bicirrhosus	2	4	2												8	0.79
	Glossogobius biocellatus													2	1	3	0.30
	Glossogobius celebius													1	2	3	0.30
	Oligolepis acutipennis													2	1	3	0.30
	Redigobius bikolanus								2	6						8	0.79
	Rhinogobius spp. (3 species)	1	7	11	30	1	13	5	1	45	4				1	119	11.79
	Sicyopterus japonicus		10	27	43	79	40	50	4	5				2	3	263	26.07
	Schismatogobius roxasi	1	4	1	1											7	0.69
	Stenogobius genivittatus													5	8	13	1.29
Kuhliidae	*Kuhlia marginata*									10						10	0.99
Leiognathidae	*Leiognathus nuchalis*										2					2	0.20
Mugilidae	*Liza macrolepis*	10		1			1			6		3	3			24	2.38
Teraponidae	*Terapon jarbua*	7	2							4		1	1			15	1.49
Total		93	133	74	104	98	75	65	20	140	53	36	34	20	64	1009	100.00

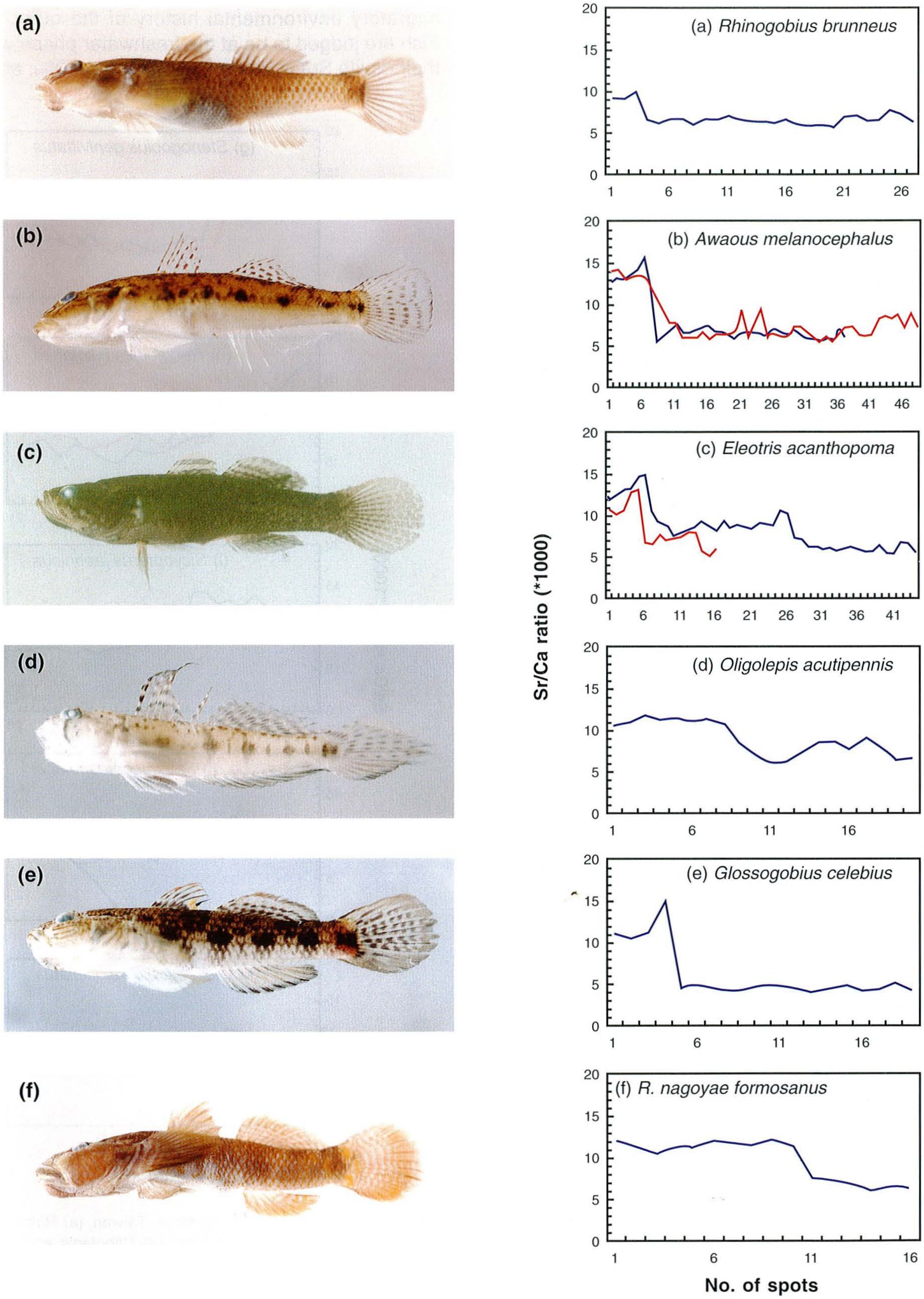

Fig. 2. (Caption see next page)

(Tzeng 1986). Sr/Ca ratios of the species ranged from 6‰ to 10‰ (Fig. 2a). The upper limit of the ratios was used as a criterion to determine the migratory environmental history of the other fish. Fish are judged to be at the freshwater phase when their otolith Sr/Ca ratios are lower than 10‰, and at

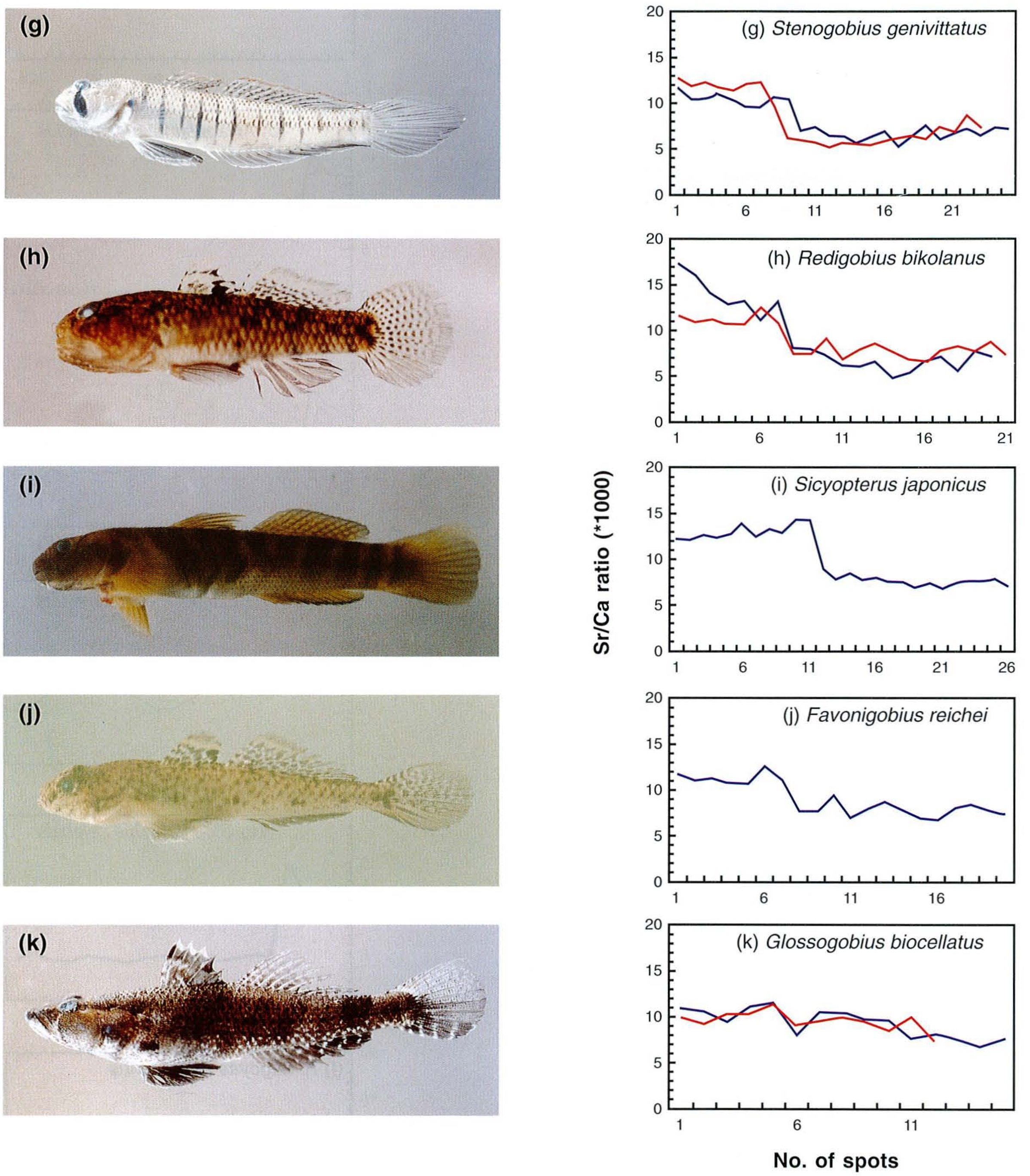

Fig. 2. (Left): Photographs showing external features of the 11 species of gobies collected in Longlong Brook, Taiwan, (a) *Rhinogobius brunneus*, total length = 5.1 cm; (b) *Awaous melanocephalus*, 8.3 cm; (c) *Eleotris acanthopoma*, 4.6 cm; (d) *Oligolepis acutipennis*, 3.8 cm; (e) *Glossogobius celebius*, 6.6 cm; (f) *R. nagoyae formosanus*, 7.4 cm; (g) *Stenogobius genivittatus*, 7.3 cm; (h) *Redigobius bikolanus*, 3.8 cm; (i) *Sicyopterus japonicus*, 10.0 cm; (j) *Favonigobius reichei*, 4.3 cm; and (k) *Glossogobius biocellatus*, 7.8 cm. (Right): Sr/Ca ratios measured from the primordium to the edge of the otoliths of the 11 species, (a)~(k). According to the changes of the ratios, these fish were classified into freshwater (a), amphidromous (b-i) and estuarine species (j, k). Two individuals were measured in (b), (c), (g), (h), and (k).

the marine phase when the ratios are greater than 10‰.

The Sr/Ca ratios in otoliths of *Awaous melanocephalus*, *Eleotris acanthopoma*, *Oligolepis acutipennis*, *Glossogobius celebius*, *R. nagoyae formosanus*, *Stenogobius genivittatus*, *Redigobius bikolanus*, and *Sicyopterus japonicus* were greater than 10‰ in the inner layer, but the ratios decreased to less than 10‰ in the outer layers of the otoliths (Fig. 2b–i). This indicates that these 8 species had migrated to a marine environment at an early life stage, but returned to fresh water at a later stage. These 8 species are thus considered to be amphidromous fishes, migrating between freshwater and marine environments. Conversely, the Sr/Ca ratios in otoliths of *Favonigobius reichei* and *G. biocellatus* were not higher than 10‰ in the inner layers, but all fluctuated around 10‰ (Fig. 2j, k), indicating that these two species are estuarine fishes, which did not experience a marine environment at an early life stage and may migrate between fresh water and the estuary.

Change of growth rate during upstream migration

Increment widths in otoliths of the amphidromous goby *Stenogobius genivittatus* were wider in the outer layers than in the inner layers, producing an obvious mark at the transition boundary (Fig. 3). The abrupt change in increment width corresponds to a dramatic decrease of the Sr/Ca ratios in otoliths of the fish. This indicates that the mark in the otolith was deposited when the fish migrated from a marine environment to fresh water. Meanwhile fish growth became faster after entering the stream. The fish changed from a pelagic form in the marine phase to a benthic form in the stream. Thus the mark may be considered to be a settlement mark.

Duration of marine larval life

Daily growth increments in otoliths from the primordium to the settlement mark represent the duration of larval life in the marine phase. Durations were different among species, lasting from 1 mo in *Oligolepis acutipennis* to 6 mo in *Sicyopterus japonicus*. Among them, *S. japonicus* was best studied, with its marine larval duration ranging from 4 to 6 mo (Table 2).

DISCUSSION AND CONCLUSIONS

Sr/Ca ratios in otoliths have been used to investigate the environmental history of gobies in Hawaii by Radtke and his colleagues (Radtke et al.

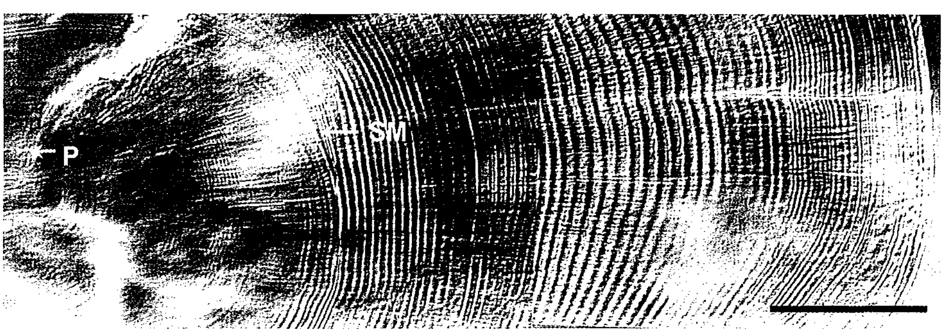

Fig. 3. Daily growth increments and settlement mark (SM) in an otolith of *Stenogobius genivittatus* (7.3 cm) collected in Longlong Brook, on 28 Oct. 1996, and the marked increase of increment width beyong SM. Scale bar = 100 mm. P = primordium.

Table 2. Duration of marine larval stage of amphidromous gobies

Species	n	Marine larval stage duration (d)
Awaous melanocephalus	2	63, 97
Eleotris acanthopoma	2	113, 129
Oligolepis acutipennis	1	35
Redigobius bikolanus	2	48, 50
Sicyopterus japonicus	97	125 to 186
Stenogobius genivittatus	2	92, 93

1988, Radtke and Kinzie 1996). Trends in Sr/Ca ratios in otoliths of gobies in a Taiwanese stream are similar to those of Hawaii. The ratios in this study are expressed as weight ratios of the elements and are approximately twice those from Hawaii, which were expressed by the ratio of atomic number. Nonetheless, the Sr/Ca ratios in otoliths of gobies from both areas are higher in inner layers than in outer layers. Because Sr concentration is higher in seawater than in freshwater environments (Tzeng and Tsai 1994), Sr/Ca ratios in otoliths of fish in seawater are thus higher than those in fresh water (Casselman 1982, Radtke et al. 1988, Kalish 1990, Secor 1992, Otake et al. 1994, Tzeng and Tsai 1994, Tzeng 1996, Tzeng et al. 1997). Accordingly, the higher Sr/Ca ratios in inner layers of otoliths of amphidromous gobies imply that the fish are swept out to sea in the larval stage and return to the freshwater environment at a later life stage. This inference is supported by the examination of Sr/Ca ratios in otoliths of juvenile *Sicyopterus japonicus* collected during their upstream migration in the river mouth (Shen 1997).

Counts of daily growth increments in inner layers of otoliths represent the larval duration in the marine phase of amphidromous fishes. The larval duration of the goby, *S. japonicus*, ranged from 4 to 6 mo, which is compatible with that of the Japanese eel *Anguilla japonica* (Tzeng 1990). The lengthy larval duration of the eel is adaptive for long-distance dispersal from the spawning ground in the ocean to the growth habitat in rivers (Tsukamoto and Umezawa 1994, Cheng and Tzeng 1996). Spawning grounds and growth habitats of the goby are both in fresh water (Tzeng 1986). Accordingly, the lengthy larval duration of the goby may be unlike that of the eel for returning from the spawning ground to the growth habitat, but may have evolved simply to increase the range of dispersal and the population size. In addition, the marine larval duration of *S. japonicus* was flexible. The difference between minimal and maximal duration was approximately 2 mo. Differences in larval duration of the goby between individuals were proposed to be related to seasonal variations in marine productivity (Cowen 1991, Bell et al. 1995, Shen 1997).

In conclusion, most gobies in the stream in northeastern Taiwan are amphidromous migratory species. The marine larval duration differs among species, lasting from 1 to 6 mo. Growth rates are reduced in the larval stage and dramatically increase after entering the freshwater environment.

Acknowledgments: This study was financially supported by the National Science Council, Republic of China (Project No. NSC 86-2311-B-002-042). The authors are grateful to Miss C.H. Wang for preparing the manuscript and to 2 anonymous reviewers for helpful comments.

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耳石微化學應用於鰕虎魚洄游環境史之研究

沈康寧[1] 李英周[1] 曾萬年[2]

為了證明溪流鰕虎魚的海陸兩側洄游現象，利用電子微探儀，分析 1995 年 9 月至 1996 年 11 月採自臺灣東北部隆隆溪的 11 種鰕虎魚耳石的微量元素。根據耳石中鍶鈣元素重量比的時間序列變化，這 11 種鰕虎魚可以歸納為三種不同的洄游環境史：1) 褐吻鰕虎 (*Rhinogobius brunneus*) 為淡水種，鍶鈣比小於 10‰，2) 雙斑叉舌鯊 (*Glossogobius biocellatus*) 及雷氏鯊 (*Favonigobius reichei*) 為河口域種，鍶鈣比在 10‰ 附近變動，3) 曙首厚唇鯊 (*Awaous melanocephalus*)，塘鱧 (*Eleotris acanthopoma*)，岩舌鰕虎 (*Glossogobius celebius*)，寡鱗鰕虎 (*Oligolepis acutipennis*)，紅鰕虎 (*Redigobius bikolanus*)，名古屋吻鰕虎 (*Rhinogobius nagoyae formosanus*)，日本禿頭鯊 (*Sicyopterus japonicus*) 以及種子鯊 (*Stenogobius genivittatus*) 等 8 種為海水與淡水之間洄游的兩側洄游性種類；其耳石鍶鈣比，內層 (10 – 17‰) 比外層 (小於 10‰) 高，顯示這 8 種魚的初期發育階段生活在海洋中，後期則回到淡水。兩側洄游性種類的海洋浮游期，範圍從鱗寡鰕虎的一個月至日本禿頭鯊的六個月不等。本研究發現耳石鍶鈣比可以用來研究鰕虎魚類的洄游環境史。

關鍵詞：鰕虎魚，兩側洄游，耳石，微化學，仔魚期持續的時間。

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