Zoological Studies **48**(5): 640-648 (2009)

Zoological Studies

Validation of Annular Deposition in Scales and Otoliths of Flathead Mullet *Mugil cephalus*

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(Accepted January 6, 2009)

Chih-Chieh Hsu and Wann-Nian Tzeng (2009) Validation of annular deposition in scales and otoliths of flathead mullet *Mugil cephalus*. *Zoological Studies* **48**(5): 640-648. Age estimation of fish is essential for understanding their life history and population dynamics, and for assessing and managing stocks. The periodicity of age marks of both scales and otoliths of flathead mullet *Mugil cephalus* was validated using cultured fish of known ages, and these were compared to those of wild ones. Annuli in scales were characterized by dense circuli in the basal area, merged circuli in the lateral-basal area, and interrupted circuli in the lateral area of the scale. Annuli in whole otoliths were characterized by an opaque zone under both reflected and transmitted light. In acid-etched sectioned otoliths, annuli were defined by dense concentric rings. Annuli of both scales and otoliths of *M. cephalus* were coincident and were deposited once a year during winter. Scales are convenient for age estimation because there is no need for special treatment. Preparing otolith sections for etching is time-consuming and costly, but the otolith microstructure and microchemical analyses can provide additional life-history information. http://zoolstud.sinica.edu.tw/Journals/48.5/640.pdf

Key words: *Mugil cephalus*, Otolith, Scale, Annulus, Age estimation.

Flathead mullet *Mugil cephalus* Linnaeus, 1785, is a euryhaline fish, widely distributed in coastal waters, lagoons, estuaries, and rivers between the latitudes of 42°N and 42°S worldwide (Thomson 1953). The population of *M. cephalus* in waters adjacent to Taiwan migrates annually from its feeding ground in coastal waters of China to the southwestern and northeastern coastal waters of Taiwan to spawn in winter (Chen and Su 1986, Huang and Su 1989, Chang et al. 2004, Hsu et al. 2007). Approximately 1 mo after spawning, juveniles of *M. cephalus* are abundantly recruited to estuarine nursing areas (Chang et al. 2004). *Mugil cephalus* is an economically important food source of capture fisheries in many countries and aquaculture worldwide (Nash and Shehadeh 1980); in particular, females are harvested for their highly valuable roe (Kuo 1995). Due to overfishing, the annual catch of wild *M. cephalus* populations in Taiwan decreased from a historic peak of approximately 6900 tons in 1980 to 193 tons in 2006 (Chang and Tzeng 2006). Therefore, sustainable management of *M. cephalus* is urgently needed. Nevertheless, successful fisheries management requires accurate age-based information.

The age of fish can be determined by various methods such as mark-recapture data, length frequency, scales, vertebrae, and otoliths. Among these, the use of scales and otoliths are the most popular (Jennings et al. 2001). Annuli in scales have been used to investigate the ages of *M. cephalus* for a long time (Tung 1959, Berg and Grimaldi 1967, Grant and Spain 1975). However, scales can be easily damaged or removed from a fish's body, and regenerated scales may cause the age to be underestimated (Tung 1959). Otoliths

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are located in the inner ear sac of teleost fish and function in balance and hearing (Lowenstein 1971). Otoliths are biomineralized aragonite crystals, mainly composed of $CaCO_3$ with an organic matrix and trace impurities of various elements (Calstrom 1963, Degens et al. 1969, Campana 1999). Otoliths are deposited alternately by calcium carbonate-rich and protein-rich layers in both annual and daily cycles that permit fish age estimations on daily and annual scales.

The age of *M. cephalus* juveniles was determined in a previous study by daily growth increments in otoliths (Chang et al. 2000), together with an otolith microchemical analysis to study the migratory environmental history of *M. cephalus* (Chang et al. 2004). Annuli in whole and sectioned otoliths of *M. cephalus* were also studied (Ibáñez-Aguirre and Gallardo-Cabello 1996, Smith and Deguara 2003, Chang et al. 2004). Cech and Wohlschlag (1975) noted that age marks in otoliths of *M. cephalus* in coastal waters of Texas, USA were deposited twice a year, in summer and winter. This differs from the conventional concept which explains that annuli in otoliths of *M. cephalus* are deposited once a year (Ibáñez-Aguirre and Gallardo-Cabello 1996, Chang et al. 2004). In Australia, the 1st annulus of otoliths of *M. cephalus* was estimated to be deposited approximately 20 mo after hatching, not 1 yr (Smith and Deguara 2003). Consequently, age estimation of *M. cephalus* remains controversial and requires validation.

In order to validate the periodicity of annuli in scales and otoliths of *M. cephalus*, structures of annuli of scales and otoliths of pond-reared *M. cephalus* of known ages were compared using different preparation methods. Moreover, age marks of pond-reared fish were also compared to those of wild ones to establish a protocol for age estimation of this fish.

MATERIALS AND METHODS

Sample collection

In total, 44 pond-reared *M. cephalus* at the ages of > 1 and 3 yr were collected from 2 fish farms in Yunlin County, southwestern Taiwan in Nov. 2006 and Sept. 2007. The maximum age of cultured mullet is 3 yr, and most wild mullet are 4 yr old. The culture water of the fish farms was taken from nearby estuaries and had salinities of 8-20 PSU. Seasonal water temperatures of the pond varied from 15°C in winter to 30°C in summer, which is similar to those of the natural environment. Mullet of known ages were reared after they were caught in the estuary at the juvenile stage approximately 1 mo after hatching. These mullet were fed commercial fish feed. In addition, it was reported that many life history events in the early stage, such as metamorphosis from the larval to the juvenile stage and habitat transition may leave a mark in otoliths (Chang et al. 2000), which may confuse validation of the age mark. Accordingly, wild juvenile mullets in the estuary adjacent to the fish farms in Dec. 2006 were also collected, and the dimensions of its otoliths were compared to both whole and sectioned otoliths of pond-cultured *M. cephalus* of known age. Moreover, age marks in otoliths of 25 migratory *M. cephalus* aged 4 yr old, which were caught in the spawning ground in southwestern waters of Taiwan in Dec. 2006, were also examined to determine if the structures of annuli in otoliths of wild fish differ from those of cultured ones. These wild-caught mullet were in the spawning stage according to their gonadal development stage (Hsu et al. 2007).

Table 1. Sampling location and date, sample size (F, female; M, male; U, undetermined), age, mean (± SD) fork length (FL, mm), and body weight (BW, g) of *Mugil cephalus* of known age collected from 2 fish farms, and wild-caught juvenile and adult *M. cephalus* from offshore and a nearby estuary in southwestern Taiwan

Sample site	Initial date of culture	Sample date	Sample size				Age	FL (mm)	BW (g)
			M	F	U	Total			
Fish farm A	Dec. 2003	28 Nov. 2006	9	12	0	21	3 yr	478.10 ± 29.71	1658.48 ± 268.42[a]
Fish farm B	Dec. 2005	23 Sept. 2007	0	0	23	23	> 1 yr	205.75 ± 19.37	91.17 ± 18.92
Offshore	-	26 Dec. 2006	15	10	0	25	4 yr	506.36 ± 18.63	1485.92 ± 175.52
Estuary	-	10 Dec. 2006	0	0	5	5	approx. 37 d	29.31 ± 1.11	A

[a]Body weight without gonad weight; A, absent.

After collection, all specimens were preserved on ice, their fork length (FL, mm) and body weight (BW, g) were measured, and the sex was determined within 24 h (Table 1).

Scale and otolith preparation for age estimation

Five scales located close to the left pectoral fin of each individual were collected for age estimation, because the size of scales close to the pectoral fin is larger and the shape is thought to be more regular and to consist of fewer regenerated scales than those in other portions of the fish body (Tung 1959, Liu and Tzeng 1972). The scales were cleaned, air-dried, and mounted on 2 glass slides after collection; then the annuli were examined under a dissecting microscope (Nikon SMZ-10) with transmitted light; and a photograph of the annuli of scales was taken with a digital camera (Nikon Coolpix 4500). The annular characteristics of scales were identified to establish a criterion for age determination.

The sagittae, the largest of 3 pairs of otoliths, were removed, cleaned, and dried in a drying oven. Annuli in whole non-sectioned otoliths were examined in a glass petri dish using both reflected and transmitted light. Under reflected light with a black background, some light could pass through the translucent zone of the otoliths and be absorbed by the background,but the translucent zone of the entire otolith was not bright. Under the same condition, the light could not pass through the opaque zone of the entire otolith and was reflected, displaying a white color. On the other hand, under transmitted light with a transparent background, since light could not pass through the opaque zone, the opaque zone was dark.

Chang et al. (2004) and Smith and Deguara (2003) separately used frontally and transversely sectioned otoliths to estimate the age of *M. cephalus*. To determine if numbers of annuli are consistent among different sectioned planes, otoliths were embedded in epofix resin (Epofix, Struers) and sectioned with a low-speed diamond saw from the transverse and frontal planes at an approximate thickness of 300 μm. The sectioned otolith was then polished using a 0.05 μm alumina suspension from 1 side of the section until the primordium was exposed, similar to the procedure reported in previous studies (Smith and Deguara 2003, Chang et al. 2004). After etching with a 5% HCl solution for 10 s to enhance the contrast of the annuli, the sectioned otolith was observed and photographed with a microscope equipped with a digital camera. In addition, however, sectioned but un-etched otoliths displayed no growth checks. Accordingly, the results of un-etched sectioned otoliths are not shown.

RESULTS

Microstructure of the annuli in scales

Patterns of circuli in the annuli varied among different portions on the scale. The circuli of the annulus in the basal area of the > 1 and 3 yr old pond-cultured and wild 4 yr old *M. cephalus* were all densely arranged (Figs. 1A-a, B-a, C-a) and merged together in the lateral-basal area (Figs. 1A-b, B-b, C-b), and old circuli were interrupted by newly deposited circuli in the lateral area of the scale (Figs. 1A-c, B-c, C-c).

Because the > 1 yr old pond-reared fish were collected in early autumn (Sept.), the marginal increment width (the distance between the edge of the scale and the last annulus) was still comparatively wide. However, the annuli of 3 yr old pond-cultured and 4 yr old wild-caught fish collected during winter (Nov. and Dec.) were almost always deposited at the margin of the scale (Figs. 1B, C). This indicates that the annulus in the scale might be deposited in winter (Nov. to Dec.).

Optical structure of annuli of the whole otolith

Annuli in whole otoliths were composed of 1 opaque zone and 1 translucent zone. The opaque zone appeared dark under transmitted light with a transparent background but bright under reflected light with a black background (Fig. 2). The optical appearance of the core was similar to the opaque zone. The numbers of opaque zones in the entire otolith of both > 1 and 3 yr old *M. cephalus* were all consistent with their true ages (Figs. 2a-d). This indicates that the opaque zone can be used as an age mark for age estimation. The annuli in the otoliths of wild-caught fish also showed a similar annular pattern (Figs. 2e, f). The growth of otoliths was asymmetrical among different growth axes, growing fastest in the anterior-posterior axis, followed by the dorsal-ventral axis, and was slowest in the distal-proximal axis of otoliths. Among the 3 growth axes of whole otoliths, annuli were clearest in the anterior-posterior axis. The 3rd annual ring (opaque zone) of pond-reared 3 yr old fish and the 4th annual ring of wild-caught 4 yr old fish were all deposited at the margin of the

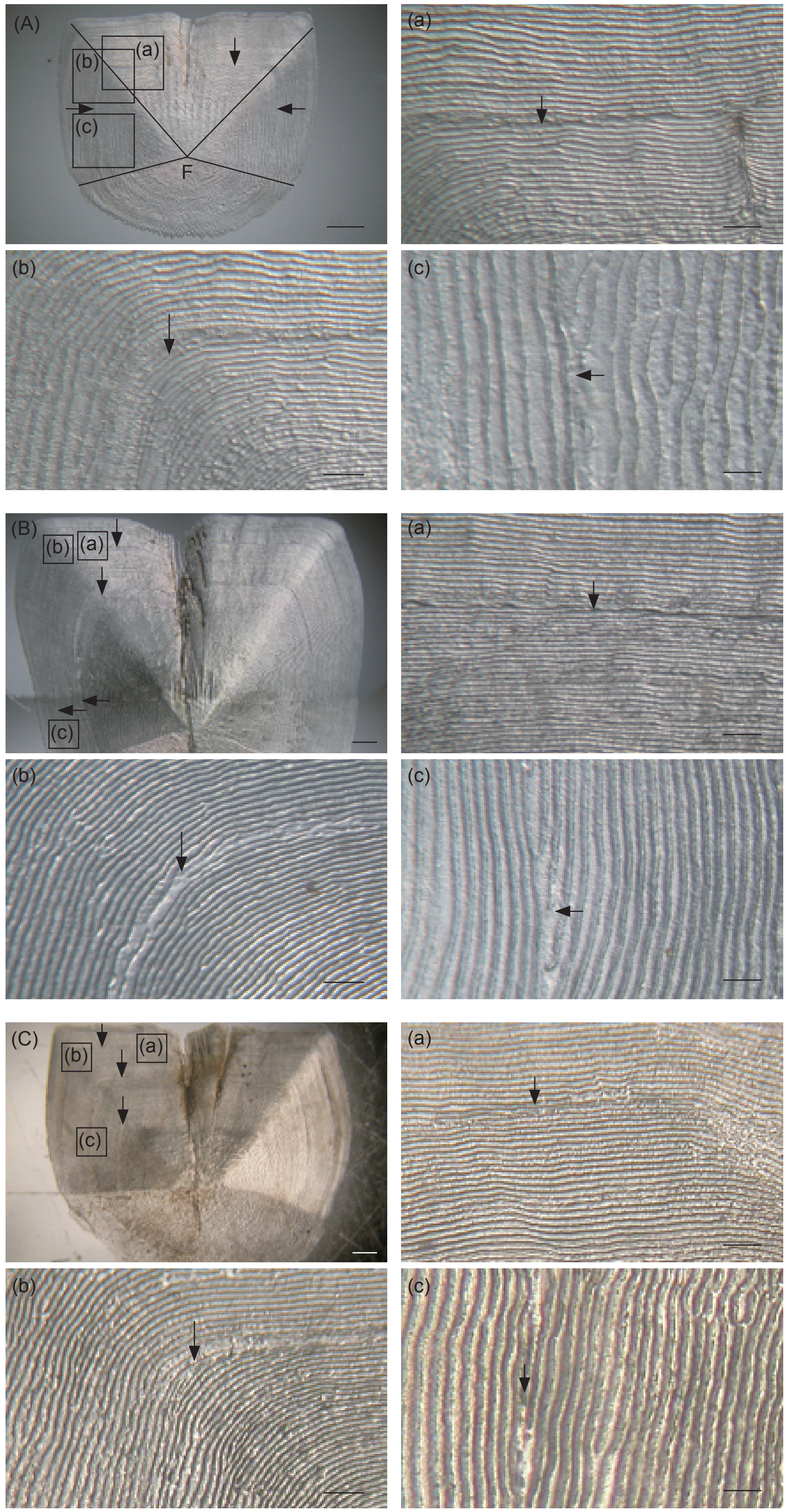

Fig. 1. Microscopic structure of annuli in the basal anterior area (a), basal-lateral area (b), and lateral area (c) in scales of a > 1 yr old (A, fork length (FL) of 203.0 mm) and pond-reared 3 yr old *Mugil cephalus* (B, FL of 468.9 mm) and wild-caught 4 yr old *M. cephalus*. (C, FL of 481.0 mm). Arrow, annuli; F, focus. Scale bar: A-C = 1 mm; a-c 250 μm.

otolith. This also indicates that annuli in otoliths were likely deposited during winter like the annuli of scales.

Microstructure of annuli and growth checks in sectioned otoliths

Annuli in sectioned otoliths were composed of multiple concentric increments in both transversely and frontally sectioned otoliths of pond-cultured and wild mullet (Fig. 3). These increments in annuli were almost always close together. In addition, growth rates of frontally sectioned otoliths differed among directions, being fastest along the anterior-posterior axis. Accordingly, annuli of the anterior-posterior axis of frontally sectioned otoliths were easy to identify, whereas annuli of the dorsal-ventral axis of transversely sectioned otoliths were easy to identify.

On the other hand, multiple concentric increments were also deposited around the core region of the otolith before the 1st annulus had formed (Fig. 3b). Comparing whole and sectioned otoliths of a > 1 yr old *M. cephalus* with that of a juvenile aged approximately 1 mo after hatching, the dimension of the multiple concentric rings in the core region of the > 1 yr old *M. cephalus* otolith corresponded to the otolith size of the juvenile (Fig. 4). This indicates that the multiple concentric rings around the core region of the otolith are not annuli.

DISCUSSION

Characteristics of annuli in scales and otoliths

This is the first study to validate the annuli of both scales and otoliths of flathead mullet *M. cephalus* using pond-reared fish of known age. Numbers of annuli were consistent between scales and otoliths of the fish. Meanwhile, patterns of annuli of scales and otoliths of pond-

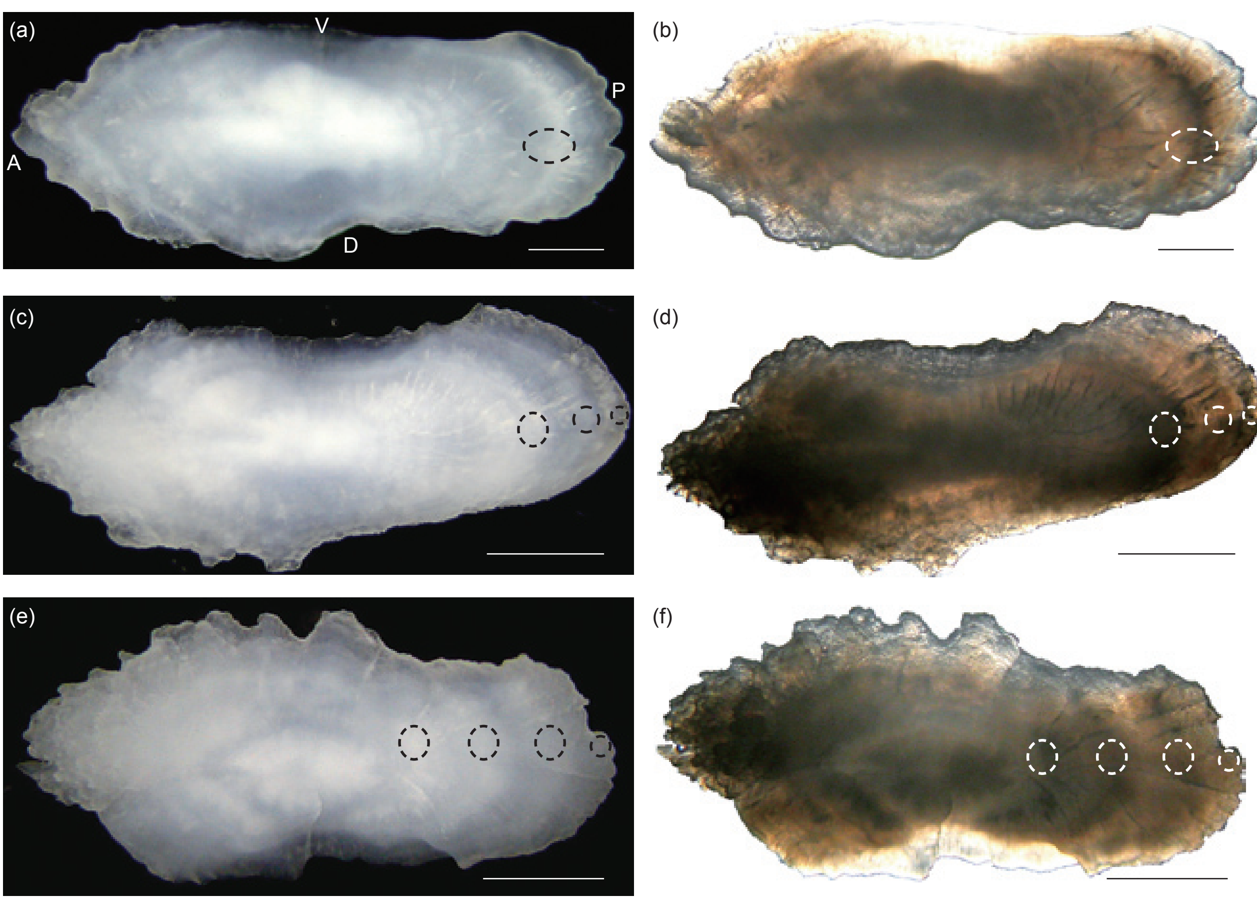

Fig. 2. Opaque and translucent zones in the whole otolith of a > 1 (a, b) and a 3 yr old (c, d) pond-reared *Mugil cephalus* and a wild-caught 4 yr old *M. cephalus* (e, f) under reflected light with a black background (a, c, e) and under transmitted light (b, d, f). Open circles indicate opaque zones (annuli). A, anterior; D, dorsal; P, posterior; V, ventral. Scale bar: a, b =1 mm; c-f = 2 mm.

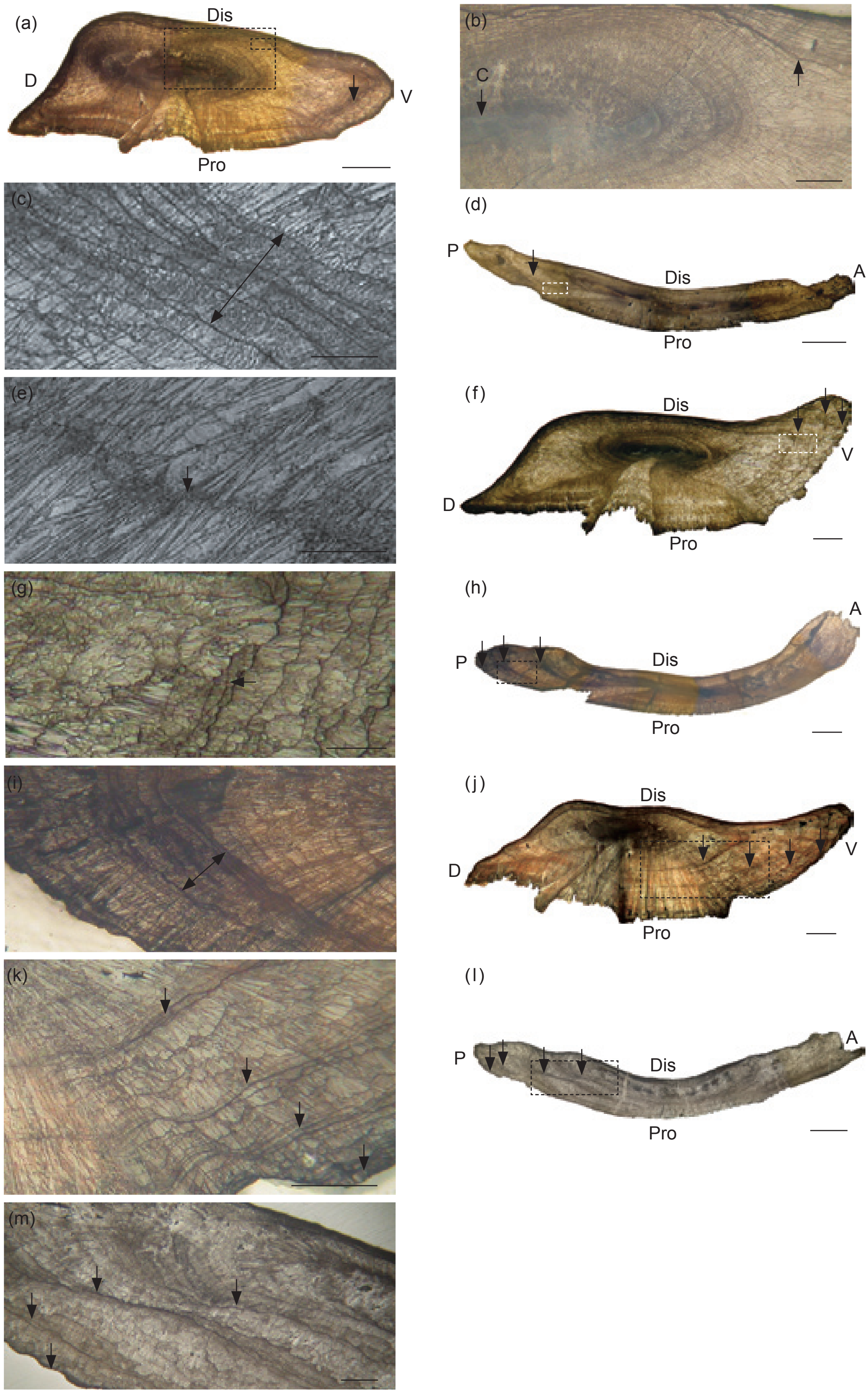

Fig. 3. Microstructure of annuli in transversely sectioned (a, f, j) and frontally sectioned otoliths (d, h, l) of pond-reared > 1 (a-e) and 3 y old (f-i) *Mugil cephalus* and wild-caught 4 yr old (j-m) *M. cephalus* under reflected light after etching with HCl. On each graph, arrows indicate the annuli and (b, c, e), (g, i), and (k, m) were magnified from open squares of (a, d), (f, h), and (j, l), respectively, to show the microstructure of the annulus. A, Anterior; C, core; D, dorsal; Dis, distal; Pro, proximal; P, posterior; V, ventral. Scale bar: a, f, j, k = 300 μm; b, c, e = 50 μm; d, h, l = 800 μm; g = 100 μm; i, m = 200 μm.

reared fish were similar to those of the wild ones although the environmental conditions which they had experienced differed. This indicates that the criterion for age determination revealed by pond-reared *M. cephalus* is applicable to wild populations. In addition, the pattern of annuli of scales of *M. cephalus* is similar to that of other fishes, such as the white croaker (Sciaenidae: *Argyrosomus agentatus*) (Liu and Tzeng 1972). This further indicates that the structure of annuli of scales might be universal for fish with ctenoid scales.

Compared to scales, the structure of annuli in otoliths of *M. cephalus* seemed to be more complicated. Patterns of annuli in otoliths differed in appearance with different treatments (sectioned or non-sectioned) and light source (reflected or transmitted light). Annuli in sectioned otoliths with HCl etching were more discernable than those in whole otoliths without treatment. This was due to

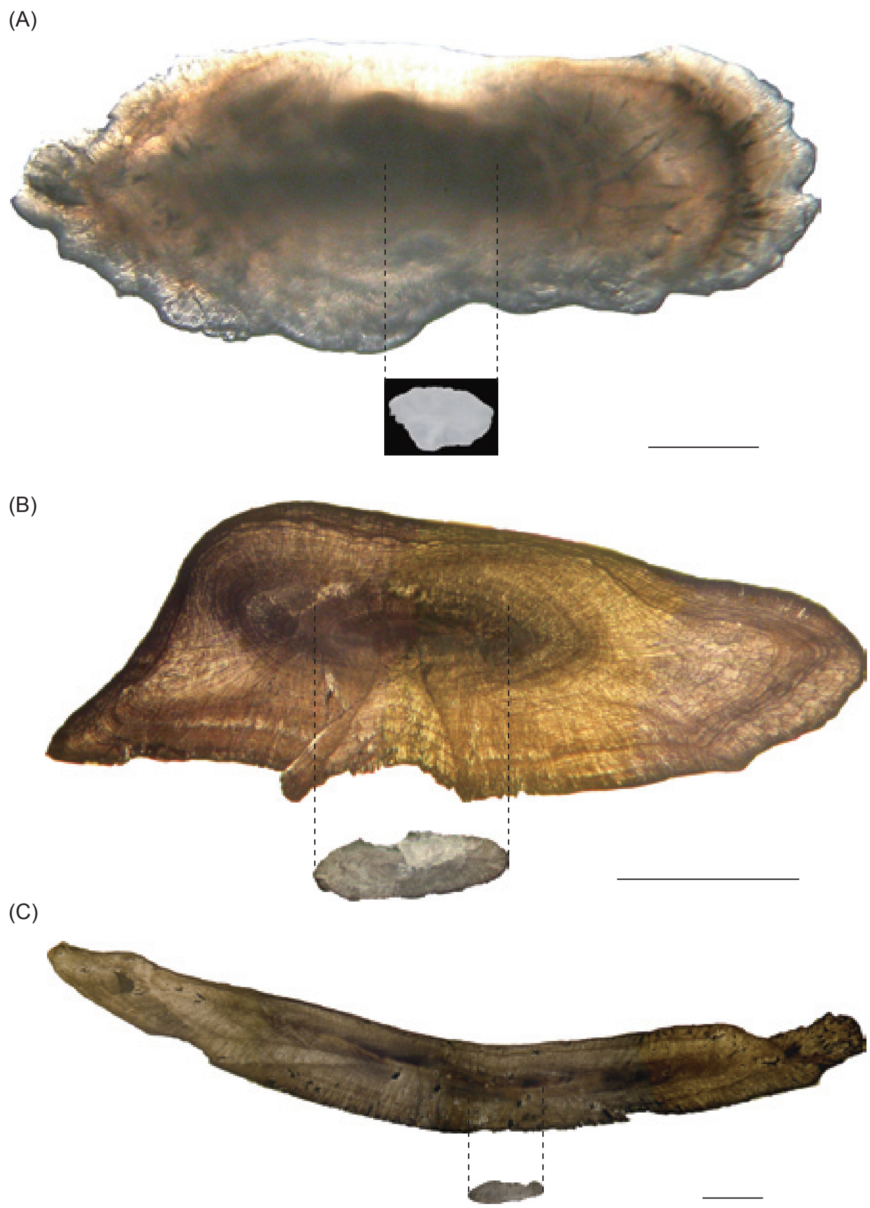

Fig. 4. Size of otoliths of juvenile (approx. 37 d old, 30.0 mm in fork length (FL)) relative to that of > 1 yr old *Mugil cephalus* (203.0 mm in FL). (A) Whole otolith, (B) transversely sectioned otolith, and (C) frontally sectioned otolith of both juvenile and > 1 yr old *M. cephalus*. Scale bar: A = 1 mm; B, C = 500 μm.

the boundary between the opaque and translucent zones not being easily distinguished in whole otoliths which may lead to difficulty in determining the exact age. In addition, annuli in frontally sectioned otoliths along the anterior-posterior axis and transversely sectioned ones along the dorsal-ventral axis were easier to read than on the other axes of otoliths. This was because annuli in otoliths are clearer in the longer than the shorter axes, and because growth rates differed among the axes of otoliths. On the other hand, the age of *M. cephalus* can be determined using either scales or otoliths, because the number of annuli was consistent between these 2 calcified structures. However, each calcified structure has its advantages and disadvantages. Scales are easily collected without sacrificing the fish, while otoliths have daily growth increment deposition which allows the determination of the age of a fish on a daily schedule (Pannella 1971, Chang et al. 2000) and can further be subjected to microchemical analysis as well (Campana 1999).

The mechanism of annular formation

The > 1 yr old mullet were collected in late summer, and the annuli were not all formed at the margins of the scales and otoliths. However, the 3 yr old pond-reared and 4 yr old wild-caught mullet were respectively collected in Nov. and Dec., and their annuli had initially formed at the margins of the scales and otoliths. This phenomenon indicates that annuli of both scales and otoliths are deposited in winter. Annuli in calcified structures are usually formed during the slow-growing period when fish are in low-temperature and poor-nutrition environments (Bilton and Robbins, 1971). Intrinsic factors, such as ontogenetic changes, the metabolic rate, maturation, and population density as well as extrinsic factors like the migratory environment, food availability, and temperature all have the potential to influence the growth of fish and the subsequent deposition rate of annuli (Panfili et al. 2002). Al-Husaini et al. (2001) indicated that the opaque zone of otoliths under transmission-light microscopy is formed in the slow-growth period, while the translucent zone is formed in the fast growth period in semitropical species. Ibáñez-Aguirre and Gallardo-Cabello (1996) indicated that opaque zones in otoliths of mullet are formed in winter or the spawning period, while fast-growth zones are formed in summer when fish feed actively and grow fast. Sufficient food was supplied for the pond-reared *M. cephalus* of known age. Accordingly, the food supply might not be the principal determinant of the annular deposition rate. Pond-reared fish, however, in this study were reared in an outdoor fish pond. The water temperatures in the fish pond seasonally fluctuate from 15°C in winter to 30°C in summer. The difference in seasonal water temperatures was approximately 15°C. Therefore, seasonal variations in water temperature are probably the primary factor determining the food uptake rate, fish growth, and subsequent formation of annuli in scales and otoliths. In other words, annular formation more closely reflects seasonal water temperature changes than the food supply. This also validates why annuli are easier to read on scales and otoliths of temperate fish compared to tropical ones.

On the other hand, an annulus-like mark was found in the core region of otoliths of pond-reared and wild *M. cephalus* before the formation of the 1st annulus. Comparing the otolith length of juveniles with the dimension of the mark in specimens of a known age, this mark possibly occurred when juveniles migrated from offshore into estuaries and/or when juveniles were transferred from the wild into the fish ponds.

Moreover, in the present study, no obvious spawning check was found in scales or otoliths of the 4-yr-old wild-caught *M. cephalus* doing spawning migration. The annuli of scales and otoliths of immature (aged 1-2 yr) and mature (aged ≥ 4 yr) mullet were deposited during winter. However, the mullet also spawn in winter, and even if the spawning check existed, it would not be easy to discriminate temperature-related annuli and a spawning-related check in scales and otoliths because they are deposited at almost the same time in winter.

Age estimate discrepancies

Annuli were consistent in both scales and otoliths, and both were determined to be suitable for aging *M. cephalus* and back-calculating the fish growth rate. Scales are more applicable due to the convenience of manipulation and the ability to avoid sacrificing individuals, which would be useful for endangered species. However, a previous study indicated that scales are not suitable for back-calculating growth rates of older *M. cephalus* because of Lee's phenomenon (Tung 1959). In addition, other previous studies indicated that annuli in otoliths are inconsistent between different preparations in some species, e.g., red mullet were under-aged by 1 yr using broken-burnt otoliths compared to whole otoliths (Polat et al. 2005), and whole otoliths might underestimate the age of threeline grunts (*Parapristipoma trilineatum*) which are older than 2 yr (Doiuchi et al. 2007). However in this study, we did not find a similar inconsistency in *M. cephalus* with different otolith preparations.

In conclusion, both scales and otoliths can be used to determine the age of *M. cephalus*. Annuli in scales can be easily identified by the pattern of circuli. The 1st opaque zone in otoliths is deposited in the juvenile stage, and it can be misinterpreted as an annulus. Sectioned otolith preparations for age estimation are time-consuming and costly but can provide information on the environmental history and other life history events through otolith microchemical analyses.

Acknowledgments: This study was financially supported by the National Science Council of Taiwan (NSC93-2313-B-002-054 and 94-2313-B-002-040 awarded to WNT). The authors are grateful to Ms. H.Y. Chen for measuring specimens and Dr. C. W. Chang for the helpful comments on this study.

REFERENCES

Al-Husaini M, S Al-Ayoub, J Dashti. 2001. Age validation of nagroor, *Pomadasys kaakan* (Cuvier, 1830) (Family: Haemulidae) in Kuwaiti waters. Fish. Res. **53:** 71-81.

Berg A, E Grimaldi. 1967. A critical interpretation of the scale structure used for the determination of annuli in fish growth studies. Mem. Ist. Ital. Idrobiol. **21:** 225-239.

Bilton HT, GL Robbins. 1971. Effects of feeding level on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada **28:** 861-868.

Campana SE. 1999. Chemistry and composition of fish otolith: pathways, mechanisms and application. Mar. Ecol.-Prog. Ser. **188:** 263-297.

Carlstrom D. 1963. A crystallographic study of vertebrate otolith. Biol. Bull. **125:** 441-463.

Cech JJ, DE Wohlschlag. 1975. Summer growth depression in the striped mullet, *Mugil cephalus*. Contrib. Mar. Sci. **19:** 92-100.

Chang CW, Y Iizuka, WN Tzeng. 2004. Migratory environmental history of the grey mullet *Mugil cephalus* as revealed by otolith Sr: Ca ratios. Mar. Ecol.-Prog. Ser. **269:** 277-288.

Chang CW, WN Tzeng. 2006. Spatial and temporal variations of grey mullet resource in the coast of Taiwan: species identification, production and aquaculture. Fish. Extens. Natl. Taiwan Univ. **18:** 25-42.

Chang CW, WN Tzeng, YC Lee. 2000. Recruitment and hatching dates of grey mullet (*Mugil cephalus* L.) juveniles in the Tanshui estuary of northwest Taiwan. Zool. Stud. **39:** 99-106.

Chen WY, WC Su. 1986. Reproductive biology of the grey mullet, *Mugil cephalus* L. of Taiwan. *In* CR Lee, ed. Study on the resource of grey mullet in Taiwan, 1983-1985. Kaohsiung, Taiwan: Kaohsiung Branch Taiwan Fisherires Research Institute, pp. 73-80.

Degens ET, WG Deuser, RL Haedrich. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. **2:** 105-113.

Doiuchi R, T Kokubo, M Ogawa. 2007. Age and growth of threeline grunt *Parapristipoma trilineatum* along the southwestern coast of Kii Peninsula, Japan. Fish. Sci. **73:** 777-783.

Grant CJ, AV Spain. 1975. Reproduction, growth and size allometry of *Mugil cephalus* L. (Pisces: Mugilidae) from North Queensland inshore waters. Aust. J. Zool. **23:** 181-201.

Hsu CC, YS Han, WN Tzeng. 2007. Evidence of flathead mullet *Mugil cephalus* L. spawning in waters northeast of Taiwan. Zool. Stud. **46:** 717-725.

Huang CS, WC Su. 1989. Studies on the fluctuations of fishing conditions for grey mullet (*Mugil cephalus* Linnaeus) from the western coast of Taiwan. J. Fish Soc. Taiwan **16:** 47-83.

Ibáñez-Aguirre AL, M Gallardo-Cabello. 1996. Age determination of the grey mullet *Mugil cephalus* L. and the white mullet *Mugil curema* V. (Pisces: Mugilidae) in Tamiahua Lagoon, Veracruz. Cienc. Mar. **22:** 329-345.

Jennings S, MJ Kaiser, JD Reynold, eds. 2001. Marine fisheries ecology. London: Blackwell Science Press, pp. 192-195.

Kuo CM. 1995. Manipulation of ovarian development and spawning in grey mullet, *Mugil cephalus* L. Isr. J. Aquacult. Bamidgeh **47:** 43-58.

Liu HC, WN Tzeng. 1972. Age and growth of white croaker, *Argyrosomus argentatus* (Houttuyn), in the East China Sea and the Taiwan Strait. J. Fish Soc. Taiwan. **1:** 20-30.

Lowenstein O. 1971. The labyrinth. *In* WS Hoar, DJ Randall, eds. Fish physiology. New York: Academic Press, pp. 207-240.

Nash EC, ZH Shehadeh. 1980. Review of breeding and propagation techniques for grey mullet, *Mugil cephalus* L. ICLARM Stud. Rev. **3:** 1-40.

Panfili J, H de Pontual, H Troadec, PJ Wright. 2002. Manual of fish sclerochronology. *In* J Panfili, H de Pontual, eds. Manual of fish sclerochronology. Brest, France: Ifremer-IRD coedition, pp. 19-27.

Pannella G. 1971. Fish otolith: daily growth layers and periodical patterns. Science **173:** 1124-1127.

Polat N, D Bostanic, S Yilmaz. 2005. Differences between whole otolith and broken-burnt otolith ages of red mullet (*Mullus barbatus ponticus* Essipov, 1927) sampled from the Black Sea (Samsun, Turkey). Turk. J. Vet. Anim. Sci. **29:** 429-433.

Smith KA, K Deguara. 2003. Formation and annual periodicity of opaque zones in sagittal otoliths of *Mugil cephalus* (Pisces: Mugilidae). Mar. Freshwater Res. **54:** 57-67.

Thomson JM. 1953. Status of the fishery of sea mullet (*Mugil cephalus* Linnaeus) in the eastern Australia. Aust. J. Mar. Freshw. Res. **2:** 41-81.

Tung IH. 1959. Age determination of the grey mullet (*Mugil cephalus* Linne). China. Fish. Monthly **180:** 2-10.