Age validation and growth of three commercially important hemiramphids in south-eastern Australia

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The method of using sectioned otoliths to estimate age in three species of garfishes (family Hemiramphidae) was validated by: (1) staining fishes with the vital stain alizarin complexone (ALC) and periodically examining their otolith growth, and (2) marginal increment analyses. Staining fishes with ALC indicated that opaque zones were formed during winter and spring, but did not become visible on the otolith edge until late spring and summer. *Hyporhamphus australis* were found to be similar to the hemiramphids of the Atlantic in having fast growth rates and a maximum observed age of 4+ years old. *Hyporhamphus regularis ardelio* and *Arrhamphus sclerolepis krefftii* were found to be more similar to the southern sea garfish, *Hyporhamphus melanochir*, in being moderately long-lived, with maximum observed ages of 7+ years old for both species. Females grew faster and attained greater fork lengths than males for each species. Sectioned otoliths showed large variation in the appearance of opaque zones between the three species studied, with those from the wide-ranging, oceanic *H. australis* appearing inconsistent and diffuse when compared to the estuarine *H. r. ardelio* and *A. s. krefftii*. This variation was also apparent from fishes kept in aquaria, suggesting that the appearance of opaque zones in otoliths of these species is largely influenced by physiology, rather than by environmental conditions.   © 2007 The Authors

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Key words: age; age validation; growth; Hemiramphidae; otolith.

INTRODUCTION

Fishes of the family Hemiramphidae are widely distributed throughout the world and are important as food and bait in many countries (www.fishbase.org). The largest fisheries occur in the western Atlantic (McBride & Styer, 2002), Southeast Asia (Sokolovsky & Sokolovskaya, 1999) and southern and eastern Australia (Kailola *et al.*, 1993). In Australia, there are 17 species from this family (colloquially known as garfishes) and they are exploited by commercial and recreational fisheries in all states (Collette, 1974). Only one species however, southern sea garfish *Hyporhamphus melanochir* (Valenciennes), has been studied in any detail. In eastern Australia, the main fisheries for

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hemiramphids occur in New South Wales (NSW) and target three species, eastern sea garfish *Hyporhamphus australis* Steindachner, eastern river garfish *Hyporhamphus regularis ardelio* (Whitley) and snub-nosed garfish *Arrhamphus sclerolepis krefftii* (Steindachner). *Hyporhamphus australis* and *H. r. ardelio* are endemic to eastern Australia and are the dominant garfishes species targeted by commercial and recreational fishers in these waters. *Arrhamphus s. krefftii* range from the northern half of NSW to southern Queensland (*c.* 23° S) (Collette, 1974). While each of these species are typical of hemiramphids in being small to medium-sized surface dwelling fishes, they each inhabit different waters. *Hyporhamphus australis* is predominantly a marine species, *H. r. ardelio* is generally confined to brackish estuarine systems and *A. s. krefftii* mainly inhabits fresh water (Collette, 1974). Each of these species is caught commercially using nets that fish the upper layers of water. *Hyporhamphus australis* are mainly caught using modified lampara nets that are deployed from small vessels in coastal waters (Stewart *et al.*, 2004) and *H. r. ardelio* and *A. s. krefftii* are mainly caught using gillnets in shallow estuarine waters. There is also a recreational hook and line fishery for these species. There are currently concerns for the stocks of *H. australis*, *H. r. ardelio* and *A. s. krefftii* due to declining catches and the apparent reliance of these fishes on estuarine systems, which are being increasingly impacted by urbanization. Unfortunately, despite more than a century of exploitation and recent concerns for the stocks, almost no research has been done on garfishes in south-eastern Australia.

Information on the age of fishes is fundamental to understanding their life histories. Estimates of size-at-age can be used to model growth, which can be used to predict how a stock will respond to exploitation and management changes. The technique of estimating fish age by counting annual marks within calcified structures (*i.e.* otoliths, scales, vertebrae and spines) has been well documented (Beamish & McFarlane, 1983; Beckman & Wilson, 1995). Of these structures, otoliths are the most frequently used (Secor *et al.*, 1995). Several previous studies on species of the Hemiramphidae have used scales (Berkeley & Houde, 1978; Sokolovsky & Sokolovskaya, 1999), whole otoliths (Ling, 1958), sectioned otoliths (Jordan *et al.*, 1998; Jones *et al.*, 2002; McBride & Thurman, 2003), and whole otoliths broken and burnt (Jones, 1990) to estimate age. Scales are easily shed when *H. australis* are handled and previous studies appear to have underestimated the age of garfishes using scales [Berkeley & Houde, 1978; *Hemiramphus brasiliensis* (L.) and *Hemiramphus balao* (Lesueur)]. Therefore, scales were not considered to be a useful structure for estimating age in the present study. The most recent and comprehensive studies to estimate the age of hemiramphids have used sectioned otoliths (Jones *et al.*, 2002; McBride & Thurman, 2003).

Prior to the present research there were no published studies that had estimated the age of any species of garfishes in eastern Australia. This is probably a result of: (1) the relatively low value of the commercial and recreational fisheries for garfishes in NSW, and (2) the difficulty in interpreting opaque zones in their otoliths. Only references to age estimation for four species from the genera *Hyporhamphus* and *Hemiramphus* [*Hyporhamphus sajori* (Temminck & Schlegel), *H. melanochir*, *H. brasiliensis* and *H. balao*] could be found despite these genera comprising a total of 33 species worldwide. These previously studied species

have been characterized by having relatively fast growth rates and being short to moderately long-lived. Maximum ages of *H. brasiliensis* and *H. balao* in Florida (U.S.A.), have been reported as 4 and 2 years, respectively (McBride & Thurman, 2003) and the maximum age of *H. sajori* in Japan as 2 years (Sokolovsky & Sokolovskaya, 1999). The southern sea garfishes (*H. melanochir*) has been reported up to 10 years of age in southern Australia (Jones, 1990; Jones *et al.*, 2002).

The objectives of this study were to: (1) develop a validated ageing method using sectioned otoliths for *H. australis*, *H. r. ardelio* and *A. s. krefftii*; (2) develop protocols for accurately converting counts of opaque zones into ages based on the month of capture and the otolith marginal increment, and (3) use the resulting size-at-age information to model and compare the growth of these species. The results are used to discuss the timing of formation and appearance of opaque zones in otoliths.

MATERIALS AND METHODS

Samples of *H. australis* were obtained each month at regional centres along the NSW coast, between Forster (32°09′ S; 152°29′ E) and Ulladulla (35°22′ S; 150°29′ E) between December 2001 and August 2003. Samples of *H. r. ardelio* were obtained from the three estuaries reporting the largest catches, Lake Illawarra (34°30′ S), Tuggerah Lakes (33°17′ S) and Wallis Lake (32°09′ S) between October 2001 and March 2004. *Arrhamphus s. krefftii* were sampled from the only major estuary in NSW where commercial fishers catch them, the Clarence River (29°26′ S) between November 2001 and February 2004. Samples of fishes were mostly obtained from commercial fishers, however fishes smaller than those generally landed were obtained on an *ad hoc* basis. Most of these smaller fishes were either by-catch from fishing for prawns (*Penaeus plebejus* Hess and *Metapenaeus macleayi* Haswell) or were caught using spotlights and dip-nets by the authors. The protocol was to collect at least 20 fishes each month from each location, however a reliance on the commercial fishery meant that this was not always possible. All fishes were measured as fork length (L_F, the distance from the upper jaw to the fork in the tail) and their sex determined from a macroscopic examination of the gonads (Hughes & Stewart, 2006).

AGE ESTIMATION

Sagittal otoliths were used to estimate age for the three species of garfishes. One of each pair of sagittal otoliths was embedded in resin and a transverse section (*c.* 0·25–0·30 mm thick) taken through the core (primordium) of the otolith, using a Buehler low speed saw with two spaced diamond blades. Sections were polished on both sides and mounted on a glass microscope slide under a cover slip. The slide was then viewed under a compound microscope using reflected light against a black background. Opaque zones were evident in sections of otoliths viewed this way and were counted along the ventral edge of the sulcus (Fig. 1).

VALIDATION OF AGEING METHOD

To examine otolith growth and the timing of opaque zone formation and appearance in the otoliths of *H. australis* and *H. r. ardelio*, a vital stain (alizarin complexone, ALC) was used to mark the otoliths of wild caught captive fishes.
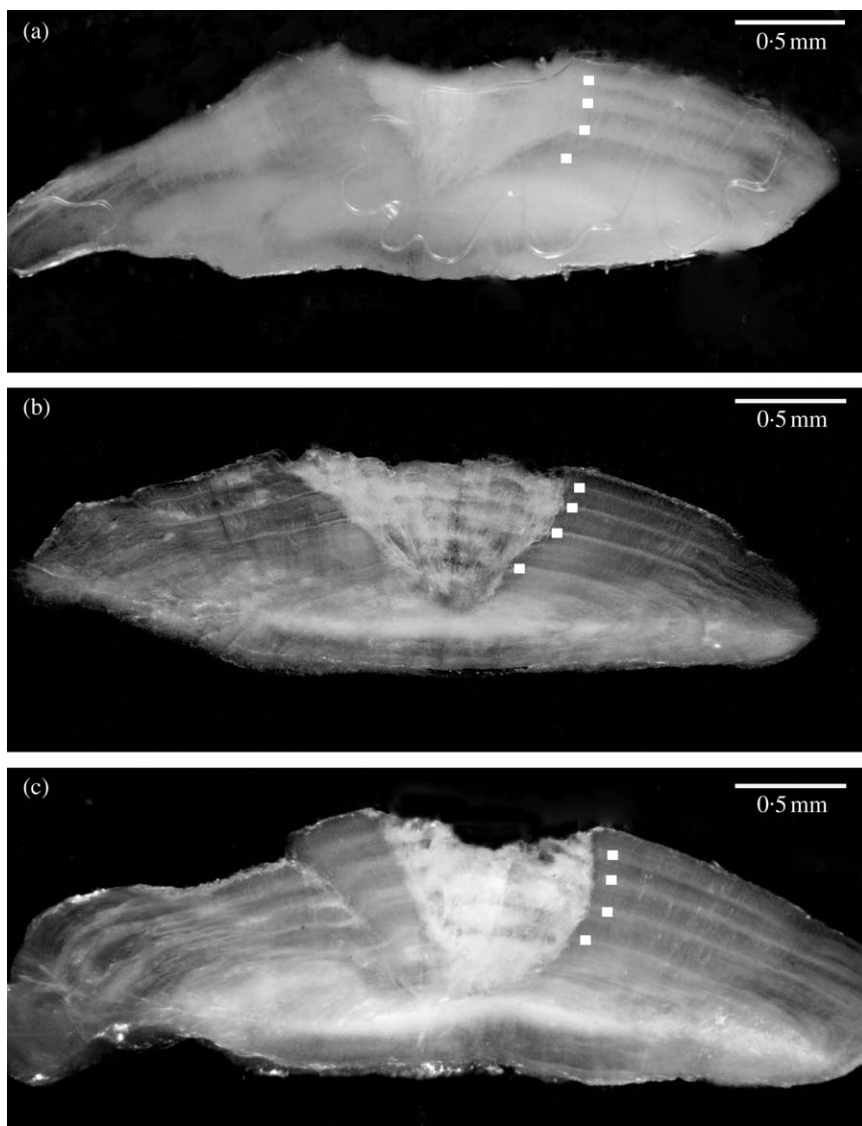
FIG. 1. Sections of (a) *Hyporhamphus australis*, (b) *Hyporhamphus regularis ardelio* and (c) *Arrhamphus sclerolepis krefftii* otoliths viewed under reflected light against a black background. □, Opaque zones on the ventral side of the sulcus. All were aged as being 4+ years old. The *H. australis* was 335 mm forklength (L_F), the *H. r. ardelio* was 239 mm L_F and the *A. s. krefftii* was 230 mm L_F.

Eighty-six *H. australis* were caught at night from the mouth of Port Hacking (34°04′ S) during January 2003, using a spotlight and dip-net operated from a research vessel. The fish were placed in an outdoor 4500 l tank, treated with formaldehyde (150 mg l^{-1} for 1 h) to remove parasites and given a mild antibiotic bath (oxytetracycline solution at 100 g per 1000 l) to reduce the risk of infection after capture. On 3 February 2003 these fish were immersed in an ALC bath at a concentration of 30 mg l^{-1} for 24 h. Surviving fish (38 died during this treatment) were kept in the 4500 l tank, with flow-through sea water at ambient temperature (15–23° C) and salinity, and were fed

twice each day on chopped pilchards *Sardinops sagax* (Jenyns) and vegetable fish-food flakes. Fish were sampled monthly to examine their otolith growth subsequent to the ALC mark.

The otoliths from all fish were examined after the experiment was terminated in April 2005. Otoliths were sectioned and viewed as described above. The ALC marks were visible under reflected light and the distances from the otolith core to the ALC mark, to any identified opaque zones, and to the otolith edge were measured. Measurements were made along the ventral edge of the sulcus using a microscope mounted video camera interfaced with a computer running 'Image Pro Plus' image analysis software. The timing of formation of opaque zones was estimated using the assumption that the growth rate of the otolith after formation of the first opaque zone was constant (Cappo *et al.*, 2000).

One hundred and twenty *H. r. ardelio* were captured at night from the upper reaches of Port Hacking during December 2002 and January 2003, using a spotlight and dip-net operated from a research vessel. These fish were treated in a similar manner to *H. australis* but were placed in a separate 4500 l tank. On 23 January 2003, these fish were given a 24 h bath in ALC at a concentration of 30 mg l^{-1} to stain their otoliths. Surviving fish (three died during the immersion) were kept in the 4500 l tank and were sampled monthly to examine their otolith growth subsequent to the ALC mark. The otoliths of these fish were analysed in the same manner as for *H. australis*.

Marginal increment analyses from wild caught fishes were used to examine the periodicity of opaque zone appearance in the otoliths of each species of garfishes. Measurements were made from the core of the sectioned otolith to each successive opaque zone and to the otolith edge along the ventral edge of the sulcus, using the equipment described above. The marginal increment was defined as follows: for fishes with no opaque zones as the distance (in mm) from the core to the otolith edge; for fishes with one opaque zone as the distance from the first opaque zone to the otolith edge as a proportion of the first complete increment, and for fishes with ≥2 opaque zones as the distance from the most recently completed opaque zone to the otolith edge as a proportion of the last completed increment. These measurements were made for all garfishes collected for age determination. The sizes of these marginal increments through time were used to describe the periodicity of opaque zone formation and also in converting counts of opaque zones into age classes.

The timing of the first increment formation was assessed using the staining experiments described above (where young-of-the-year, YOY, fishes were used) and by periodically sampling very small fishes from the wild that were judged to be YOY. These otoliths were sectioned as described above and their sizes and internal structure used to support the interpretations of what constituted the first annual zone.

PRECISION

Two hundred otoliths from each species were re-read at least 3 weeks after their first read to examine the precision of the estimates of counts of opaque zones. The coefficient of variation (CV) for the two readings for each otolith was calculated and an average across all otoliths for each species obtained after the method described in Kimura & Lyons (1991) and Campana (2001).

DEVELOPMENT OF AN AGEING PROTOCOL

Ages were assigned for each species based on a birth date (set at the middle of the spawning season; Hughes & Stewart, 2006), the number of opaque zones counted, the state of the otolith edge (either wide, medium or narrow as defined in the results) and the month of capture. The classification of edge width was based on the distribution of marginal increment data and was designed to separate those fishes that had newly counted opaque zones in any month from those that did not.

GROWTH RATE DETERMINATION

The von Bertalanffy growth function was fitted to the size-at-age data for the three species: $L_t = L_\infty[1 - e^{-k(t-t_0)}]$, where L_t is the L_F at age t, L_∞ is the asymptotic L_F, k is the rate at which the curve approaches the L_∞ and t_0 is the hypothetical age of the fish at zero length. The von Bertalanffy growth curves for each sex were compared, across equal age ranges, using the analysis of residual sums of squares (ARSS) method (Chen *et al.*, 1992). The mean sizes of males and females in different age classes were compared using *t*-tests.

RESULTS

VALIDATION OF AGEING METHOD

Experimental fishes were not measured (to minimize handling), however, 46 *H. australis* died within 2 days of being treated with ALC. These fish appeared to be representative of the sizes of the survivors and ranged between 11 and 24 cm L_F. Of the fish that died, those with otoliths containing one opaque zone were, on average, 20·5 cm L_F.

An ALC mark was present as a thin red band in all but one *H. australis* (Fig. 2). Four *H. australis* had no opaque zones when stained and 35 had one complete opaque zone (Fig. 2). Opaque zones subsequent to the ALC mark were first identified in January 2004. In fish that already had one opaque zone when stained, the mean $\pm$ s.e. distance from the opaque zone to the ALC mark was $0·15 \pm 0·01$ mm. Measurements from the ALC mark to the otolith edge showed that otoliths grew, on average, 0·68 μm day^{-1}. The position of the ALC mark relative to the centre of opaque zones indicated that for *H. australis* the first opaque zones were formed, on average, during the previous winter (June), the second opaque zones were formed during the following spring (November) and the third opaque zones were formed during the winter (July) after. Fish sampled between January and July 2004 averaged 28·5 cm L_F and those sampled in April 2005 averaged 32·5 cm L_F. The largest fish was sampled in April 2005 and measured 34·4 cm L_F.

The monthly marginal increments for *H. australis* with one opaque zone showed considerable individual variation, but with a repeated seasonal pattern of lower values in December (mean $\pm$ s.e. $0·112 \pm 0·010$ and $0·115 \pm 0·006$ in 2001 and 2002, respectively) steadily increasing to around June before decreasing again (Fig. 3). Marginal increments are at a minimum when opaque zones are recently completed, and this pattern indicates that opaque zones were completely formed by summer. The marginal increments of these recently completed first opaque zones were <0·3.

There were only 32 *H. australis* that had two or more opaque zones in their otoliths and this number of fish was insufficient to show any seasonal pattern in marginal increment. The otoliths from six very small (<10 cm L_F and <3·4 g) *H. australis* sampled in December 2001, assumed to be from the most recent spawning event (July to December; Hughes & Stewart, 2006), were entirely opaque. Slightly larger (12–18 cm L_F and 6–25 g) fish sampled in March showed 14% with totally opaque otoliths (one of seven fish) and 100% of similar-sized (14–18 cm L_F) fish sampled in April had translucent edges (eight fish).
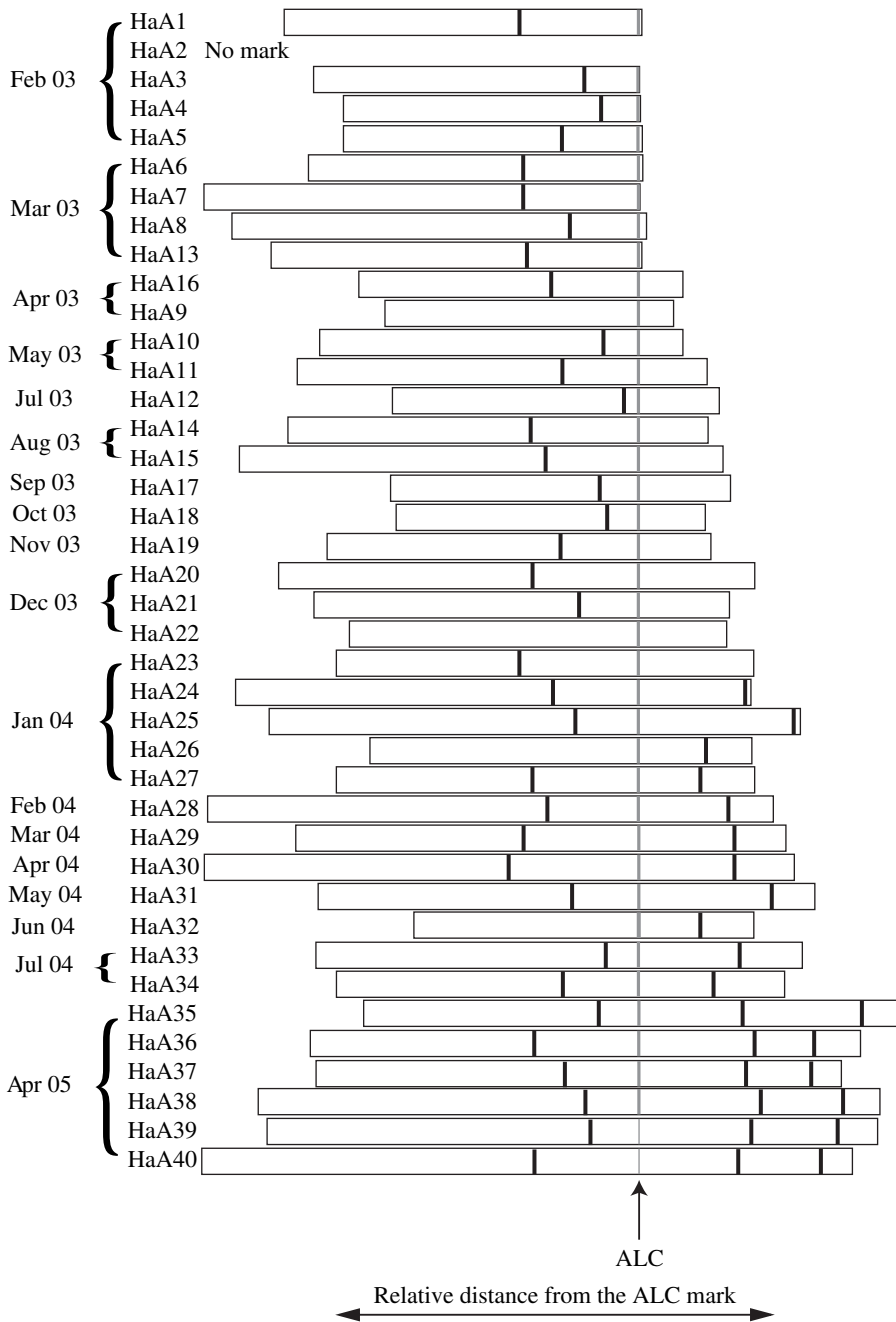
FIG. 2. Relative distances from the otolith core to the alizarin complexone mark (ALC) and opaque zones (▌) taken along the ventral edge of the sulcus in sectioned otoliths of *Hyporhamphus australis*. Marking with alizarin complexone occurred in February 2003. The dates indicate when fish were removed from the experiment and sacrificed.
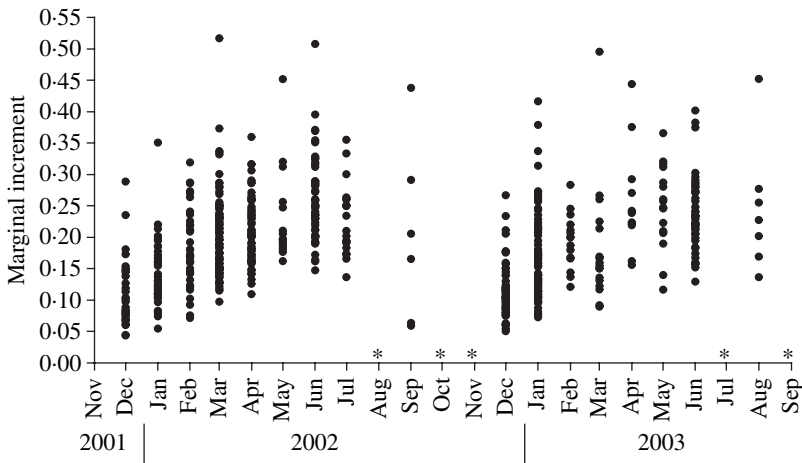
FIG. 3. Marginal increment plot for *Hyporhamphus australis* with one opaque zone. *, No fish were sampled in these months.

Sixty-one *H. r. ardelio* treated with ALC died within the first month. These fish were generally representative of the sizes of the survivors and were between 5 and 16 cm L_F (mean $\pm$ s.e. $= 7 \pm 2$ cm).

A total of 80 *H. r. ardelio* otoliths were examined and of these, 79 had ALC marks in their otoliths. One fish had one opaque zone when stained, the other 78 had no opaque zones at the start of the experiment (Fig. 4). Opaque zones were identified on the edge of otoliths of some fish in January 2004 and in all fish sampled by April 2004. The mean $\pm$ s.e. distance from the ALC mark to the subsequent opaque zone was 0.14 ± 0.01 mm. Measurements from the ALC mark to the otolith edge showed that otoliths grew, on average, 0.56 μm day^{-1}. The position of the ALC mark relative to the centre of opaque zones indicated that for *H. r. ardelio*, the first opaque zones were formed, on average, during early spring (September).

The monthly marginal increments for *H. r. ardelio* showed considerable variation but there was a repeated seasonal pattern of lower values in summer (November to January) (Fig. 5). November to March were the months where some fish had identifiable opaque zones near their otolith edges and others did not. Marginal increments for recently completed opaque zones in these months were <0.6 and <0.4 in fish aged 1+ and ≥2+ years old, respectively.

The mean $\pm$ s.e. distance from the core to the middle of the first opaque zone for all *H. r. ardelio* with one or more opaque zones was 0.517 ± 0.002 mm. Fifteen small fish (<110 mm L_F and <6.8 g), assumed to be from the previous year's spawning (Hughes & Stewart, 2006), were sampled between March and July 2003. All of these fish had otoliths with translucent otolith edges and distances from the core to edge of 0.393 ± 0.019 mm.

The monthly marginal increments for *A. s. krefftii* showed considerable variation but with a repeated seasonal pattern of lower values in spring and summer (October to January) (Fig. 6). October and November were the months when some fish had identifiable opaque zones near their otolith edges and
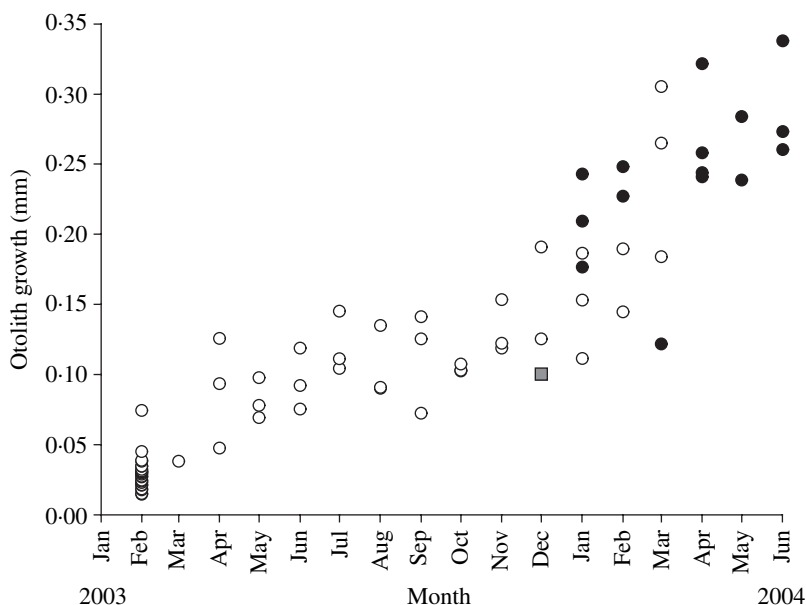
FIG. 4. Otolith growth by month for *Hyporhamphus regularis ardelio* stained with alizarin complexone in January 2003 and kept in captivity: ○, fish that had no opaque zones when stained and had not formed an opaque zone when sampled; ●, fish that had no opaque zones when stained that had an identified opaque zone after the alizarin complexone mark when sampled; ■, the single fish that had one opaque zone when stained and it had not formed an additional opaque zone when sampled.

others did not. Marginal increments for recently completed opaque zones in these months were <0·2 and <0·4 in fish aged 1+ and ≥2+ years old, respectively. Many months were not sampled, however, the pattern is consistent with annual periodicity of opaque zone formation.

PRECISION

Ages were assigned to all *H. australis* otoliths examined and comparative readings showed total agreement for 84·5%, agreement to ±1 year for 15% and agreement to ±2 years for 0·5%. The CV, averaged across all ages, was 0·190. Precision in assigning counts of opaque zones to *H. r. ardelio* otoliths was high, with ages being assigned to all otoliths examined. Comparative readings showed total agreement for 94% and agreement to ±1 year for the remaining 6%. The CV, averaged across all ages, was 0·032. Ages were assigned to all *A. s. krefftii* otoliths examined and comparative readings showed total agreement for 79% and agreement to ±1 year for the remaining 21%. The CV, averaged across all ages, was 0·088.

DEVELOPMENT OF AGEING PROTOCOLS

The classification of edge width was based on the distributions of marginal increment measurements (Figs 3, 5 and 6) and varied between species (Table I).
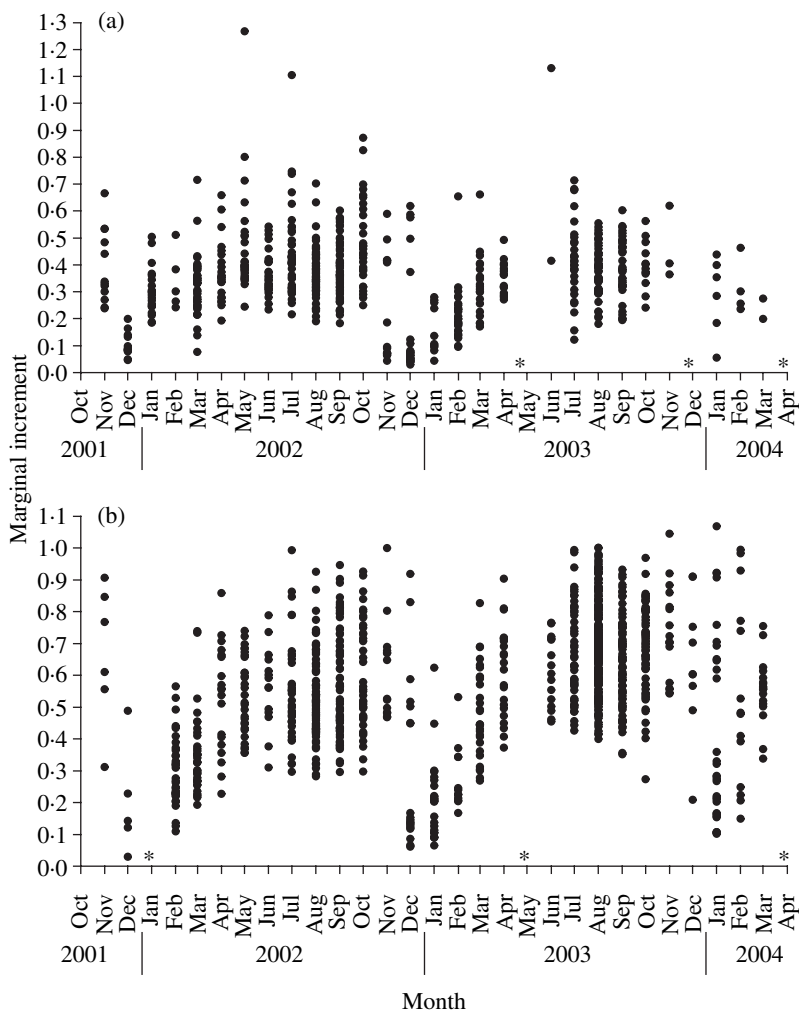
FIG. 5. Marginal increment plots for *Hyporhamphus regularis ardelio* with (a) one opaque zone and (b) two or more opaque zones. *, No fish were sampled in these months.

The models used to convert counts of opaque zones into age classes are provided in Fig. 7.

Based on the above determinations the following algorithm was applied to convert counts of opaque zones into ages: $A_D = 365N^* + D_c$, where A_D is the age in days, N^* is the number of opaque zones modified by edge width and capture month and D_c is the days from nominated birth date to capture.

GROWTH RATES

Male and female *H. australis* grew at a similar rate until age 1+ years, after which male growth was slower than female growth. There was no significant difference in the mean size of female or male *H. australis* aged 0+ years (*t*-test,
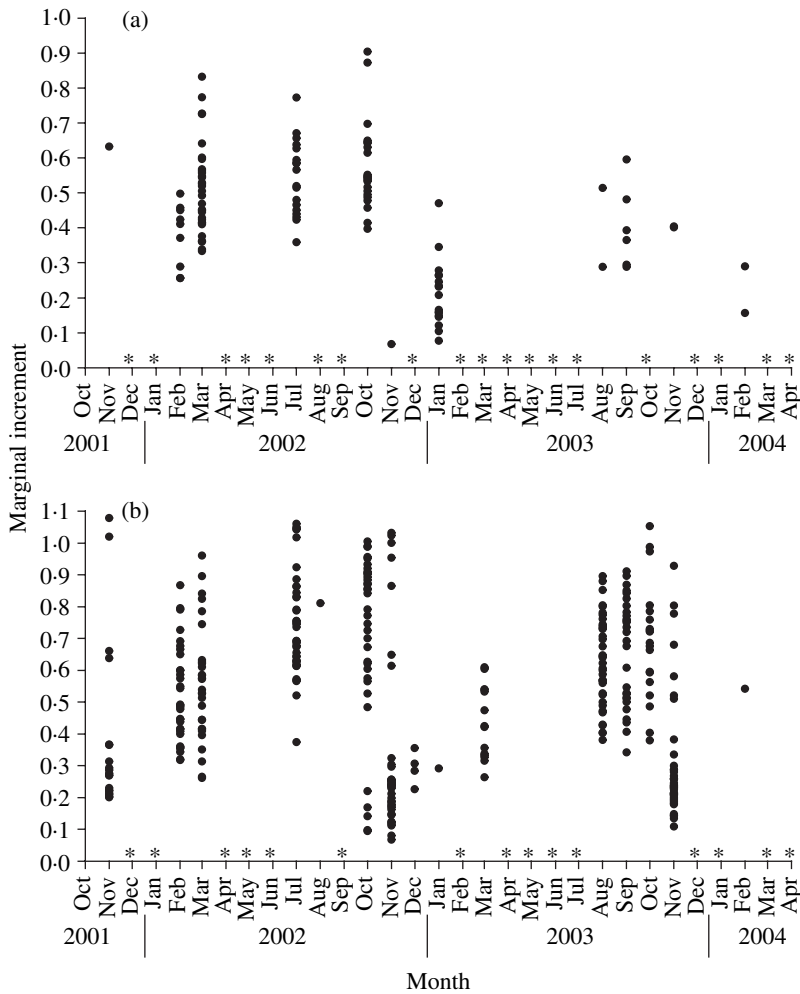
FIG. 6. Marginal increment plots for *Arrhamphus sclerolepis krefftii* with (a) one opaque zone and (b) two or more opaque zones. *, No fish were sampled in these months.

d.f. = 319, P = 0·4), but significant differences between those aged 1+ years (*t*-test, d.f. = 634, P < 0·001) and 2+ years (*t*-test, d.f. = 48, P < 0·001). Males and females had significantly different von Bertalanffy growth curves (ARSS, $F_{3,1016}$, P < 0·001) and the data were therefore kept separate [Fig. 8(a)]. Three fish were estimated to be >3 years old and one to be >4 years. These 3 and 4 year-old fish were all females.

Female *H. r. ardelio* were, on average, larger than males for all age classes 1+ to 7+ years (*t*-tests, d.f. = 581, 488, 274, 125, 77, 25, P < 0·001 for the 1+ to 6+ year age classes and d.f. = 16, P < 0·05 for the 7+ year age class). There was no difference in the mean size of females and males aged <1 year (*t*-test, d.f. = 35, P > 0·05). ARSS indicated significant differences in growth curves between males and females ($F_{3,853}$, P < 0·001), with females growing faster

TABLE I. Classification of otolith edge widths for *Hyporhamphus australis*, *Hyporhamphus regularis ardelio* and *Arrhamphus sclerolepis krefftii*

Number of opaque zones	Edge status								
	H. australis			*H. r. ardelio*			*A. s. krefftii*		
	Wide	Medium	Narrow	Wide	Medium	Narrow	Wide	Medium	Narrow
	MI	MI	MI	MI	MI	MI	MI	MI	MI
0	≥0·6 mm	<0·6 mm	N/A	≥0·45 mm	<0·45 mm	N/A	≥0·45 mm	<0·45 mm	N/A
1	≥0·3	0·1–0·3	≤0·1	≥0·6	0·2–0·6	≤0·2	≥0·6	0·2–0·6	≤0·2
>1	≥0·7	0·1–0·7	≤0·1	≥0·4	0·2–0·4	≤0·2	≥0·65	0·4–0·65	≤0·4

MI, is marginal increment; N/A, not applicable.

and attaining larger sizes than males [Fig. 8(b)]. Both females and males had estimated maximum ages of 7+ years.

Female *A. s. krefftii* were, on average, larger than males for all age classes 1+ to 6+ years (t-tests, d.f. = 134, 168, 97, 29, 15, 3, $P < 0.001$ in each case). There was no difference in the mean size of females and males aged <1 year (t-test, d.f. = 5, $P > 0.05$). ARSS indicated significant differences in growth between sexes ($F_{3,460}$, $P < 0.001$), with females growing faster than males and attaining larger sizes [Fig. 8(c)]. Females were estimated up to 7+ years old and males up to 6+ years old.

DISCUSSION

Marginal increment analyses showed repeated seasonal patterns for each species, consistent with the annual formation of opaque zones in their otoliths. These patterns, in combination with staining fishes with ALC, provide an insight into the timing of formation and appearance of opaque zones in the otoliths of these species. Assuming growth rate of the otolith after formation of the first opaque zone to be constant (Cappo *et al.*, 2000), then the present results indicated that for *H. australis* the first opaque zones were formed during the previous winter (June), the second opaque zones were formed during the following spring (November) and the third opaque zones were formed during the winter (July) after. Similarly, for *H. r. ardelio*, the middle of the first opaque zones was formed during early spring (September). Results from the marginal increment data, however, showed that opaque zones were not generally identifiable until late spring and summer. These observations support the model of Francis *et al.* (1992) that opaque zones in otoliths are not identifiable as being fully formed until sufficient material has been deposited outside of them. This model may also explain some of the variation in timing of appearance of opaque zones, with the fastest growing individuals having opaque zones identified earlier in the year (Stewart *et al.*, 1999). This has important implications when converting counts of opaque zones into age classes as the fastest growing individuals in any age class may be assigned to the age class above, effectively decreasing the estimated mean size-at-age and underestimating growth. The protocols developed for converting counts of opaque zones to age classes
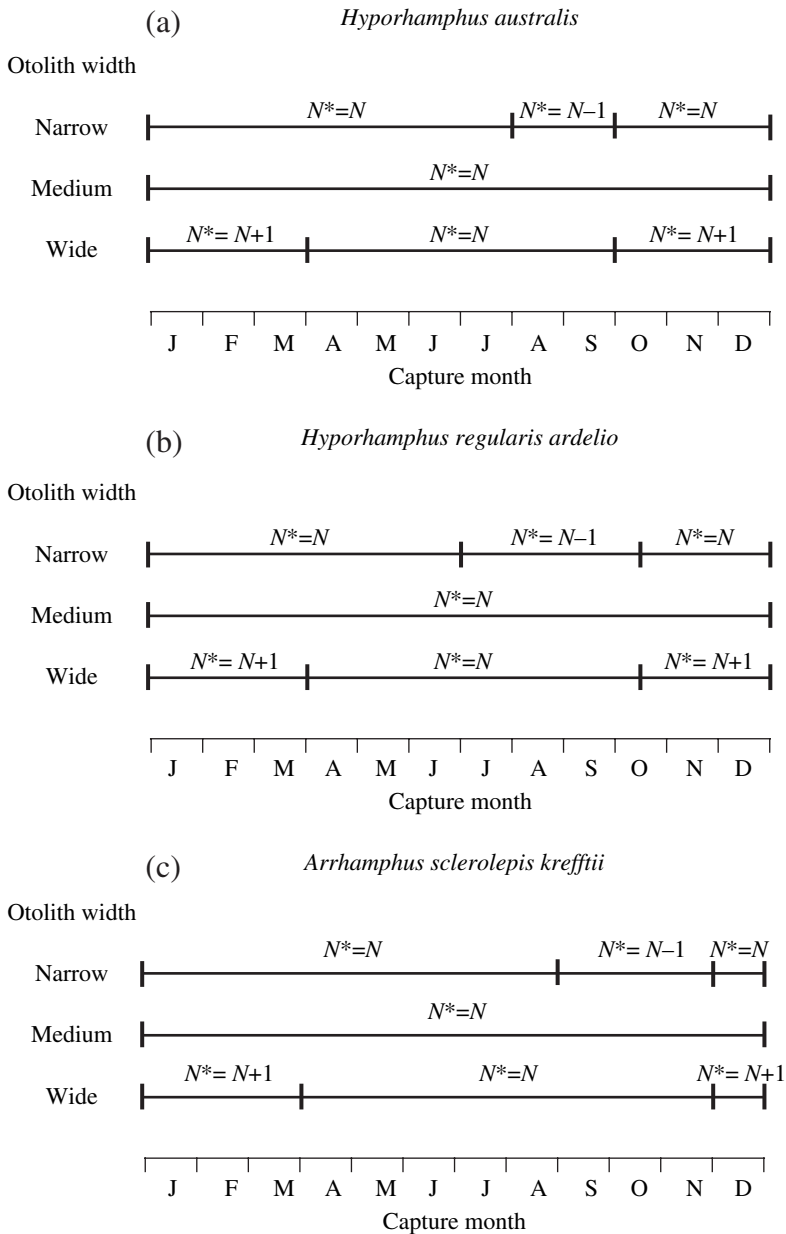
(a) *Hyporhamphus australis*

Otolith width

FIG. 7. Models used to determine the age class ($N*$) of (a) *Hyporhamphus australis*, (b) *Hyporhamphus regularis ardelio* and (c) *Arrhamphus sclerolepis krefftii* based on the number of opaque zones (N), month of capture, width of the otolith edge and assigned birth date.

based on the marginal increment, number of opaque zones and month of capture were designed to minimize errors caused by such variation.

Information on the timing of spawning (Hughes & Stewart, 2006), the validation studies above, and observation of the otoliths from very young *H. australis*
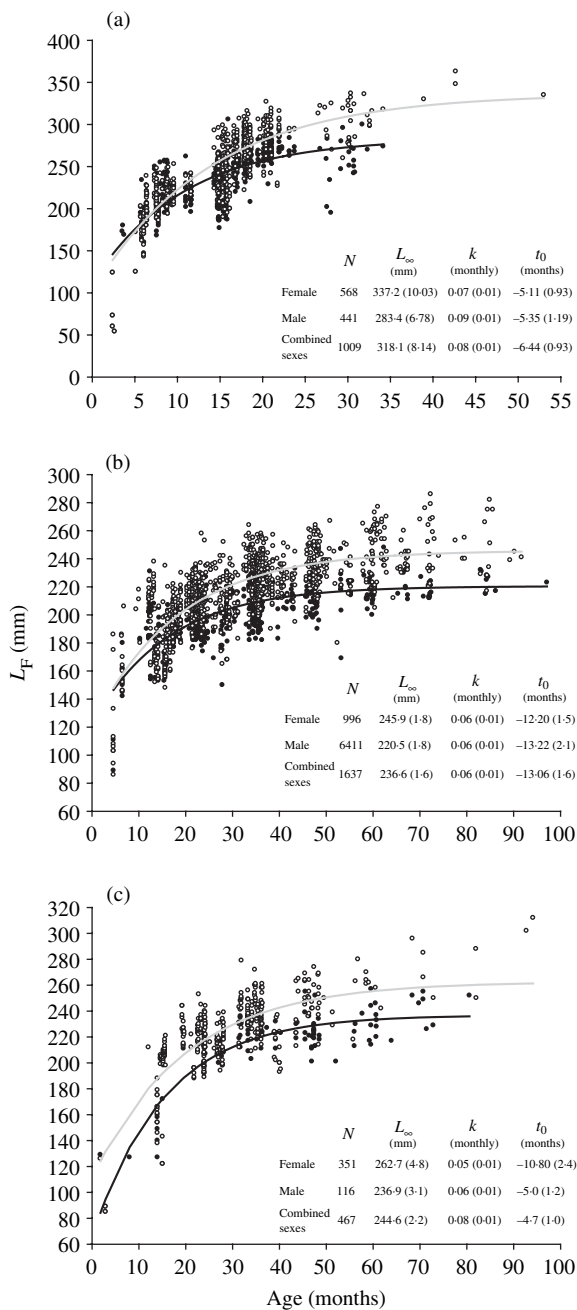
FIG. 8. Size (fork length)-at-age data for male (●) and female (○) (a) *Hyporhamphus australis*, (b) *Hyporhamphus regularis ardelio* and (c) *Arrhamphus sclerolepis krefftii* with fitted von Bertalanffy growth curves. The coefficients with s.e. in parentheses are given (L_∞, asymptotic length; k, the rate at which the curve approaches L_∞; t_0, age at L_F zero).

and *H. r. ardelio* have allowed the development of a model to describe early oto-lith growth in these species. *Hyporhamphus australis* and *H. r. ardelio* hatch in late winter and spring and have entirely opaque otoliths. Translucent material is deposited outside of this opaque core area during the following summer and autumn and the first annulus is formed during the following winter. This first annulus does not become visible on the otolith edge until spring and sum-mer when the fish is aged between 12 and 18 months old. The distance of the first annulus from the core was variable in both species (*e.g.* 0·31–0·81 mm for *H. australis*) and may reflect the relative time during the spawning season that the fish was hatched (*i.e.* fish with wide first increments were hatched early, July, while fish with narrow first increments were hatched late, December). A similar observation has been made for *H. melanochir* in southern Australia (Jones *et al.*, 2002). Unfortunately, the very small measurement distances involved and the errors associated with sectioning (*i.e.* sectioning at slightly oblique angles or not through the otolith core) meant that it was not possible to further refine estimates of age based on the distance from the core to the first annulus.

Growth rates of *H. australis*, *H. r. ardelio* and *A. s. krefftii* were typical of the Hemiramphidae. The mean size-at-age estimate for *H. australis* after 1 year of *c.* 230 mm L_F for *H. australis* was larger than that for *H. r. ardelio* (*c.* 178 mm L_F), *A. s. krefftii* (*c.* 175 mm L_F) and for the closely related *H. melanochir* (*c.* 160–180 mm L_F; Jones *et al.*, 2002). McBride & Thurman (2003) reported the average size of both *H. brasiliensis* and *H. balao* aged 1+ years to be between 225 and 250 mm L_F, while the size estimates for all *H. australis* aged 1+ years old were between 252 and 262 mm L_F. Females of each species grew faster and reached larger sizes than males, although there were no differences in the mean sizes of females and males <1 year old (their age at sexual matu-rity; Hughes & Stewart, 2006) in any species. Jones *et al.* (2002) also reported differences in growth between female and male *H. melanochir*, with females growing faster and tending to reach larger sizes than males, however the differ-ences in mean size-at-age found by these authors do not appear to be as large as for *H. australis*. Berkeley & Houde (1978) and McBride & Thurman (2003) also reported that female *H. brasiliensis* and *H. balao* from waters off Florida (U.S.A.) attained a larger average size-at-age than males.

The maximum sizes observed during this study (363 mm L_F for *H. australis*, 286 mm L_F for *H. r. ardelio* and 312 mm L_F for *A. s. krefftii*) were close to the maximum reported for these species (398, 280 and 277 mm standard length, L_S, respectively; Collette, 1974). The measurement of L_F is slightly larger than that of L_S, however, the 312 mm L_F *A. s. krefftii* sampled in this study was still larger than any previously reported. Given that fishes were sampled close to the max-imum sizes recorded, the maximum ages estimated are likely to be close to maximum ages possible for these species. The maximum age of 4+ years for *H. australis* is considerably younger than that estimated for the closely related and morphologically similar *H. melanochir* of 10 years in southern Australia (Jones, 1990; Jordan *et al.*, 1998). Recent work on similar species in Florida (U.S.A.) reported maximum ages of 4 years for *H. brasiliensis* and 2 years for *H. balao* (McBride & Thurman, 2003). It appears as though *H. australis* are more similar in growth rate and maximum age to the hemiramphids of the Atlantic than to *H. r. ardelio*, *A. s. krefftii* and *H. melanochir*. This may

be due to differences in habitat and distribution. *Hyporhamphus australis* are more similar to the hemiramphids reported by McBride & Thurman (2003) in being largely oceanic, whereas *H. r. ardelio*, *A. s. krefftii* and *H. melanochir* are more estuarine and inshore species (Ling, 1958; Jones, 1990; Jones *et al.*, 1996).

There was large variation in the internal structure of sectioned otoliths between each of the three species studied here, and also with that reported for other hemiramphids. Sectioned otoliths of *H. r. ardelio* and *A. s. krefftii* were similar in appearance to each other and displayed obvious alternating translucent and opaque zones (Fig. 1). The opaque zones were thinner and more definite than those reported for *H. melanochir* (Jones *et al.*, 2002), *Hemiramphus* spp. (McBride & Thurman, 2003) and also *H. australis* in the present study. Conversely, sectioned otoliths of *H. australis* were very difficult to interpret, with patterns of alternating translucent and opaque zones being unclear and inconsistent (Fig. 1). Opaque zones in *H. australis* are wider than the intervening translucent zones and are quite diffuse. It was only after examining *H. australis* that were stained with ALC and kept in aquaria that it became possible to confidently interpret their otoliths. The inconsistent and confusing appearance of *H. australis* otoliths meant that the precision of re-reading them was relatively low, with an average CV of 0·19, which is at the higher end of the generally reported range (Campana, 2001). There was total agreement for 169 of 200 otoliths that were re-read (84·5%), however, precision was considered poor because of the small number of age classes observed. Measurements from the core to each successive opaque zone revealed that discrepancies between first and second readings were always due to identification of the first annulus. Jones *et al.* (2002) reported average CV of <0·03 for *H. melanochir*, which may reflect the differences in appearance of the opaque zones between *H. australis* and *H. melanochir*. Sectioned otoliths of *H. r. ardelio* and *A. s. krefftii* were more easily interpreted and this was reflected by the high degree of precision re-reading them (94% agreement with an average CV of 0·032 for *H. r. ardelio*, and 79% agreement with an average CV of 0·088 for *A. s. krefftii*). These values are well within the range of CV reported for many other species (Campana, 2001).

The process of otolith formation and factors affecting variation in their internal structure are not fully understood (Wright *et al.*, 2002), however, it is thought that opaque zones may form as a result of a combination of physiological (Fowler, 1995), exogenous (Fowler & Doherty, 1992) and endogenous (Romanek & Gauldie, 1996) factors. The thin but distinct opaque zones in the otoliths of *H. r. ardelio* and *A. s. krefftii* are probably due to these species being restricted to their estuaries and experiencing lower water temperatures during winter. These fishes also spawn in winter and spring (Hughes & Stewart, 2006) and available energy during this time is probably used for reproduction rather than for growth. *Hyporhamphus australis*, however, are a wide-ranging oceanic species that associate with temperate waters of a relatively consistent temperature, and previous studies have reported that the otoliths of fish that live in areas where seasonal influences are reduced often display less distinct annuli (Fowler & Doherty, 1992; Fowler, 1995; Smith & Deguara, 2003). *Hyporhamphus australis* stained with ALC in the present study were kept in tanks with flow-through water of ambient temperature that varied from *c.* 23° C in summer to *c.* 15° C during winter, yet their otoliths displayed indistinct zones

similar to those from wild fish. Otoliths from fish with fast specific growth rates often have complex structures, thought to result from their opportunistic feeding and large variations in growth rates within and between seasons (Morales-Nin & Panfili, 2005), and the inconsistent patterns observed in the otoliths of *H. australis* may largely be a result of their fast and variable growth rate.

Counts of opaque zones from sectioned otoliths of *H. australis*, *H. r. ardelio* and *A. s. krefftii* can be used to determine age and to model growth. All three species are typical of the Hemiramphidae in having fast growth rates. Otoliths of *H. r. ardelio* and *A. s. krefftii* showed discrete annuli that were easily counted and these two species appear to be more similar to the southern *H. melanochir* in being moderately long-lived. Otoliths of *H. australis* displayed more diffuse annuli that were difficult to interpret, and this species appears to be more similar to the oceanic hemiramphids of the Atlantic in having an observed maximum age of 4 years. Future work using daily increment counts would be useful in understanding seasonal and interannual variations in growth rates for these species. Daily age estimates may also improve estimates of early growth that have been limited to date because of the prolonged (*c*. 6 months) spawning periods for these species.

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