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Preliminary Study on Growth of Juvenile Orange-Spotted Grouper *Epinephelus coioides* Collected Off Northwest Peninsular, Malaysia

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Abstract: Growth of juvenile orange-spotted grouper *Epinephelus coioides* from a coastal reef and mangrove estuary in Northwest Peninsular Malaysia were investigated using size (TL) and daily age information obtained from otolith (lapillus) daily increment analysis. Increments in the lapillus of known-age hatchery-produced fish were deposited daily (following a 12 day delay in deposition onset) after hatching. The juveniles, collected from the coastal reef at Langkawi Island (25.9-48.0 mm TL) and the Merbok mangrove estuary (90.0-232.0 mm TL), Malaysia, were estimated to be 31-53 and 85-217 days old, respectively. Back-calculation of hatching dates showed that *E. coioides* spawned throughout most of the year in the region, probably due to the year-round constant water temperatures (> 27°C). Age and size information also indicated that the juveniles entered the mangrove estuary from the coastal area at ca. 90 mm TL (> 85 days old), growing to over 200 mm TL over a more than 4 month (maximum) period in the estuary, and disappeared by ca. 230 mm TL (maximum age 217 days old). Growth rates (mm per day) of the juveniles from the mangrove estuary hatched in each quarter were 0.84 mm in January-March, 0.90 mm in April-June, 0.77 mm in July-September and 0.90 mm in October-December, not significantly different each other, and the growth rate of aggregate juveniles from the mangrove estuary being 0.91 mm. The growth pattern of *E. coioides* during some 7 months after hatching (from hatching to disappearance from the mangrove estuary), estimated from the age-length relationship in both hatchery-produced and wild fish (including juveniles from the Langkawi coastal area and Merbok mangrove estuary), closely fitted the Gompertz growth formula (L = 214.26.exp[-3.89.exp(-0.016T)] (r = 0.97)). It was estimated that *E. coioides* grew to ca. 90-100 mm TL by ca. 90 days old, the growth thereafter becoming slower.

Keywords: *Epinephelus coioides*, juveniles, growth, mangrove estuary, otolith, Malaysia

Abstrak: Tumbesaran anak kerapu orange-spotted *(Epinephelus coioides)* dari terumbu karang dan kawasan paya bakau di bahagian timur-laut Semenanjung Malaysia telah dikaji menggunakan saiz (TL) dan maklumat umur harian yang diperolehi dari analisa peningkatan otolith (lapillus) harian. Peningkatan dalam lapillus ikan hatceri yang diketahui umurnya disimpan setiap hari (selepas lewat 1-2 hari dalam onset deposition) selepas menetas. Anak-anak yang ikan dikutip dari kawasan terumbu pantai di Pulau Langkawi (25.9-48.0 mm TL) dan muara Sg. Merbok (90.0-232.0 mm TL), Malaysia masing-masing dianggarkan berumur 31-53 and 85-217 hari. Kiraan semula tarik penetasan menunjukkan *E. coioides* membiak di sepanjang tahun di kawasan ini, berkemungkinan disebabkan oleh suhu air yang konstan sepanjang tahun (> 27°C). Umur dan saiz juga menunjukkan anak-anak ikan memasuki muara paya bakau dari kawasan pantai pada anggaran 90 mm TL (berumur > 85 hari) dan meningkat sehingga lebih 200 mm TL dalam masa lebih dari 4 bulan (maksimum) di kawasan muara dan lenyap apabila mencapai 230 mm TL (maksimum umur 217 hari). Kadar tumbesaran (mm per hari) anak-anak ikan dari kawasan muara paya bakau yang menetas pada setiap suku adalah 0.84 mm pada Januari-Mac, 0.90 mm pada April-Jun, 0.77 mm pada Julai-September dan 0.90 mm pada Oktober-Disember, dengan tiada perbezaan yang ketara setiap satu. Kadar tumbesaran anak-anak agregat dari muara paya bakau pulau adalah 0.91 mm. Corak tumbesaran *E. coioides* selepas 7 bulan menetas (dari mula menetas sehingga hilang dari kawasan paya bakau), dianggarkan dari perhubungan umur-panjang dalam kedua-dua produk hatchery dan ikan liar (termasuk anak ikan dari pantai Langkawi dan muara Sg. Merbok), sangat padan dengan formula tumbesaran Gompertz (L = 214.26.exp[-3.89.exp(-0.016T)] (r = 0.97)). Dijangkakan *E. coioides* membesar sehingga lebih kurang 90-100 mm TL dalam masa lebih kurang 90 hari dengan pertumbuhan yang lebih perlahan selepas itu.

Introduction

Groupers, particularly *Epinephelus* species, are exposed to considerable fishing pressure worldwide in temperate and tropical areas due to their high marketable value. Consequently, their stock levels are thought to have declined drastically in recent years, as reported for *E. guttatus* (Sadovy *et al.*, 1992), *E. coioides* (Grandcourt *et al.*, 2005), *E. akaara* (Yoseda *et al.*, 2006) and *E. itajara* (Lala *et al.*, 2009). This situation has led to recent progress in commercial aquaculture, seed production (Doi *et al.*, 1997; Kohno *et al.*, 1997; Ahmed *et al.*, 2000; Teruya *et al.*, 2008) currently being practiced in several countries, including Japan (Tucker, 1999), Taiwan (Liao *et al.*, 2001) and the Philippines (Marte, 2003), and stock assessment/management conducted for a number of species of *Epinephelus* (Bullock *et al.*, 1992; Sadovy *et al.*, 1992; Grandcourt *et al.*, 2005).

The orange-spotted grouper *Epinephelus coioides* commonly occurs in subtropical and tropical coastal areas in the Indo-West Pacific, from the Red Sea to South Africa, eastward to Palau and Fiji, north to the Ryukyu Islands and south to the Arafura Sea and Australia (Russell and Houston, 1989). Records from the Mediterranean coast of Israel (Heemstra and Randall, 1993) have been attributed to the movement of fish from the Red Sea through the Suez Canal (Randall, 1995). The species grows to over 100 cm in total length (Smith, 1986) and is an important fishery target in a number of countries, including Malaysia. Its tendency to form spawning aggregations is considered to predispose *E. coioides* to over-exploitation (Domeier and Colin, 1997).

Epinephelus coioides juveniles are known to utilize brackish water mangrove estuaries as nursery grounds (Sheaves, 1995; Mumby *et al.*, 2004; Matsuura, 2007), as do their congeners, *E. striatus* (Eggleston, 1995) and *E. goliath* (Frias-Torres 2006; Lara *et al.*, 2009). Juveniles subsequently return to coastal areas for further growth and reproduction (Kailola *et al.*, 1993; Eggleston, 1995; Sheaves and Molony, 2000; Mous *et al.*, 2006; Koenig, 2007; Pina-Amargós and González-Sansón, 2009). In Malaysia and Indonesia, wild juveniles of ca. 100200 mm in total length (unpublished data) caught from mangrove estuaries have formed the basis of recent commercial cage culture operations (Mous *et al.*, 2006; Matsuura, 2007) rather than hatchery-produced seed. This practice is now seen to be potential factor reducing recruitment success to coastal populations of the species. Furthermore, maintaining the stock levels of predatory fishes such as *E. coioides*, which have high food web status (Evers *et al.*, 2009), is a necessary action for the conservation of mangrove estuary biodiversity. Accordingly, investigations on the biology of such wild grouper juveniles (including hatching periods, growth, and size at entering and departing mangrove estuaries) are now seen as necessary for future conservation and management. However, such has received little attention in Malaysia as yet, although some congeners have been considerably studied in other counties, e.g., *E. striatus* (Eggleston, 1995; Collin *et al.*, 1997) and *E. itajara* (Brusher and Schull, 2008; Lala *et al.*, 2009).

For analyzing the growth of 0 year-old fish, it is essential to have information on the age of the fish in days. However, investigations on otolith increments have not been carried out thus far in *Epinephelus coioides*, although the age determination in days using otolith daily increments in a number of fish species has progressed since Pannella (1971). Therefore, the daily periodicity of otolith increment formation is firstly to be validated, and subsequent research on fish growth in the wild using age information in days from otolith are required. The present study aims to elucidate the hatching periods, growth and size of *E. coioides* juveniles, particularly in a mangrove estuary situation using daily age data from otolith increment analysis, as part of the project "Studies of the biology and sustainable management of commercially important fish species in mangrove estuaries and related coastal waters on the northwest coast of Peninsular Malaysia" of the Japan International Research Center for Agricultural Sciences, Japan and, the Fisheries Research Institute, Malaysia.

Materials and methods

Validation of periodicity of otolith increment formation

The periodicity of otolith increment formation was validated using hatchery-produced *Epinephelus coioides* larvae (hatched on 5th October 2009 (day 0); reared in a rectangular concrete tank (2.5 m × 2.0 m × 1.0 m containing 5 t seawater) at SEAFDEC-AQD (Aquaculture Department, Southeast Asian Fisheries Development Center), Iloilo, the Philippines. Water temperature was kept between 27.5 and 30.2° C. rotifers and mysids being fed daily during rearing. Nine or 10 fish each on days 12 (3.7-5.7 mm TL, *n* = 10), 36 (12.1-16.1 mm TL, *n* = 9) and 43 (15.6-21.1 mm TL, *n* = 10) after hatching were collected and immediately preserved in 70% ethanol. After fish total lengths (TL, mm) were measured, the otoliths (lapilli) of all specimens were removed under a dissecting microscope and mounted in epoxy resin on glass slides. The otoliths were ground using sandpaper (# 1500) and lapping films (6 and 9 μm mesh). The ground otolith surfaces were occasionally etched by 0.1 N hydrochloric acid to emphasize increment contrast. Otolith increments were observed and counted under an optical microscope with transmitted light (× 200-400) using the otolith daily ring measurement system (RATOC System Engineering Co. Ltd., Japan), and the maximum radius (μm) of each otolith measured. The preparation and increment observations of otoliths were undertaken in the Japan International Research Center for Agricultural Sciences (JIRCAS), Japan.

Fish sample collection from the wild

A total of 230 *Epinephelus coioides* juveniles were collected from the western coastal reef of Langkawi Island during November 2007, and February, June, July and December 2008 (25.9-48.0 mm TL, n = 25), and from the Merbok mangrove estuary during June 2007 to May 2008, except for October-November 2007 and April 2008 (90.0-232.0 mm TL, n = 205) (Table 1, Fig. 1). Collection gears used were four vault-shaped net cage traps (55 cm diameter × 170 cm length, 6 cm of mesh size) and four cyrindrical net cage traps (12 cm diameter × 120 cm length, 1.5 cm of mesh size), the former being used on the western coastal reef of Langkawi Island and the latter in the Merbok mangrove estuary (Fig. 1). The nets were set overnight on each day of sampling on the bottom of each sampling site. Fish collected from Langkawi Island comprised already settled juveniles that were found inside the PVC pipes (20-34 mm of diameter) forming the frame of the traps, and from Merbok mangrove estuary, those found within the traps. Water depth was less than 20 m in both areas. After fish collection, they were frozen or preserved in 70% ethanol and the otoliths removed following TL measurement in the Fisheries Research Institute, Penang, Malaysia. Otolith treatments and increment observations were undertaken in the JIRCAS, following the methods described above for hatchery-produced fish.

Table 1: Years, months and locations of wild fish collections, and numbers and size ranges of fish

Year	Months of collection	Locations of collection	Number of fish	Range of total lengths (mm)
2007	June	MME*	14	92.0 - 172.0
	July	MME	18	96.0 - 197.0
	August	MME	26	93.0 - 232.0
	September	MME	24	95.0 - 170.0
	October	CRL **	1	28.9
	November	MME	26	101.0 - 1430
	December	MME	21	93.0 - 167.0
	January	MME	25	90.0 - 160.0
2008	February	CRL	1	26.1
	March	MME	26	90.0 - 201.0
		MME	25	122.0 - 193.0
	May	CRL	3	28.6 - 33.5
	July	CRL	15	25.9 - 48.0
	December	CRL	5	32.4 - 39.3

*MME: Merbok mangrove estuary (5°37'43.40"N – 5°38'28.20"N, 100°23'59.14"E – 100°25'14.54"E)
**CRL: Coastarl reef of Langkawi Island (6°10'33.36"N – 6°25'24.05"N, 99°38'25.69"E – 99°43'23.77"E)

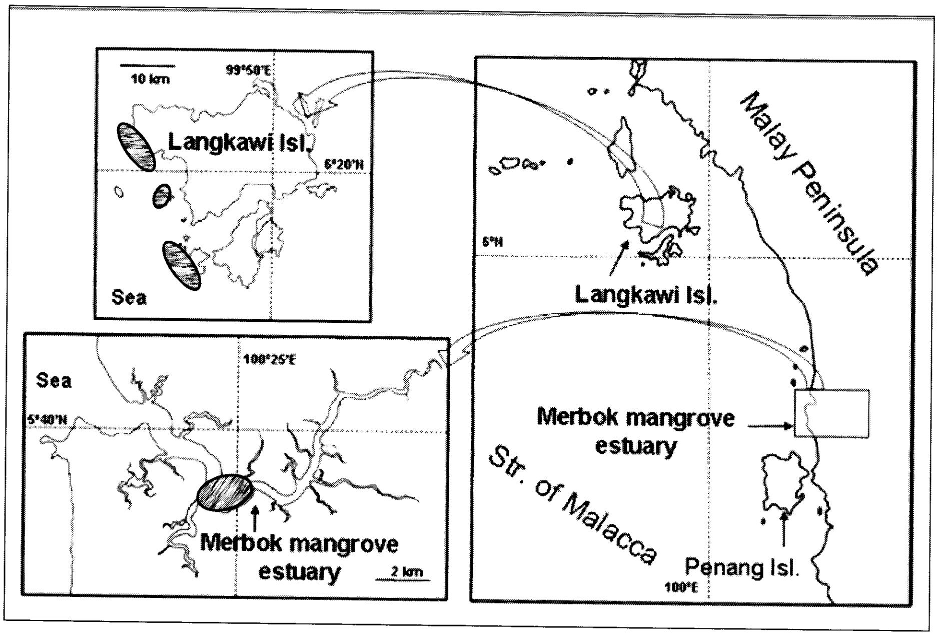

Figure 1: Sampling sites on the west coast of Langkawi Island and in the Merbok mangrove estuary. *Grey circles* indicate fish collection sites

Results

Otolith increment formation in hatchery-produced fish

The total lengths of hatchery-produced fish were 4.3 ± 0.6 (mean ± SD) mm (n = 10) on day 12, 14.1 ± 1.5 mm (n = 9) on day 36 and 18.0 ± 1.8 mm (n = 10) on day 43 after hatching. The otoliths (lapilli) were almost round in day 12 larvae, becoming fan-shaped with fish growth (Fig. 2). The increments and core portion of the otoliths were observable in all specimens (n = 29) (Fig. 2). A distinctive ring, interpreted as the hatch check, was deposited ca. 5 μm from the otolith core (Fig. 2). The relationship between the age in days (D) and number of increments (I) was linearly regressed by a following formula (Fig. 3);

$$I = 1.03D - 1.47 \ (R^2 = 0.99, n = 29) \quad (1)$$

The slope of the regression was not significantly different from 1 (ANCOVA, $p > 0.05$), indicating that the otolith increments were deposited daily and that the actual age in days could be estimated as the number of increments plus 12.

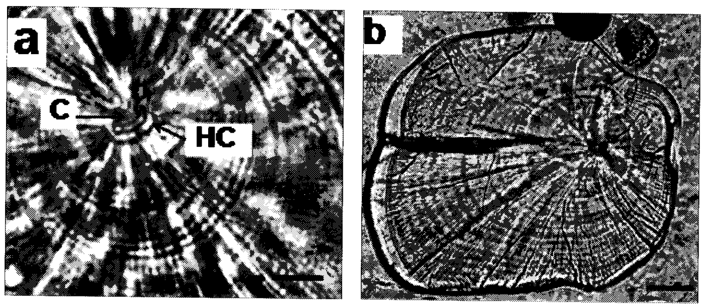

Figure 2: Otoliths (lapilli) of hatchery-produced orange-spotted grouper *Epinephelus coioides*. **a** core portion of otolith in day-36 juvenile (14.6 mm SL), **b** entire otolith in day-43 juvenile (17.3 mm SL). **C** otolith core, **HC** hatch check. *Scale bars* 10 μm in **a**, 40 μm in **b**

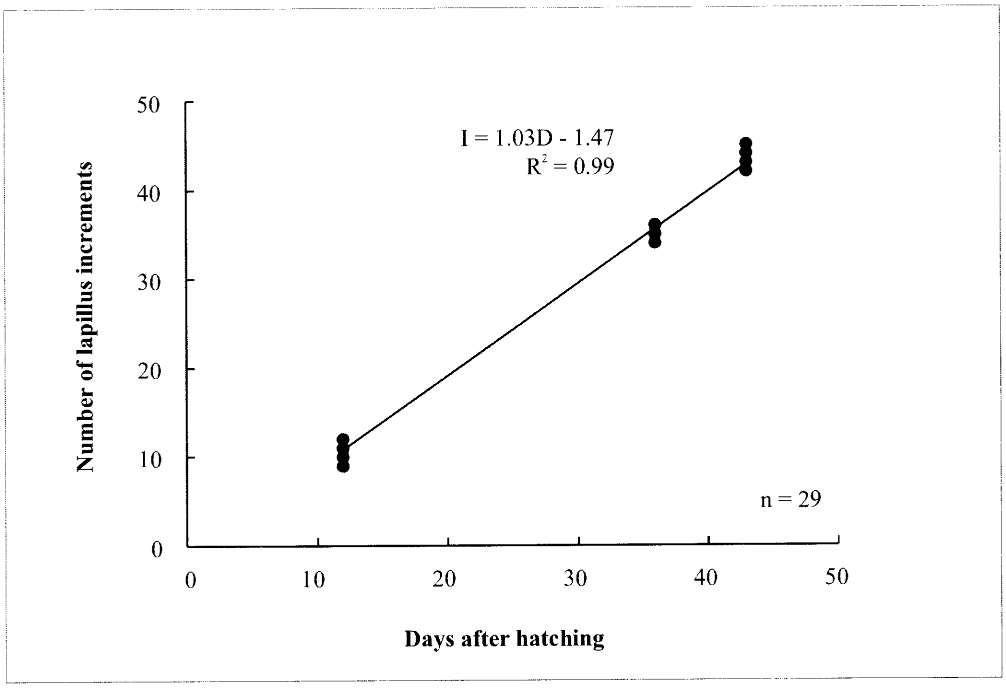

Figure 3: Relationship between days after hatching and number of otoliths (lapilli) increments in larval and juvenile hatchery-produced orange-spotted grouper *Epinephelus coioides*

Size distributions and estimation of daily age and hatching periods of the wild juveniles

The frequency distribution of total lengths of the *Epinephelus coioides* juveniles collected from Langkawi Island (25.9-48.0 mm TL, n = 25; Fig. 4) and those from Merbok mangrove estuary are shown in Fig 5 (90.0-232.0 mm TL, n = 205), respectively. Modal changes of total lengths from the latter area suggested the intermittent entries of juveniles > ca. 90 mm TL to the estuary, where they were considered to sojourn for about 4 months in maximum (Fig. 5).

The otoliths (lapilli) in juveniles from these areas were larger and more oblong fan-shaped than those in the hatchery-produced larvae and juveniles, and had observable increments (Fig. 6). Relationship between total length (L, mm) and otolith radii (OR, µm) of aggregate fish samples of the present study, including laboratory-produced and wild fishes, was logarithmically regressed by a following formula (Fig. 7);

$$OR = 164.2 \, Log(L) - 284.8; (R^2 = 0.96, n = 259) \quad (2)$$

Otolith increment counts in the juveniles of Langkawi Island (25.9-48.0 mm TL, n = 25) were 29-51, and real ages being estimated as 31-53 days old (plus 1-2), following the regression (1) (Fig. 3). Juveniles from the Merbok mangrove estuary (90.0-232.0 mm TL, n = 205) were equally estimated to be 85-217 days old since the increment counts were 83-215.

Based on this back-calculation of hatching dates for the wild juveniles, the spawning period of *E. coioides* in the area of this study was estimated as occurring almost throughout the entire year (Fig. 8).

In addition, in order to verify if the growth trend of juveniles in the mangrove estuary varied seasonally, the fish were divided into the following 4 seasonal hatching groups; January-March (Group-I), April-June (Group-II), July-September (Group-III) and October-December (Group-IV). Relationships between daily age (T) and total length (L, mm) in each group were linearly regressed as follows; L = 0.84·T + 32.9 (R^2 = 0.56, n = 35; Fig. 9a) for Group-I, L = 0.90·T 11.5 (R^2 = 0.69, n = 46; Fig. 9b) for Group-II, L = 0.77·T + 35.5 (R^2 = 0.68, n = 42; Fig. 9c) for Group-III and L = 0.90·T + 15.0 (R^2 = 0.86, n = 82; Fig. 9d) for Group-IV. The results indicated growth rates (increase in length per day) in each hatching group of 0.84, 0.90, 0.77 and 0.90 mm, respectively, the growth rates between the groups being not significantly different (ANCOVA, p>0.05). The growth of all juveniles in the estuary was linearly regressed as L = 0.91·T + 15.0 (R^2 = 0.77, n = 205; Fig. 9e).

Duration of sojourn in the mangrove estuary and growth model

Based on the age information for *E. coioides* juveniles collected from the Merbok mangrove estuary (85-217 days old, n = 205), the maximum duration of their sojourn in the estuary was estimated to be a little more than 4 months (217 - 85 = 132 days). Monthly changes in the mode of the total length frequency distributions also suggested the sojourn duration as ca. 4 months (maximum), the size at recruitment to the mangrove estuary being greater than 90 mm TL, with specimens disappearing by ca. 230 mm TL (Fig. 5).

The growth pattern of the aggregated larvae and juveniles using the age and total length data for both hatchery-produced and wild fish closely fitted the Gompertz formula; L = 214.26·exp[-3.89·exp(-0.016·T)] (r = 0.97, n = 259; Fig. 10), where L and T denoted total length and daily age, respectively. The relative growth (Lr = Lt Lt-1; Lt denotes the TL at age t days old), calculated on the basis of the above Gompertz formula, illustrated that this species grew to ca. 90-100 mm TL by ca. 90 day-old, the growth thereafter becoming slower (Fig. 10). The results showed that *E. coioides* grew to 190-200 mm TL during the first ca. 7 months after hatching.

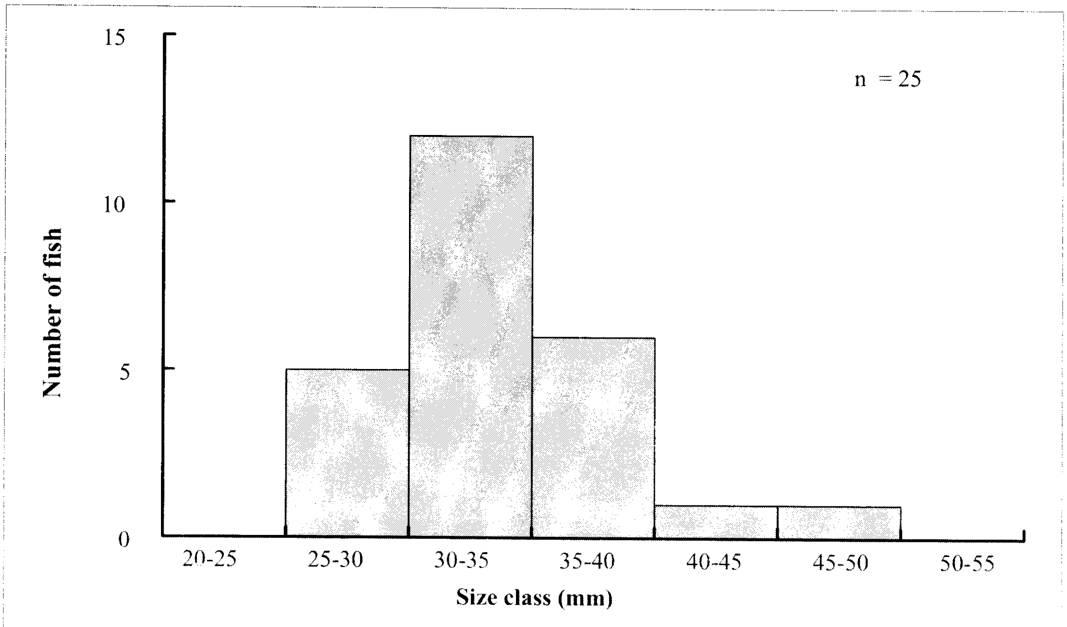

Figure 4: Frequency distribution of total lengths (mm) in wild orange-spotted grouper *Epinephelus coioides* juveniles collected from the coastal reef (west coast) of Langkawi Island from November 2007 to December 2008

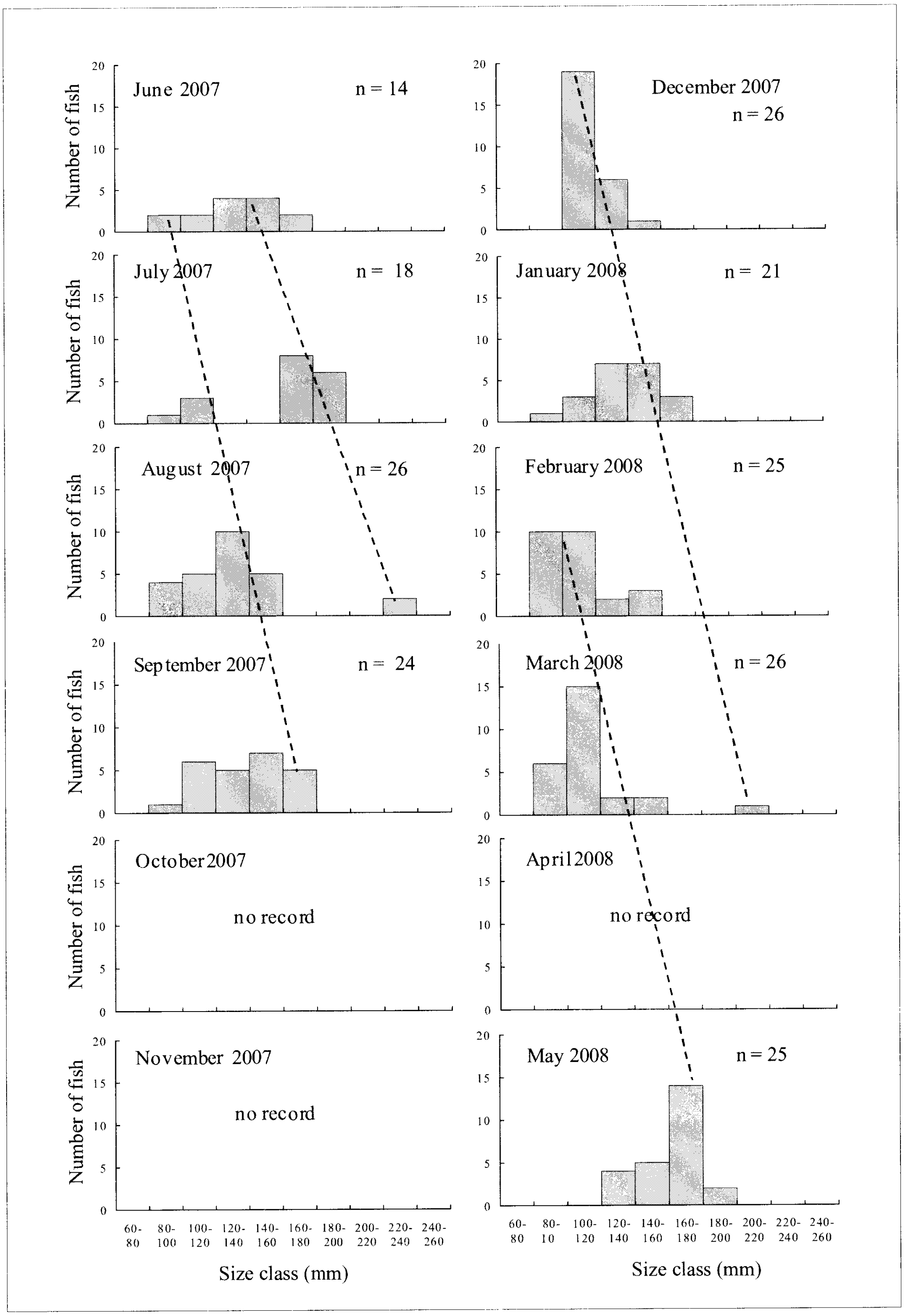

Figure 5: Monthly frequency distributions of total length (mm) in wild orange-spotted grouper *Epinephelus coioides* juveniles collected from the Merbok mangrove estuary from June 2007 to May 2008. *Dotted lines* indicate modal changes

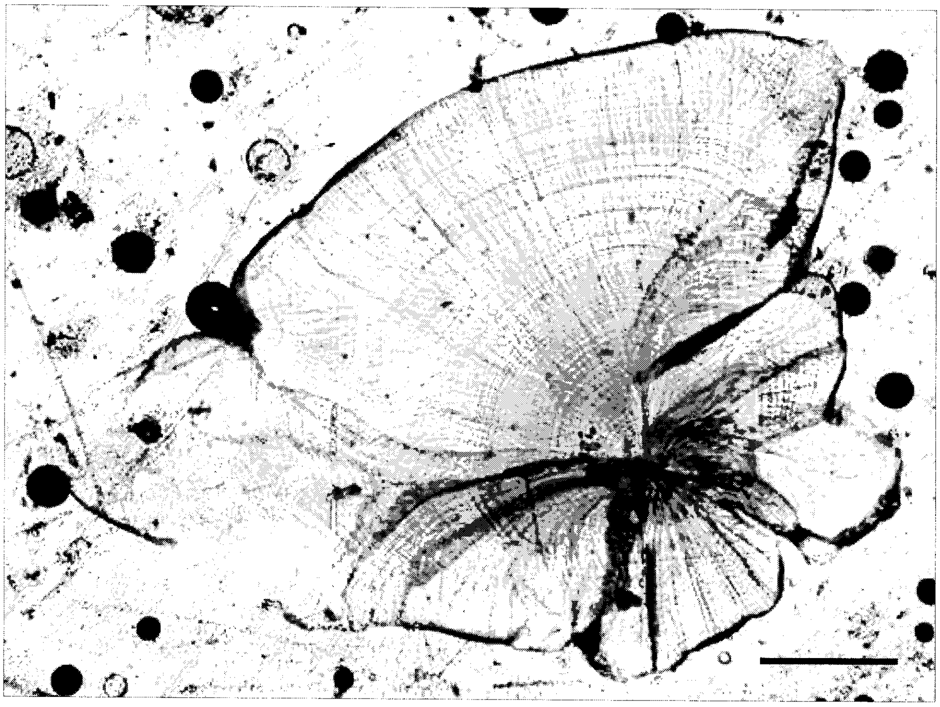

Figure 6 : Otolith (lapillus) of a wild-caught juvenile *Epinephelus coioides* (130 mm SL). *Scale bar* 200μm

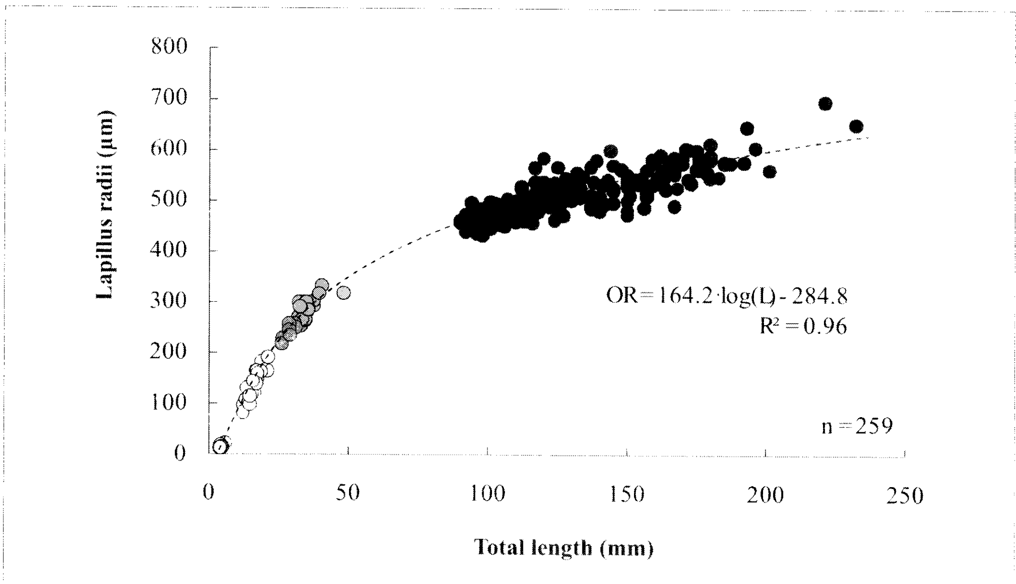

Figure 7: Relationship between fish total length (mm) and lapillus radii (μm) in larval and juvenile orange-spotted grouper *Epinephelus coioides*. *Open circles*- hatchery-produced fish, *grey circles*- juveniles from the coastal reef, *solid circles*-mangrove estuary

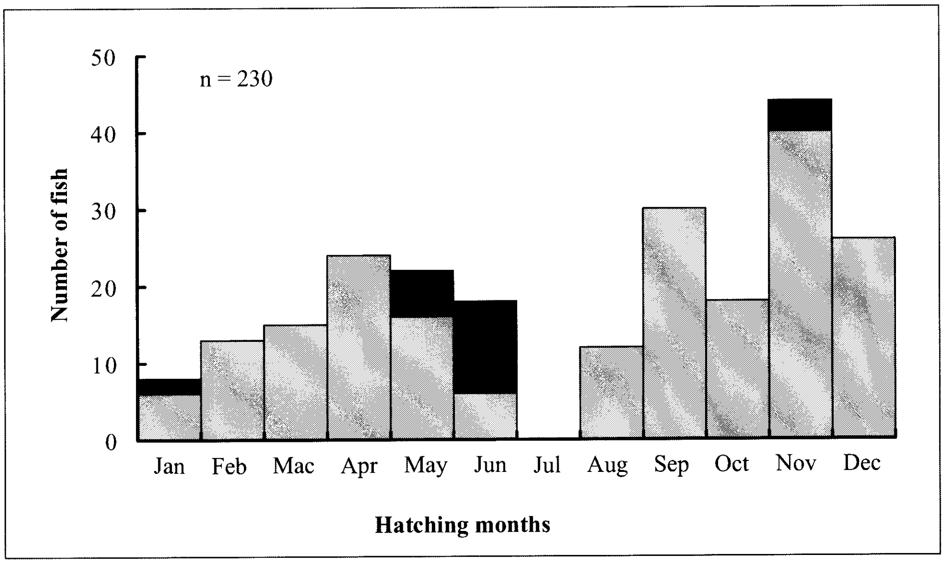

Figure 8: Frequency distribution of hatching months of wild orange-spotted grouper *Epinephelus coioides* juveniles from June 2007 to May 2008. *Grey bars* indicate months in 2007, *solid bars* 2008

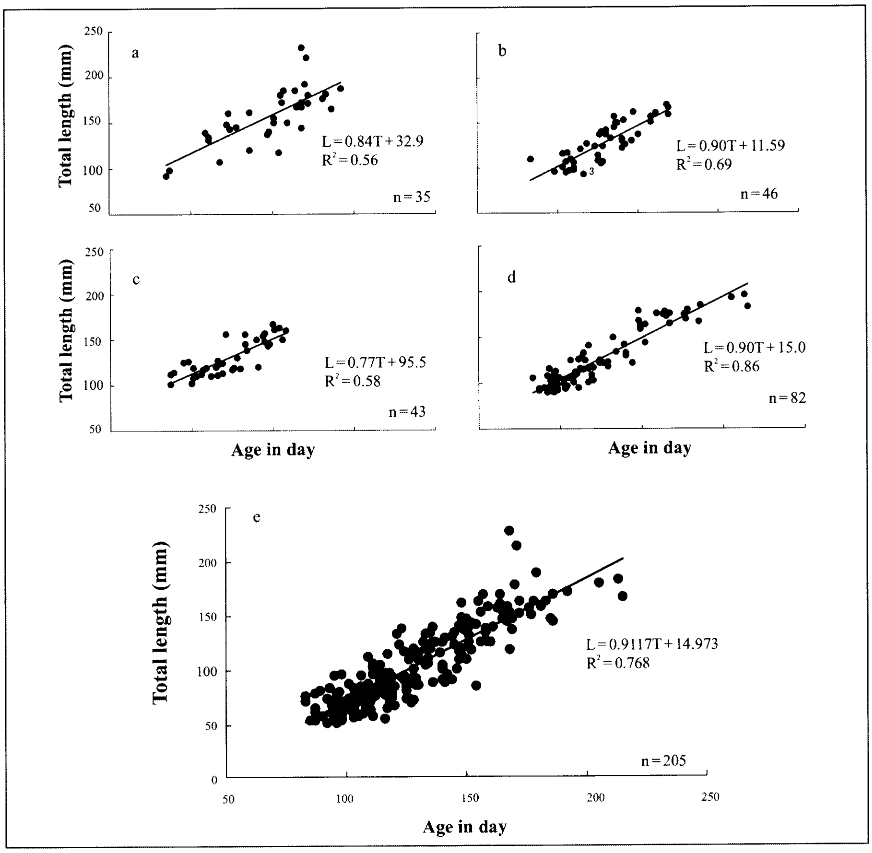

Figure 9: Seasonal growth trends wild orange-spotted grouper *Epinephelus coioides* juveniles hatched in four seasonal periods. **a** January to March, **b** April to June, **c** July to September*, **d** October to December, **e** all specimens from mangrove estuary. *no July hatchings recorded

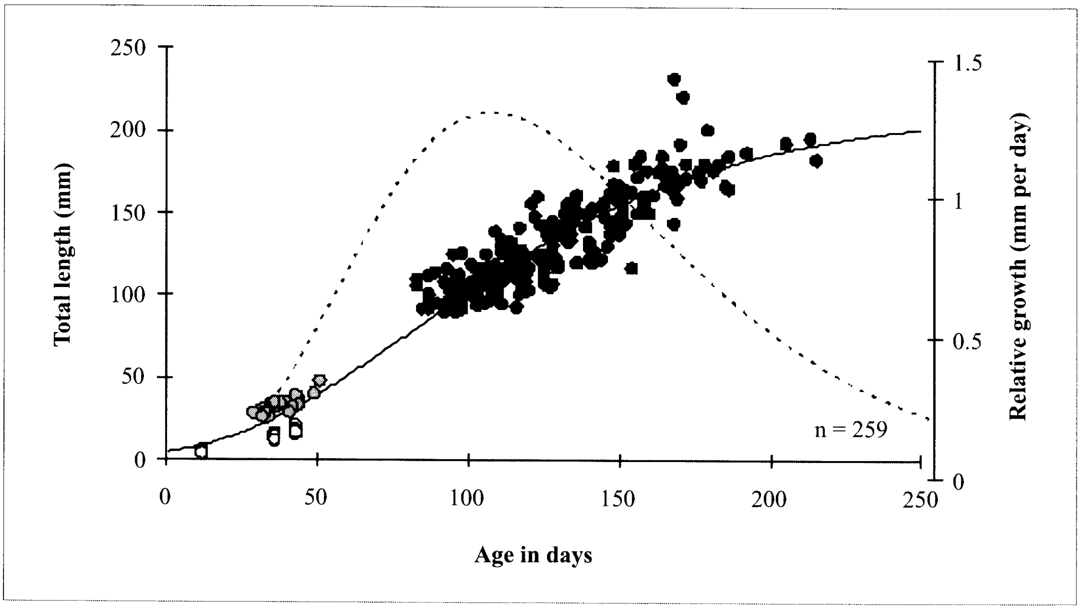

Figure 10: Growth pattern (solid line fitted by Gompertsz growth model) and the instantaneous growth (dotted line) of the aggregated larval and juvenile orange-spotted grouper *Epinephelus coioides*. *Open circles*- hatchery-produced fish, *grey circles*- juveniles from the coastal reef, *solid circles*- mangrove estuary

Discussion

In the present study, the otolith (lapillus) increment was elucidated to be the daily increments with 12 days of delay after hatching in first increment formation in *Epinephelus coioides* (Fig. 3). In addition, the fish sizes at entering to and disappearing from the mangrove estuary were estimated to be ca. 90 mm TL and 230 mm TL, respectively, with growth rate to be ca. 0.91 mm TL in the estuary and the sojourn of about 4 month in maximum (Figs. 5, 9).

Furthermore, it was elucidated that the growth pattern of larvae and juvenile was well fitted by a Gompertz growth model, the fish growing to > 200 mm TL in 7 months post-hatching, and the species bred almost throughout a year (Figs. 8, 10). These results are the preliminarily contributory data for future stock management of *E. coioides* in the mangrove estuary as well as the considerations on biodiversity conservation in the estuary.

Lower otolith increment numbers than actual age in days have been reported in other fishes due to a delayed start in first increment formation (initial deposition at onset of feeding) (Tsuji and Aoyama, 1984; Hayashi *et al.*, 1989). In *E. coioides*, the onset of feeding has been reported as 23 day after hatching (Ordonio-Aguilar *et al.*, 1995), indicating subsequent daily formation of increments in the *E. coioides* individuals examined here. Larval *Lateolabrax japonicus* have lapillus increment counts which tend to be lower than sagittal counts because of too concentrated/narrow increment structure around the core (Islam *et al.*, 2009). However, in the present study, the core portion was clearly observable in the ground lapillus (Fig. 2), confirming the latter as the appropriate structure for aging purposes in *E. coioides*. In many other fish species, the sagitta has been the most often employed otolith for daily age estimation, but the morphological complexity of the sagitta in *E. coioides*, particularly the three-dimensionally bent shape, was problematic (Yamamoto, unpublished data) as in some other fish taxa, for example *Genypterus blacodes* (Ophidiidae) (Morioka and Machinandiarena, 2001), despite being a much greater size than the lapillus.

The frequency distribution of hatching months estimated in the present study confirmed that *E. coioides* spawns almost all year round in the region (Fig. 8). By contrast, it has been reported that *E. coioides* in the Arabian Gulf had a limited spawning period in April, based on gonad analysis of wild females (Grandcourt *et al.*, 2005). The fish collection area in the present study was located at lower latitude (56°N, Fig. 1) and characterized by constantly high water temperatures throughout the year. On the other hand, the survey area in the Arabian Gulf was situated 24-25°N, being subject to greater seasonal temperature fluctuations. The differing water temperature conditions in the two areas may be responsible for an extended spawning period in the former area and a limited spawning period in the latter, although gonad analysis of Malaysian specimens should be undertaken before any further conclusions be drawn on the reproductive biology of local *E. coioides* populations.

In other *Epinephelus* species, e.g., Nassau grouper *E. striatus* (Colin *et al.*, 1997) and goliath grouper *E. itajara* (Lara *et al.*, 2009), the deposition of a settlement mark in the otoliths (lapillus) has been reported, but such a mark was not observed here in *E. coioides* (Fig. 6), making accurate estimations of settlement size and age impossible. However, the sizes and ages of the small juveniles collected from the coastal reef of Langkawi Island (25.9-48.0 mm TL, 31-53 day-old; Table 1, Fig. 4) were close to previously-reported settlement sizes and ages in *E. striatus* (mean 31.7 mm TL, Eggleston 1995) and *E. itajara* (ca. 30-40 mm SL, 30-80 day old, Lara *et al.*, 2009), both occurring in the Caribbean Sea. This suggested that *E. coioides* juveniles collected from the coastal reef in this study were in the developmental phase shortly after settlement (post-settlement). Lara *et al.* (2009) estimated the subsequent growth rate after settlement in *E. itajara* as 0.99 mm per day, that rate being coinciding somewhat with the rate obtained here (0.91 mm per day; Fig. 9e).

Small crustaceans (e.g. Natantia and *Acetes* spp.) have been reported as important dietary organisms for *Epinephelus* juveniles in the Merbok mangrove estuary and neighboring Matang mangrove estuary, based on stomach contents (Ogawa, 2004) and stable isotope analyses (Hui, 2009). Since it was reported that the water temperature was stable in high level (> 27°C) all year around in the Merbok mangrove estuary (Hanamura *et al.*, 2007), the growth of *E. coioides* may be influenced by the abundance of such dietary organisms rather than the water temperature fluctuations. Although no significant difference in the seasonal growth rates of juveniles from the mangrove estuary was observed in the present study, the abundance of the above dietary organisms occasionally showed great fluctuations (Hanamura *et al.*, 2007; 2008).

Accordingly, further studies on the simultaneous correlations between the population dynamics of the dietary crustaceans and fish growth should be made for better understandings of dynamics of *E. coioides* population in the estuary.

The occurrence of post-settlement juveniles on the coastal reef observed in the present study (25.9-48.0 mm TL, 31-53 day-old; Fig 4) indicated that larval and juvenile *E. coioides* probably migrate onshore after hatching in order to detect settlement and nursery habitats, subsequently entering the mangrove estuary and growing to 190-200 mm TL (by ca. 7 months after hatching) (Figs. 5, 10), an example of ontogenetic habitat shift (Eggleston, 1995).

Although various micro habitats reported for *E. striatus* settlement include areas dominated by seagrass and sponges in addition to the coastal reef (Dahlgren and Eggleston, 2001), the migration and growth patterns of *E. coioides* observed in this study were similar to those reported for *E. striatus* (Eggleston, 1995) and *E. itajara* (Koenig *et al.*, 2007). Ogawa (2004) reported the occurrence of *E. coioides* juveniles (as *E. bleekeri*) smaller than 80 mm TL (minimum size 54 mm TL) and larger than 240 mm TL (maximum size 387 mm TL) in 2001-2002 in the Merbok mangrove estuary, a size range much greater than determined in the present study. However, the proportions of these smaller/larger specimens (< 80 mm TL and > 240 mm TL, respectively) reported by Ogawa (2004) were only 1.7% (22 of 1280) and 0.9% (11 of 1280), respectively, the size range of most individuals (97.4%, 1247 of 1280) coinciding closely with those in the present study (90-232 mm TL). Therefore, the size range observed here was considered to be reasonably indicative of the general *E. coioides* juvenile population in the Merbok mangrove estuary.

The movement of *E. coioides* juveniles into the mangrove estuary at ca. 90 mm TL (about 3 months after hatching) and subsequent departure ca. 4 months later demonstrated that the mangrove estuary is an important nursery ground for *E. coioides* juveniles before recruitment to the coastal fishery population, as has been shown for other reef fishes, including *E. malabaricus*, *L. russelli* (Sheaves, 1995) and *Lutjanus johnii* (Kiso and Mahyam, 2003). These findings are important for future stock assessment and management of *E. coioides* juveniles in the mangrove estuaries, in so far as they relate to the recruitment success of the species to the coastal fishery population. On the other hand, similarly-sized juveniles (100-200 mm TL) having been occasionally reported to occur in the coastal reef (unpublished data) may indicate a juvenile population that remains in coastal areas after settlement. Clearly, further investigations on detailed distributions of larvae and juveniles caused by ontogenetic habitat shift as ecological changes (Eggleston, 1995) of *E. coioides* and the migration patterns of *E. coioides* are needed.

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References

Ahmed, T.A., El-Dankour, S.M. and El-Zahar, C.R. 2000. Growth and survival of the grouper *Epinephelus coioides* (Hamilton) at different loading rates in tanks. *Aquaculture Research,* **31**: 603-608.

Brusher, J.H. and Schull, J. 2008. Non-lethal age determination for juvenile goliath grouper *Epinephelus itajara* from southwest Florida. *Endang Species Research,* **7**:205-212.

Bullock, L.H., Murphy, M.D., Godcharles M.F. and Mitchel M.E. 1992. Age, growth, and reproduction of jewfish *Epinephelus itajara* in the eastern Gulf of Mexico. *Fisheries Bulletin US*, **90**: 243-249.

Colin, P.L., Laroche, W.A., Brothers, E.B. 1997. Ingress and settlement in the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae), with relationship to spawning occurrence. *Bulletin Marine Science,* **60**: 656-667.

Dahlgren, C.P. and Eggleston, D.B. 2001. Spatio-temporal variability in abundance, size and microhabitat associations of early juvenile Nassau grouper *Epinephelus striatus* in an off-reef nursery system. *Marine Ecology Progress Series*, **217**: 145-156.

Doi, M., Toledo, J.D., Golezl, M.S.N., De Los Santos, M. and Ohno 1997. Preliminary investigation of feeding performance of larvae of early orange-spotted grouper, *Epinephelus coioides*, reared with mixed zooplankton. *Hidrobiologia*, **358**: 259-263.

Domeier, M.L. and Colin, P.L. 1997 Tropical reef fish spawning aggregations: defined and reviewed. *Bulletin Marine Science,* **60**: 698-726.

Eggleston, D.B. 1995. Recruitment in Nassau grouper *Epinephelus striatus:* post-settlement abundance, microhabitat features, and ontogenetic habitat shifts. *Marine Ecology Progress Series*, **124**: 9-22.

Evers, D.C., Graham, R.T., Perkins, C.R., Michener. R. and Divoll, T. 2009. Mercury concentrations in the goliath grouper of Belize: an anthropogenic stressor of concern. *Endang Species Research.* doi: 10.3354/esr00158

Frias-Torres, S. 2006. Habitat use of juvenile goliath grouper *Epinephelus itajara* in the Florida Keys, USA. *Endang Species Research*, **2**: 1-6

Grandcourt, E.M., Al Abdessalaam, T.Z., Francis, F. and Al Shamsi, A.T. 2005. Population biology and assessment of the orange-spotted grouper, *Epinephelus coioides* (Hamilton, 1822), in the southern Arabian Gulf. *Fish Research,* **74**: 55-68.

Hanamura, Y., Siow, R. and Chee, P.E. 2007. Abundance and spatio-temporal distribution of *Acetes* shrimp (Crustacea, Decapoda, Sergestidae) in the Merbok and Matang mangrove estuaries, Peninsular Malaysia. *Malaysian Fisheries Journal*, **6**: 16-25.

Hanamura, Y., Siow, R. and Chee, P.E. 2008. Reproductive biology and seasonality of the Indo-Australasian mysid *Mesopodopsis orientalis* (Crustacea: Mysida) in a tropical mangrove estuary, Malaysia. *Estuarine, Coastal and Shelf Science*, **77**: 467-474.

Hayashi, A., Yamashita, Y., Kawaguchi, K. and Ishii, T. 1989. Rearing method and daily ring of Japanese sardine larvae. *Nippon Suisan Gakkaishi*, **55**: 997-1000.

Heemstra, P.C. and Randall, J.E. 1993. Groupers of the world (family Serranidae, subfamily Epinephelinae): an annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO species catalogue Vol. 16, Rome.

Hui, A.T.Y. 2009. The structure and trophodynamics of the fish community in estuaries of Matang mangrove forest reserve, Peninsular Malaysia. Master's thesis, The Institute of Biological Sciences, Faculty of Science, University of Malaya. Kuala Lumpur

Islam, Md. S., Ueno, M and Yamashita, Y. 2009. Otolith microstructure of Japanese sea bass larvae and juveniles: interpretation and utility for ageing. *Journal of Applied Ichthyology*, **25**: 423-427.

Kailola, P.J., Williams, M.J., Stewart, P.C., Reichelt, R.E., McNee, A. and Grieve, C. 1993. Australian Fisheries Resources. Bureau of Resource Sciences, Canberra.

Kiso, K. and Mahyam, M.I. 2003. Distribution and feeding habits of juvenile and young John's snapper *Lutjanus johnii* in the Matang mangrove estuary, west coast of Peninsular Malaysia. *Fisheries Sciences,* **69**: 563568.

Koenig, C.C., Coleman, F.C., Eklun, A.M., Schull, J. and Ueland, J. 2007. Mangroves as essential nursery habitat for goliath grouper (*Epinephelus itajara*). *Bulletin of Marine Sciences*, **80**: 567-585.

Kohno, H., Ordonio-Aguilar, R.S., Ohno, A. and Taki, Y. 1997. Why is grouper larval rearing difficult?: an approach from the development of the feeding apparatus in early stage larvae of the grouper, *Epinephelus coioides*. *Ichthyology Research*, **44**: 267-274.

Lara, M.R., Schull, J., Jones, D.L. and Allman, R. 2009. Early life history stages of goliath grouper *Epinephelus itajara* (Pisces: Epinephelidae) from Ten Thousand Islands, Florida. *Endang Species Research*, **7**: 221-228

Liao, I.C., Su, H.M. and Chang, E.Y. 2001. Techniques in finfish larviculture in Taiwan. *Aquaculture*, **200**: 1-31.

Marte, C.L. 2003. Larviculture of marine species in Southeast Asia: current research and industry prospects. *Aquaculture*, **227**: 293-304.

Matsuura, T. 2007. Comparative analysis of pattern of fishcage culture using marine/brackish water in three Southeast Asian countries. *In*: Nakamura K (ed). *Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas* (2005), JIRCAS Working Report No. 56, 135-143 pp.

Morioka, S. and Machinandiarena, L. 2001. Comparison of daily increment formation pattern between sagittae and lapilli of ling (*Genypterus blacodes*) larvae and juveniles collected off Argentina. *New Zealand Journal of Marine Freshwater Research*, **35**: 111-119.

Mous, P., Sadovy, Y., Halim, A. and Pet, J.S. 2006. Capture for culture: artificial shelters for grouper collection in SE Asia. *Fish and Fisheries*, **7**: 58-72.

Mumby, P.J., Edwards, A.J., Arias-Gonzlez, J.E., Lindeman, K.C., Blackwell, P.G., Gall, A., Gorczynska, M.I., Harborne, A.R. and Pescod, C.L. 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature*, **427**: 533-536.

Ogawa, Y. 2004. Migration, growth and feeding habit of John's snapper *Lutjanus johnii* and duskytail grouper *Epinephelus bleekeri* in Merbok mangrove brackish river. *In*: Ogawa Y, Ogata Y, Maeno Y, Shimoda T, Fujioka Y, Fukuda Y (Eds) Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas, JIRCAS Working Report No. 35, 110 pp.

Ordonio-Aguilar, R., Kohno, H., Ohno, A., Moteki, M. and Taki, Y. 1995. Development of grouper, *Epinephelus coioides*, larvae during changeover of energy sources. *J. Tokyo Univ, Fish.*, **82**: 103-108.

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

Pina-Amargós, F. and González-Sansón, G. 2009. Movement patterns of goliath grouper *Epinephelus itajara* around southeast Cuba: implications for conservation. *Endang Species Res.*, **7**: 243-247.

Randall, J.E. 1995. Coastal fishes of Oman. University of Hawaii Press, Honolulu.

Russell, B.C. and Houston, W. 1989. Offshore fishes of the Arafura Sea. *Beagle*, **6**:69-84.

Sadovy, Y., Figuerola, M. and Román, A. 1992. Age and growth of red hind *Epinephelus guttatus* in Puerto Rico and St. Thomas. *Fish Bulletin US*, **90**: 516-528.

Sheaves, M. 1995. Large lutjanid and serranid fishes in tropical estuaries: are they adults or juveniles? *Marine Ecology Progress Series*, **129**: 31-40.

Sheaves, M. and Molony, B. 2000. Short-circuit in the mangrove food chain. *Marine Ecology Progress Series*, **199**: 97-109.

Smith, M.M. 1986. Smiths' sea fishes. Springer-Verlag, Berlin

Teruya, K., Yoseda, K., Oka, M., Nishioka, T., Nakano, S., Mori, K., Sugaya, T. And Hamasaki, K. 2008. Effects of photoperiod on survival, growth and feeding of seven band grouper *Epinephelus septemfaciatus* larvae. *Nippon Suisan Gakkaishi*, **74**: 645-652.

Tsuji, S. and Aoyama, T. 1984. Daily growth increments in otoliths of Japanese anchovy larvae *Engraulis japonica*. *Bulletin of Japanese Society Science Fisheries*, **50**: 1105-1108

Tucker, J.W. Jr. 1999. Grouper Aquaculture. Southern Regional Aquaculture Center Publication No. 72, Stoneville

Yoseda, K., Teruya, K., Sugaya, T. and Sekiya, S. 2006. Effect of delayed initial feeding on larval feeding, early survival, and growth of red spotted grouper *Epinephelus akaara* larvae. *Nippon Suisan Gakkaishi*, **72**: 702-709.