

Growth and Reproductive Status of the Spotted Scat *Scatophagus argus* in Mangrove Estuary in Matang Mangrove Forest Reserve, Malaysia

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Abstract

We analyzed the gonadal development and age of the spotted scat (*Scatophagus argus*) in the Matang Mangrove Forest Reserve, Malaysia. Histological analysis of gonads indicated that few females and males in the mangrove estuarine system were sexually mature, with few specimens having sizes greater than the typical maturation size (> 140 mm in total length for females, > 110 mm in total length for males). These observations suggested that *S. argus* do not mature sexually in the mangrove estuary. Growth analysis using age information from otolith increments suggested that *S. argus* moved or are carried into the mangrove estuary during the late tholichthys larval stage at a size of about 12 mm-14 mm in total length (ca. 37 days old), and that the fish use the estuary as a nursery ground before sexual maturation. The period of sojourn in the estuary is for a maximum of ca. 290 days for females by the size of ca. 160 mm-180 mm in total length and ca. 155 days for males by the size of ca. 110 mm in total length. After these nursery periods, grown specimens reaching sexual maturity are considered to leave the estuary habitats.

Discipline: Fisheries

Additional key words: Daily age, gonad analysis, growth, mangrove estuary

Introduction

The mangrove estuary of the Matang Mangrove Forest Reserve (MMFR), Malaysia is home to a fish community comprised of a diverse species. Many species are reported to spawn offshore of the MMFR, and the developing larvae and juveniles move or are carried by tidal currents to inshore areas or estuarine waters in mangrove forest areas (Gillanders et al. 2003, Blaber 2007, Sarpedonti & Chong 2008, Yamamoto et al. 2010). Amongst the community of fish, the spotted scat (*Scatophagus argus*) (Perciformes: Scatophagidae) is highly dominant on a biomass basis (Hui 2009) and an important index species of the ichthyofauna in the area. This species is also an important resource that is consumed as food, and kept as a commercial ornamental

species as well throughout Southeast Asia (Musikasung et al. 2006, Gupta 2016), including areas around the MMFR.

A previous study on the ontogenetic migration of *Scatophagus argus* in the MMFR, conducted using information from carbon and nitrogen isotope ratios, suggested that this species recruits into the mangrove estuary of the MMFR from outer coastal areas at the size of < 20 mm in total length (TL), and then uses the estuary as a nursery ground to grow to a size of ca. 160 mm TL (Morioka et al. 2020). However, aspects of the growth and reproductive development of the species during its sojourn in the estuary remain unclear; thus, information about such aspects will contribute to further clarification of the species' dynamics, as well as the conservation and management of the *S. argus* resource in the estuary of the

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MMFR.

An analysis of daily growth increments in fish otoliths can provide an estimate of the timing of larval or juvenile recruitment of *Scatophagus argus* into the mangrove estuary in the early life stage, as well as their growth patterns during their sojourn there. The same technique was used in a similar study of orange-spotted grouper (*Epinephelus coioides*) juveniles in the Merbok mangrove estuary of Peninsular Malaysia (Yamamoto et al. 2010).

In the present study, we determined the age in days using otolith increments and conducted gonad analysis of *Scatophagus argus* collected from the MMFR estuarine system, in order to clarify aspects of their growth and reproduction during their utilization of the estuary for better understanding of their life history in detail.

Materials and methods

1. Study area and sample collection site

The Matang Mangrove Forest Reserve (MMFR) (Fig. 1) is situated on the northwestern coast of Peninsular Malaysia and considered the world's best managed mangrove forest (Jusoff & Taha 2009). The reserve is the

largest single tract of mangrove forest in Peninsular Malaysia (40,151 ha), measuring 52 km between its far ends and 13-km wide in the middle. The MMFR is a riverine forest type mangrove that is inundated during most spring high tides, whereas water is mainly confined to channels during neap tides. Figure 1 shows the sample collection site in the northern part of the MMFR. The Sangga Besar River basin ranged between 4 and 6 m in depth, with the coastal and mudflat areas being shallower (1 m-3 m). Mean water temperature in the study area was 29.6°C (range between 26.6 and 31.1°C). Mean salinity (\pm standard deviation) of the bottom water was high at 24.5 ± 1.89 ppt at the river mouth area and low in the inner estuary area, ranging from 18.2 ± 7.4 ppt to 22.5 ± 2.1 ppt (Okamura et al. 2010).

Fish were caught using a 2-m-wide otter-trawl net or cast net. From November 2010 to October 2011, fish were collected monthly for analyses of otolith increments, stomach contents and the gonadosomatic index (GSI), along with other samples being collected for additional analysis of the gonads (i.e., GSI and histological analysis) in September and December 2012, and in March and June 2013. The total length (TL, mm) and body weight (BW, g) of all specimens were measured before other analyses.

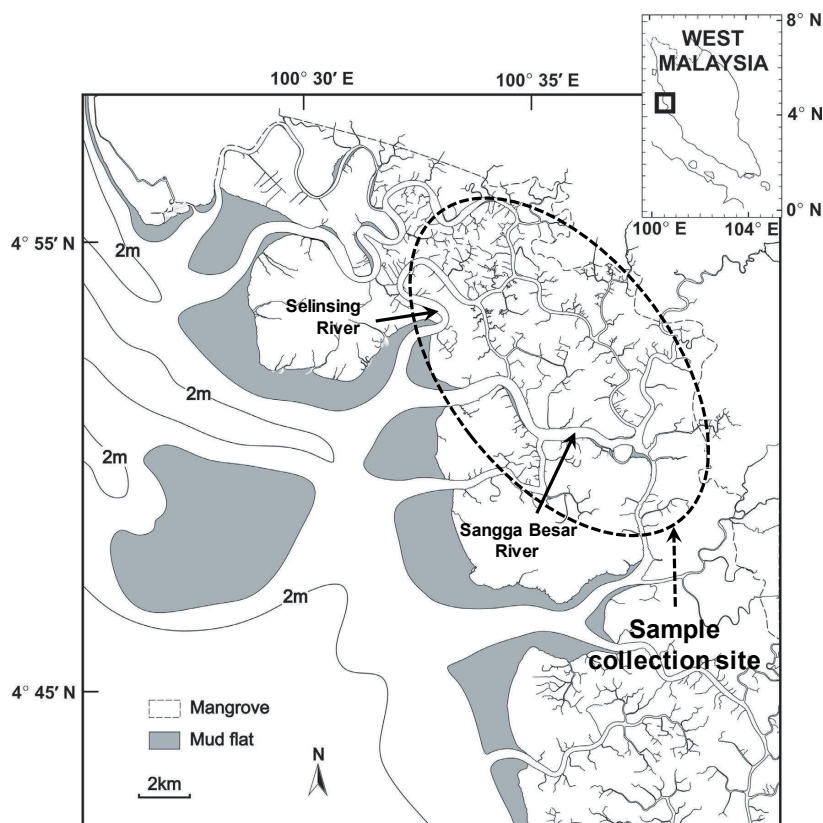


Fig. 1. Sample collection site for spotted scat (*Scatophagus argus*) in the Matang Mangrove Forest Reserve (MMFR), Malaysia

2. Histological observations of gonads

A total of 205 specimens of *Scatophagus argus* were collected over several days, including on 29 September (47.0 mm-170.0 mm TL, n = 47), 11 December 2012 (43.0 mm-154.0 mm TL, n = 54), 12 March (45.0 mm-159.0 mm TL, n = 53), and 6 June 2013 (43.0 mm-153.0 mm TL, n = 51), and then preserved in 10% formalin immediately after collection. After sex identification by microscopic observations of the ovaries or testes, the gonadosomatic index [gonad weight (g) / body weight (g) × 100%, GSI] and the sex ratio (number of females to number of males) were obtained. Thereafter, the gonads of 112 specimens (43.0 mm-170.0 mm TL) randomly selected from 205 specimens were histologically analyzed. For preparing the stained histological sections, the gonads were first dehydrated in a progressive series of ethanol dilutions and then embedded in paraffin wax, which was cut into 5- μ m sections using a microtome. The paraffin sections were mounted onto glass slides, dewaxed in xylene, and then dehydrated by immersion in the series of ethanol dilutions. Lastly, the sections were stained with hematoxylin-eosin for histological observations of the gonads, which were classified according to sex and maturation stage (Sasaki & Igarashi 1974, Kaneko et al. 1984, Doiuchi & Yoshimoto 2009, Li et al. 2015).

3. Otolith treatments for daily age analysis

A total of 297 *Scatophagus argus* specimens (11.9 mm-176.0 mm TL) randomly collected from the sampling site (Fig. 1) from November 2010 to October 2011 were dissected and the gonads were observed under a binocular microscope to identify the sex by microscopic observations of the ovaries or testes. The otoliths (lapilli) were then removed under a dissecting microscope and mounted in epoxy resin (twin pack epoxy adhesive) on glass slides. The lapilli were ground using sandpaper (#1500) and lapping films (3-, 6- and 9- μ m mesh) for observation of otolith micro increments. To emphasize the contrast of increments, the ground otolith surfaces were occasionally etched using 0.1 N hydrochloric acid. Otolith increments were observed and counted under an optic microscope with transmitted light (200×-1,000×), and the maximum radius (μ m) of each otolith was measured. The Otolith Daily Rings Measurement System (Ratoc System Engineering, Co., Ltd.) was used for otolith increment analysis. Although the daily periodicity of otolith micro increment deposition has not yet validated in *S. argus*, it has been demonstrated in a number of other fish species (Campana & Neilson 1985), including species distributed in a mangrove estuary, e.g., the orange-spotted grouper (*Epinephelus coioides*) (Yamamoto et al. 2010) and the mangrove rivulus (*Rivulus marmoratus*)

(Sakakura & Noakes 2000). Hence, we hypothesize that the otolith increments have formed on a daily basis in the species. The relationship between the daily age and total length of *S. argus* was estimated separately for females and males, using a nonlinear least-squares method with the application of Gompertz growth curves: $L_t = L_\infty \cdot \exp[-C \cdot \exp(-Kt)]$ using EXCEL solver (Microsoft Office 365 ProPlus), where L_t , L_∞ , C , K , and t denote the estimated length at age t , asymptotic length at which growth is zero, growth coefficient, instantaneous growth rate at $t = 0$, and age in days, respectively.

Results

1. Sex ratio and gonad development

Among the 205 specimens of *Scatophagus argus* used for determinations of the sex ratio and gonadosomatic index (GSI), there were 145 females (43.0 mm-170.0 mm TL) and 60 males (43 mm-104 mm TL) (Fig. 2), showing a significant bias in the sex ratio towards females at 1 : 0.41 (chi-squared test, $P < 0.01$). Moreover, the sex ratio among fish > 80 mm TL was more distinctively biased towards females as 1 : 0.10 (chi-squared test, $P < 0.01$), and the ratio of fish > 110 mm TL was 1 : 0; however, the sex ratio of fish < 80 mm TL was 1 : 0.93, not significantly biased from 1 : 1 (chi-squared test, $P > 0.05$) (Fig. 2). The GSIs varied between 0.00436 and 7.53 in females, but most females (93.1%, 135 of 145) showed low GSIs (≤ 1) throughout the four quarterly sampling periods (Fig. 3). In males, GSIs were generally very low, ranging from 0.00339 to 0.356, and most males (95%, 57 of 60) had GSIs of < 0.01 (Fig. 3).

Among the 205 fish mentioned above (145 females and 60 males), the gonads (ovaries or testes) of 71 females

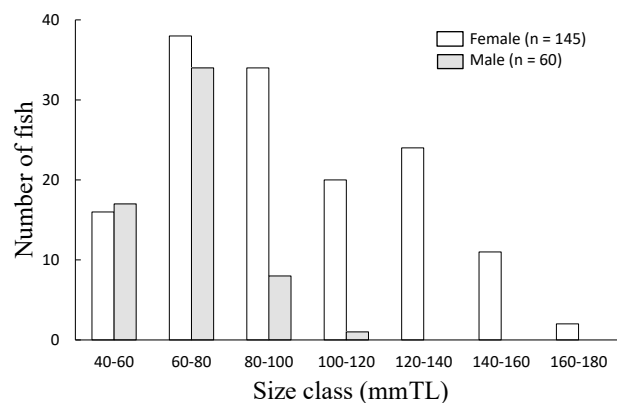


Fig. 2. Length-frequency distributions (mm TL) of *Scatophagus argus* collected in September and December 2012, and in March and June 2013 from the MMFR, Malaysia, for which gonads were histologically analyzed

White bars: females, dark bars: males

(50.0 mm-172.0 mm TL) and 41 males (43.0 mm-104.0 mm TL) were histologically observed. In most females with low GSIs (≤ 1 in 64 of 71 females), oocytes were in a resting stage (Fig. 4 a, b); the most developed oocytes were observed in specimens with higher GSIs (1.81-7.14), in which the oocytes were in the vitellogenic stage (Fig. 4 c), but no mature oocytes were observed. Among males with low GSIs (≤ 0.01 in 14 of 41 males), spermatogonia in testes were in the proliferation stage (Fig. 4 d); the most developed testes were observed in specimens with a somewhat higher GSI (0.01-0.14) and were in the early maturation stage (Fig. 4 e).

2. Growth of *Scatophagus argus* and its sojourn in the mangrove estuary

The daily growth increments in the lapilli of *Scatophagus argus* were clearly visible and thus countable under an optic microscope with transmitted light ($\times 200$ -400) (Fig. 5). The ages of the sample specimens varied from 26 to 327 days old (11.9 mm-176.0 mm TL, n = 297). The minimum age (26 days) was observed in a specimen of 13.2 mm TL having unknown sex; the maximum ages of females and males of 160.0 mm TL and 97 mm TL were 327 days and 190 days, respectively. In the 14 smallest specimens ranging from 11.9 to 14.9 mm TL (mean 13.7 ± 1.74 mm TL) with estimated ages of 26-45 days (mean 37.4 ± 6.74 days), characteristics of the tholichthys larval stage (e.g., protective sheath-like bony

plates covering the head) still remained.

The relationship between the number of lapillus growth increments (daily ages) and total length was regressed for females and males using Gompertz growth curves (Fig. 6; both models included the 55 sex-unknown specimens):

$$Lt = 161.74exp(-3.99exp(-0.015t))$$

$$(R^2 = 0.91, n = 221) \text{ for females,}$$

$$Lt = 117.68exp(-4.09exp(-0.019t))$$

$$(R^2 = 0.87, n = 131) \text{ for males.}$$

The hatching months of *Scatophagus argus* were back-calculated from the daily ages of each specimen, and spanned the entire year (Fig. 7), thereby suggesting that *Scatophagus argus* breeds throughout the year.

Discussion

As previously reported by Morioka et al. (2020) in their investigation of the ontogenetic migration of *Scatophagus argus* based on an analysis of the carbon and nitrogen isotope ratios, small-sized *S. argus* (< 20 mm TL) were found to enter the mangrove estuary from outer coastal areas, grow in the estuary to approximately 100 mm-120 mm TL, and then return to the river mouth area at larger sizes (>120 mm TL). *S. argus* has a tholichthys larval stage that is pelagic, reportedly at 6

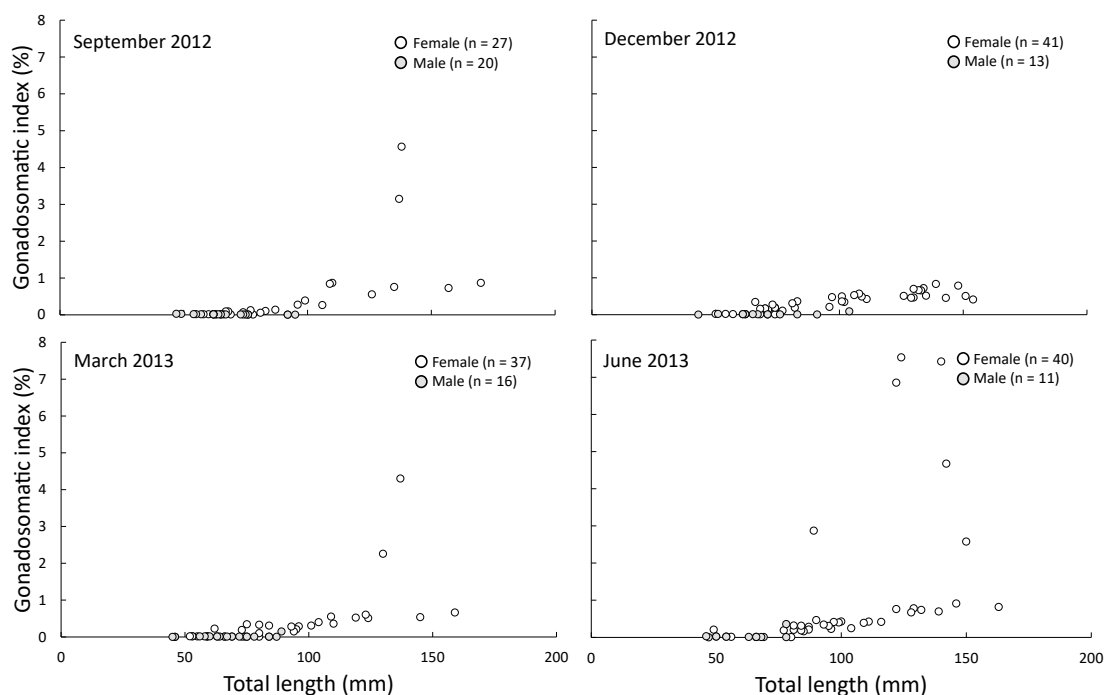


Fig. 3. Relationship between total length (mm) and gonadosomatic index (%) for *Scatophagus argus* collected quarterly (in September and December 2012, March and June 2013) in the MMFR, Malaysia

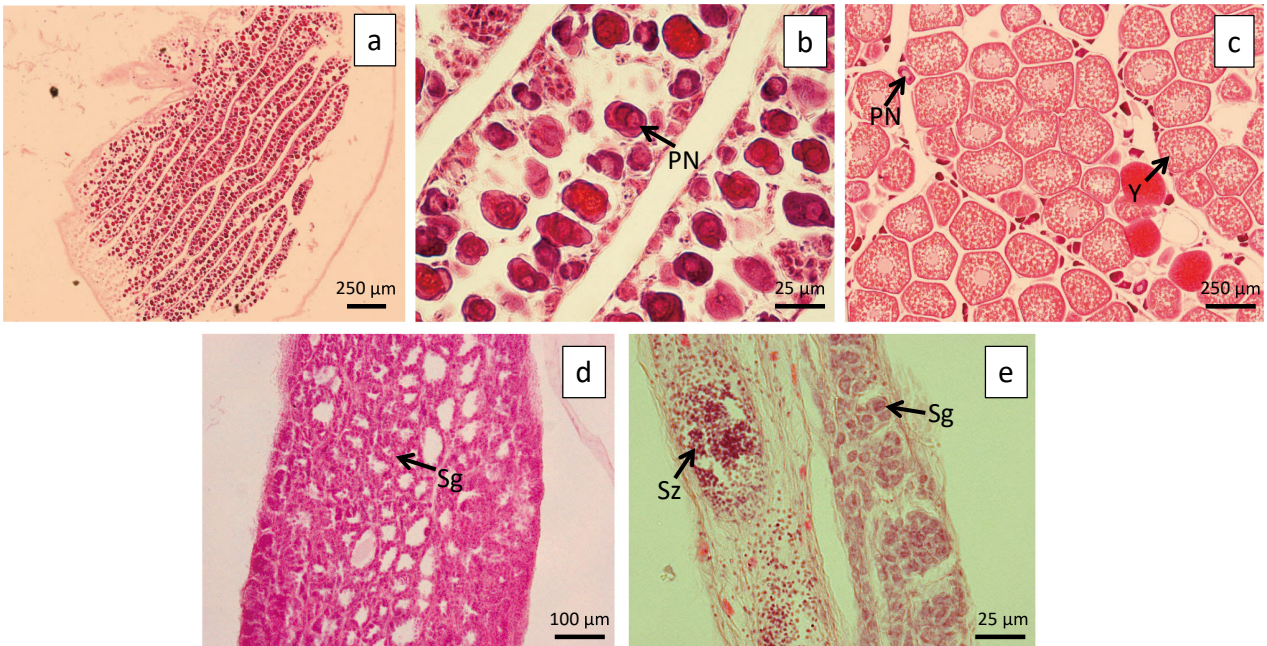


Fig. 4. Microscopic images of the histological sections of testes and ovaries of *Scatophagus argus* collected from the MMFR, Malaysia

a ovary in resting stage (66 mm TL), b extended image of a, c ovary in vitellogenic stage (150 mm TL), d testis in proliferation stage (61 mm TL), e testis in early maturation stage (75 mm TL).

PN: peri-nucleolus oocytes

Y: yolk stage oocytes

Sg: spermatogonia

St: spermatid

Sz: spermatozoa

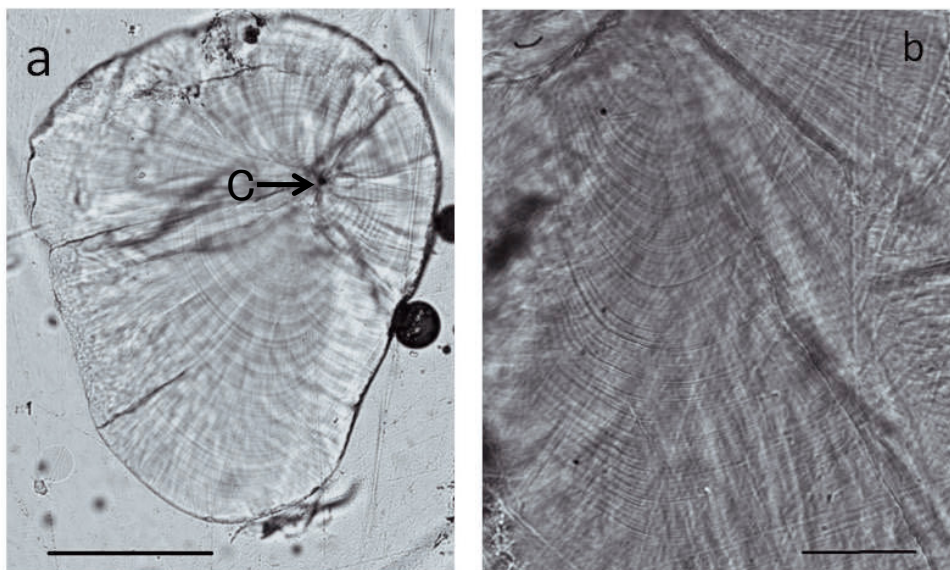


Fig. 5. Lapillus of *Scatophagus argus* (a, 16.1 mm TL; b, 35.0 mm TL) collected in the MMFR, Malaysia

C: otolith core

Scale bars: 100 µm in a and 50 µm in b

mm-12 mm TL (Barry & Fast 1992), and the 14 smallest specimens obtained in the present study [13.7 ± 1.74 mm TL, 37.4 ± 6.74 days old] still had characteristics of the tholichthys larval stage (e.g., protective sheath-like bony plates covering the head: Barry & Fast 1992). These individuals can thus be considered as being in a transitioning phase between the planktonic/pelagic larval and settlement juvenile stages, as is known to occur in chaetodontids (Harmelin-Vivien 1989). These observations illustrate that the recruitment of *S. argus* into the mangrove estuary of the MMFR takes place at the late phase of the tholichthys larval stage, with *S. argus* probably being carried in by tidal current at approximately 30-43 days after hatching.

Although *Scatophagus argus* can exceed 300 mm TL (maximum size > 350 mm TL: Pethiyagoda 1991), the mean size of the species in the MMFR was recorded as 89.0 ± 25 mm in standard length (Hui 2009) with a maximum size of ca. 160.0 mm TL (Morioka et al. 2020). Meanwhile, the size at sexual maturity of the species was reported to be >140 mm TL for females and >120 mm TL for males, with a GSI of 7%-8% for females and approximately 1.5% for males (Gandhi et al. 2014). However, the present study observed no specimens with fully mature oocytes and the most developed oocytes were in the vitellogenic stage observed in a female of 150.0 mm TL (Fig. 4). Similarly, in males, fully mature testes were not observed during consecutive one-year sampling, and the most developed testes were in the early maturation stage observed in a male of 104 mm TL (Fig. 4). These findings strongly suggested that *S. argus* reach sexual maturation after moving out of the mangrove estuary to the outer coastal areas of the MMFR, and also suggested that the mangrove estuary functions as a

nursery ground for *S. argus*. Ontogenetic migration of juvenile fishes in the mangrove estuary as their nursery ground has been reported in various species, such as John's snapper (*Lutjanus johnii*) and the Caroun croaker (*Johnius carouna*) based on isotopic signatures (see Tanaka et al. 2011, 2012) in the MMFR, and the orange-spotted grouper (*Epinephelus coioides*) in the neighboring Merbok mangrove forest area through daily age-growth analyses (see Yamamoto et al. 2010). In other areas, the nursery function of the mangrove estuarine habitat has also been proven in juveniles of various other species, including the bluestriped grunt (*Haemulon sciurus*), schoolmaster snapper (*Lutjanus apodus*) (see Mumby 2006), yellowtail snapper (*Ocyurus chrysurus*), and striped parrotfish (*Scarus iserti*) (see Nagelkerken et al. 2001) in Caribbean mangrove areas.

The estimated hatching months observed in this study (Fig. 7) indicated that *Scatophagus argus* reproduces throughout the year as commonly reported in other tropical species, including the orange-spotted spinefoot (*Siganus guttatus*) (see Hara et al. 1986) and orange-spotted grouper (*Epinephelus coioides*) (see Yamamoto et al. 2010). Such an elongated breeding period over the year may be enabled by the consistently high water temperatures year-round in this area.

Based on the overall findings here and the information on ontogenetic migration reported by Morioka et al. (2020), we reasonably estimated the growth, migratory patterns, and reproductive status of *Scatophagus argus* in the MMFR as follows: 1) *S. argus* is considered to breed outside the estuary area throughout the year with continuous larval recruitment into the mangrove estuary; 2) the recruited larvae are in a transitional phase between the pelagic tholichthys and

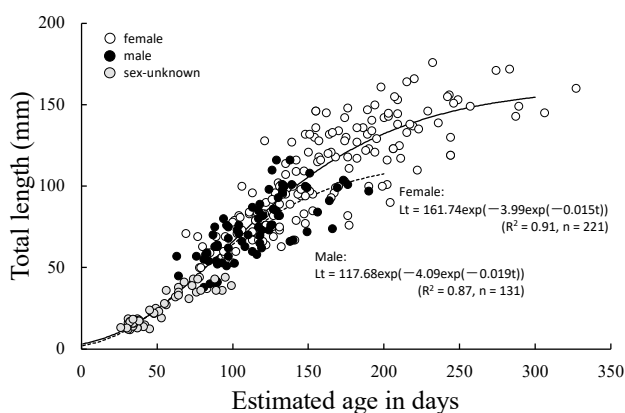


Fig. 6. Relationship between estimated age in days and total length (mm) of *Scatophagus argus* collected in the MMFR, Malaysia
Solid and dotted lines indicate growth curves of female and males, respectively.

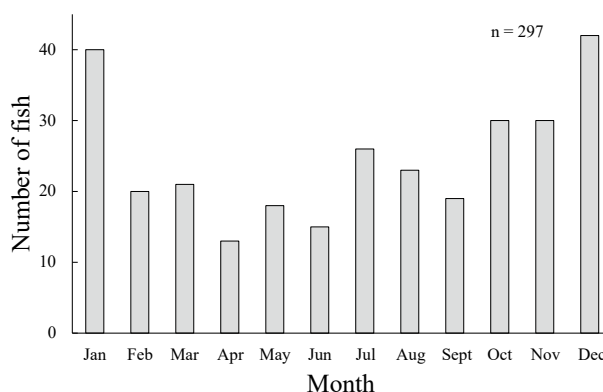


Fig. 7. Frequency distribution of hatching months of *Scatophagus argus* collected monthly from November 2010 to October 2011 in the MMFR, based on the estimated age in days of each specimen

benthic juvenile stages, and enter the mangrove estuary at approximately 12.0 mm TL (30-43 days old), probably carried by the tide (Forward & Tankersley 2001); and 3) *S. argus* stayed and grew in the estuary area for a maximum of ca. 290 days for females sized 160 mm-180 mm TL, and ca. 160 days for males sized ca. 110 mm TL (Fig. 6) before sexual maturation. The above estimated periods as the maximum age observed here are 327 days old for females and 195 days old for males minus 37 days, as the estimated average age at recruitment. Several previous studies have considered the factors of migration from the inner mangrove estuary to outer coastal areas, such as the onset of maturation (Grol et al. 2014), requirement of larger prey (Huijbers et al. 2015), a larger body size relatively safe against predation risk as growth in the estuary (Levin et al. 1997), and the tidal/lunar cycle (Dubuc et al. 2019). Considering the maturation size of *S. argus*, particularly the smaller maturation size in males than that in females (Gandhi et al. 2014), and size distributions of females/males in the estuary (Fig. 2), the migration of *S. argus* toward outer coastal areas is presumably induced by the onset of maturation.

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