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Collection and aging of greater amberjack *Seriola dumerili* larvae and juveniles around the Penghu Islands, Taiwan

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Age of *Seriola dumerili* larvae

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Abstract

In order to investigate the early life history of *Seriola dumerili*, we first validated otolith daily increments using reared-fish (11-51 days after hatching). Four larval and early-juvenile *S. dumerili* were collected in May and July 2015 around the Penghu Islands, Taiwan (23.45-23.70 °N, 119.40-119.70 °E) by surface larval net towing but not from drifting seaweeds. *Seriola dumerili* were caught at thermal front, and total lengths and ages ranged 7.4-42.5 mm and 18-56 days, respectively. Our results indicate that the hatching dates of *S. dumerili* were April to June and larvae may have been accumulated in frontal zone before juvenile phase.

Key words: Greater amberjack, *Seriola dumerili*, aging, otolith, early life history

Introduction

The greater amberjack *Seriola dumerili* (family Carangidae) is distributed widely from temperate to tropical waters around the world (Taki et al. 2005), and is an important species both for fishery and aquaculture in Japan (Nakada 2002). Spawning and morphological development of artificially-raised *S. dumerili* were described in Japan (Masuma et al. 1990; Tachihara et al. 1993; Kawabe et al. 1996; Kawabe et al. 1998), and its spawning season in the wild was estimated to be from winter to summer in western Atlantic (Fahay 1975; Wells and Rooker 2004b; Sedberry et al. 2006; Harris et al. 2007) and June and July in Mediterranean Sea (Raya and Sabatés 2015). Juveniles of *S. dumerili* in 25-297 mm standard length (SL) associate with floating objects such as drifting seaweeds (Nakata et al. 1988; Badalamenti et al. 1995; Massutí et al. 1999; Wells and Rooker 2004a, b). Based on an alizarin complexone marking experiment, it was confirmed that *S. dumerili* (136-193 mm SL) deposits otolith increments on a daily basis (Wells and Rooker 2004b). However, there is no information regarding deposition of otolith increments in larval and early-juvenile stages of this species in the wild, which is necessary to determine spawning season from hatching date and early life history of this species. Information of larval and early-juvenile *S. dumerili* is limited in the wild, with at most 15 individuals (less than 0.81 ind./1000 m^3) in Mediterranean Sea (Raya and Sabatés 2015), 11 individuals in the South Atlantic Bight (Fahay 1975) and less than 0.01 ind./1000 m^3 in the northern Gulf of Mexico (Ditty et al. 2004) have been collected. As for Asian waters, information of larval and early-juvenile *S. dumerili* is also limited, with few collection records from the basin of Tsushima Warm Current (TWC) and off southern Korea from July to August (Uchida et al. 1958), in the Pacific coast of Japan from March to September (Okiyama 2014), and in the coastal waters of Taiwan (Liu 2001) and in the northeast of Taiwan (Chen et al. 2012).

In order to facilitate understanding of the early life history of *S. dumerili* in Asian waters, firstly, we validated the otolith daily increments of artificially-raised *S. dumerili* larvae and juveniles. Next, we collected larval and early-juvenile *S. dumerili* around the Penghu Islands, Taiwan (Fig. 1), and investigated hydrographic conditions, larval fish densities of collection sites, and the age and spawning season of this species.

Materials and methods

Validation for Otolith Daily Increments

Artificially-raised *S. dumerili* were obtained from a private hatchery (Tawaki Suisan, Ltd.), in Kumamoto prefecture, Japan. Fish were reared at 24 °C and rotifers were fed until day 23 after hatching, followed by *Artemia* between day 20-35, frozen copepods between day 31-41 and dry pellets from day 33. Fish were randomly sampled from the rearing tank on day 11 (n=5), 32 (n=5) and 51 (n=4), and all samples were kept frozen until analysis. Total lengths (TL, mm) of fish from each age group were measured and pairs of sagittal otoliths were extracted under a dissecting microscope. According to the method of Sakakura and Tsukamoto (1997), otoliths were embedded in epoxy resin lying on their sides on a glass slide, except samples on day 11 which were embedded in transparent nail polish. The otoliths were observed after grinding using sandpaper (#1000) and lapping film (9 μm and 3 μm). Growth increments were counted under a light microscope at a magnification of ×1000 using an oil immersion lens. Counting of growth increments started from a conspicuous dark mark which delimited the core of the otolith. The largest radius of sagittal otolith was also measured using a digital microscope (Keyence, VH6300).

Field collection

Cruises around the Penghu Islands, Taiwan (Fig. 1, Fig. 2, 23.45-23.70 °N, 119.40-119.70 °E) were made during 13-15 May, 2-4 June, 28-29 July and 25-27 August 2015 by R/V Hai-an (42 tonnes) of Taiwan Fishery Research Institute. During 09:00-14:00 (at National Standard Time), drifting seaweed and frontal zone were visually observed and drifting seaweeds were scooped together with associated fishes by a hand net (Φ45 cm, 3 mm mesh) from the side of stationary ship, since sea surface was close to scoop drifting seaweeds from the deck. In this paper, we defined "frontal zone" as the area of surface water convergence including oceanic front created by the gradient of water temperature and/or salinity, and slick created by Langmuir circulation or internal wave. Surface tows of a larval net (Φ1.3 m, 0.33 mm mesh) from stern were conducted (10 min. with towing speed at 2 knot) in frontal zones and other areas (Fig. 2a). In order to keep towing at surface layer, a small spherical float was attached to the outer part of the opening of a larval net. A HYDRO-BIOS flow meter was placed at the opening of a larval net to measure the volume of water filtered. Larval and juvenile fishes were preserved in 95% ethanol solution. At each sampling station, vertical profile of water temperature was measured by a conductivity-temperature-depth profiler (CTD; SBE-19 plus, Sea-Bird Electronics, Bellevue, WA) from the sea surface to a depth at 5 m above the bottom.

Sample analysis

Larval and juvenile fishes were counted and *S. dumerili* was identified according to Okiyama (1988; 2014). Then, total length (± 0.1 mm TL) and wet weight (mg) were measured with a caliper and an electronic balance, respectively. In the same manner of reared-fish, otoliths of each wild fish were examined and hatching dates were back-calculated. Horizontal distribution of sea surface temperature (SST, °C) from *in situ* CTD data was summarized and plotted by Ocean Data View (version 4.6.2).

Data analysis

Comparison of regression lines for otolith increments related to the days after hatching in reared-fish and the line of Y=X was conducted with the analysis of covariance (ANCOVA). Exact Wilcoxon rank sum test was used for comparison of larval and juvenile fishes density between frontal zones and other stations. Statistical analysis was carried out using R. version3.1.3 (R Development Core Team 2015) supplied with the exactRankTests package (Hothorn and Hornik 2015) and p-values < 0.05 were considered significant in all analyses.

Results

Validation of otolith daily increments

Total lengths of artificially-raised *S. dumerili* at each age group were 4.5 ± 0.5 mm, 16.7 ± 1.8 mm and 54.8 ± 3.6 mm (mean ± standard deviation) in day 11 (n=5), 32 (n=5) and 51 (n=4), respectively. The relationships between age (x_{day}, days after hatching) and otolith radius (y_{rad}, μm), and between TL (x_{TL}, mm) and otolith radius (y_{rad}, μm) of reared-fish, were described by the following equations: $y_{\text{rad}} = 13.49 \cdot e^{(0.08 x_{\text{day}})}$ (*n*=14, *r*=0.99, Fig. 3a), and $y_{\text{rad}} = 11.71 \cdot x_{\text{TL}} - 18.49$ (*n*=14, *r*=0.98, Fig. 3b), respectively. The linear regression between age (x_{day}, days after hatching) and number of otolith increments (y_{inc}) was equated as $y_{\text{inc}} = 0.99 \cdot x_{\text{day}} + 0.23$ (*n*=14, *r*=0.99, Fig. 3c). The regression line for otolith increments related to age in reared-fish was not significantly different from the line of Y=X (ANCOVA, *n*=14, *df*=1, *F*=1.04, *p*=0.32).

Collections of larvae

Frontal zones marked with the accumulation of bubbles and/or a distinct sea surface line were visually observed around the Penghu Islands and they were found to be created by gradient of water temperature but not by the difference of salinity, especially in May and July (Fig. 2). Some of frontal zones were not correlated with water temperature gradient (e.g. A11, C2, Fig. 2). We caught a total of 898 of larval and juvenile fishes from 30 hauls of surface towing of a larval net. During our study period, dominant families (monthly mean ind./100 m^3) from surface towing were Carangidae (0.54-0.17; mostly doublespotted queenfish *Scomberoides lysan*), Exocoetidae (4.82-0.01) and Coryphaenidae (0.37-0.05). Density of larval and juvenile fishes collected by surface towing was not different between frontal zones and other stations in May (exact Wilcoxon rank sum test, *n*=10, *df*=1, *W*=7, *p*=0.71). Drifting seaweeds were found in four stations only in May (Fig. 2). A total of 144 fish juveniles associated with drifting seaweeds were collected, and *Siganus* spp. (54.2%) and threadsail filefish *Stephanolepis cirrhifer* (27.8%) were dominant. A total of four *S. dumerili* were caught by surface towing of a larval net, but not from drifting seaweeds. All *S. dumerili* were caught at frontal zones (Fig. 2a, c) and SST of collection sites ranged from 24.9 to 27.4 °C (Table 1). In the station A11, two *S. dumerili* were collected in May. Total lengths and ages of *S. dumerili* were 7.4, 9.8, 13.7 and 42.5 mm, and 18, 26, 36 and 56 days, respectively (Table 1). The relationship between age (x_{day}, days after hatching) and TL (y_{TL}, mm) of reared-fish was described by the following equation: $y_{\text{TL}} = 2.26 \cdot e^{(0.06 x_{\text{day}})}$, and growth rate of wild fish was lower than that of reared-fish (Fig. 3d).

Discussion

We confirmed that deposition of otolith increments of *S. dumerili* is daily basis in larval and early-juvenile stages. Wells and Rooker (2004b) reported that *S. dumerili* deposit otolith increments on a daily basis in juvenile stage (136-193 mm SL) by a series of alizarin complexone marking experiment. Thus, age determination using sagittal otolith is valid for larval and early-juvenile stages of this species. Growth rate of wild fish was lower than that of reared-fish. Sakakura and Tsukamoto (1997) reported that growth rate of wild early-juvenile yellowtail *S. quinqueradiata* was lower than that of reared-fish, which coincided with our results of *S. dumerili*.

We could collect larval and juvenile *S. dumerili* only around the Penghu Islands, Taiwan in the present study. Larval and juvenile *S. dumerili* had been caught in the coastal waters of Taiwan (Liu 2001, 15 ind.) and in the northeast of Taiwan in June (Chen et al. 2012, 0.13 ind./m^3), indicating that *S. dumerili* in the early life stages distribute around Taiwan. However, *S. dumerili* was not collected by surface towing of a larval net from our two preliminary surveys in 2015. The first was the cruise to cover the north-eastern part of Taiwan (a total of 15 hauls, 2-5 May; 25.0-26.5 °N, 120.5-123.0 °E) by the R/V Fishery Researcher 1 and the second was the cruise to cover the south-western part of Taiwan (a total of 11 hauls, 25-27 July; 22.5-23.5 °N, 118.6-119.8 °E) by the R/V Fishery Researcher 2, Taiwan Fishery Research Institute, Council of Agriculture. In the southern U.S., the spawning area of *S. dumerili* is estimated around the shelf-edge reef sites of 50-100 m depth (Sedberry et al. 2006). Our sampling stations around the Penghu Islands are mostly shallower than 50 m depth (Fig. 1b, Fig. 2), and the spawning area of *S. dumerili* is presumably located in the open water area off the Penghu Islands deeper than 50 m depth in our study period. In May and July 2015, frontal zones were created by the gradient of water temperature around the Penghu Islands. These frontal zones may be formed between coastal water around the Penghu Islands and the water mass intruded from the open sea. It is possible that some of frontal zones were created by Langmuir circulation or internal wave, because the number of CTD data was small to fully explain the formative factors of frontal zones. Further study is needed to understand formative factors of frontal zones around the Penghu Islands. Since larval and juvenile *S. dumerili* were collected only in frontal zones in our study, they may be spawned in the open sea side and eggs or larvae were accumulated in the frontal zones around the Penghu Islands, where total density of larval and juvenile fishes was not different between frontal zones and other stations. Raya and Sabatés (2015) reported that distribution of larval *S. dumerili* was limited by the position of thermal front in the northwestern Mediterranean Sea, which is similar to our study. SST of frontal zones in May ranged from 24.9 to 25.5°C (Table 1), and frontal zones were suitable temperature for larval and juvenile *S. dumerili* because Raya and Sabatés (2015) mentioned that temperature preference of *S. dumerili* larvae is in between 24 and 25 °C. *Seriola dumerili* was not found with drifting seaweeds in this study. Juveniles of *S. dumerili* have been reported to associate with floating objects at 25-297 mm SL (Nakata et al. 1988; Badalamenti et al. 1995; Massutí et al. 1999; Wells and Rooker 2004a, b). Since our specimens were mainly in larval or early-juvenile stage, they may stay in frontal zone until they reach the body size at associating with drifting seaweeds.

The time from fertilization to hatching is only 36 to 45 h in *S. dumerili* at 23.1-23.7 °C (Masuma et al. 1990), so our back-calculated hatching dates indicated that spawning of this species occurred in April and June 2015 around the Penghu Islands. In this study, individuals with similar age were caught in May 2015, suggesting that concurrent spawning event occurred in April around the Penghu Islands. Taking into account the hatching date in 2015 (April and early June), *S. dumerili* may spawn from spring to early summer around the Penghu Islands. In Asian waters, the spawning season of *S. dumerili* is estimated from November to March in South China Sea (off Hainan Island, China to Viet Nam) and from May to June around Japan (Hamada and Soyano 2009). Spawning season of *S. dumerili* around Taiwan seems to be between that of South China Sea and Japan. Previous studies in other waters also showed that the majority of spawning of *S. dumerili* occur between winter to summer: February to April by hatching-date analysis off Galveston, Texas (Wells and Rooker 2004b), January to June with peak spawning in April and May in South Atlantic (Sedberry et al. 2006) and off the Southeastern U.S. Atlantic (Harris et al. 2007), and in winter in the western Atlantic (Fahay 1975) and in June and July in Mediterranean Sea (Raya and Sabatés 2015). Synthesizing these evidences, spawning season of this species may range from winter to summer in Asian waters.

It is reported that densities of larval *S. dumerili* were higher by surface towing than oblique towing and no significant difference in day/night was detected in Mediterranean Sea (Raya and Sabatés 2015). Since number of *S. dumerili* in our study is not enough to reveal its early life history such as distribution and growth rate, in the future study it is needed to increase number of samples including verification of the distributional layer due to diel vertical migration by modifying sampling methods.

Acknowledgments

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台湾澎湖島周辺で採集されたカンパチ***Seriola dumerili***仔稚魚の日齢

長谷川隆真・葉信明・陳君如・郭慶老・河邊玲・阪倉良孝

短報ICRE-D-16-00069R1

カンパチ*Seriola dumerili*の初期生態を調べるために，まず人工種苗（11-51日齢）を用いて耳石日周輪のバリデーションを行った。次に，2015年5月および7月に台湾澎湖島周辺（23.45-23.70 °N, 119.40-119.70 °E）で稚魚ネットの表層曳きにより計4尾のカンパチ仔稚魚を採集した。これらは流れ藻には附随していなかった。カンパチ仔稚魚は水温フロントで採集され，その全長は7.4-42.5 mm，18-56日齢であった。以上の結果からカンパチの孵化日は4月から6月で，カンパチの仔稚魚はフロントに集積されると推測された。

（長谷川・阪倉：〒852-8521　長崎県長崎市文教町 1-14　長崎大学大学院水産・環境科学総合研究科；葉・陳・郭：20246　基隆市和一路 199 號　行政院農業委員会水産試験所；河邊：〒851-2213 長崎県長崎市多以良町 1551-7　長崎大学大学院水産・環境科学総合研究科附属環東シナ海環境資源研究センター）

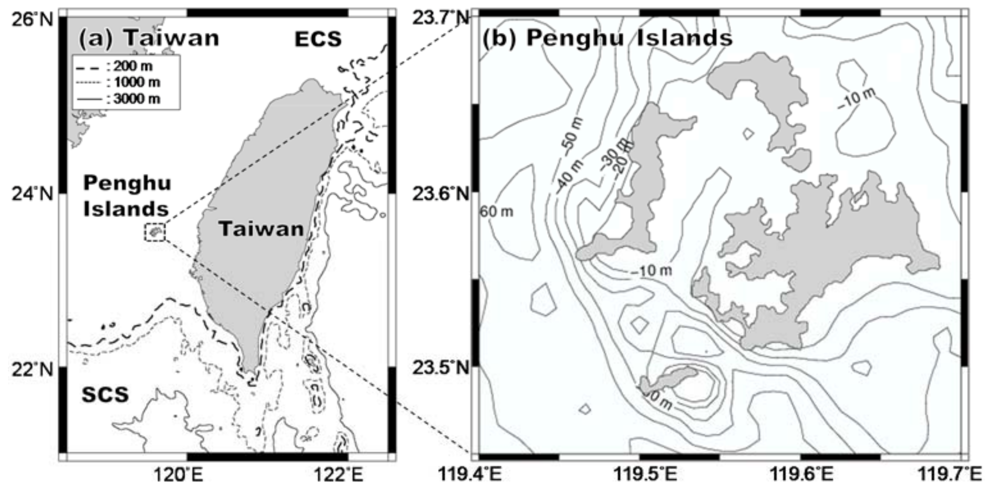
Figure captions

Fig. 1 Map showing (a) geographical location of study area and bathymetric chart around Taiwan, and (b) study area and the bathymetry in meters (*thin solid* contours with numbers) around the Penghu Islands. ECS is the East China Sea and SCS is the South China Sea

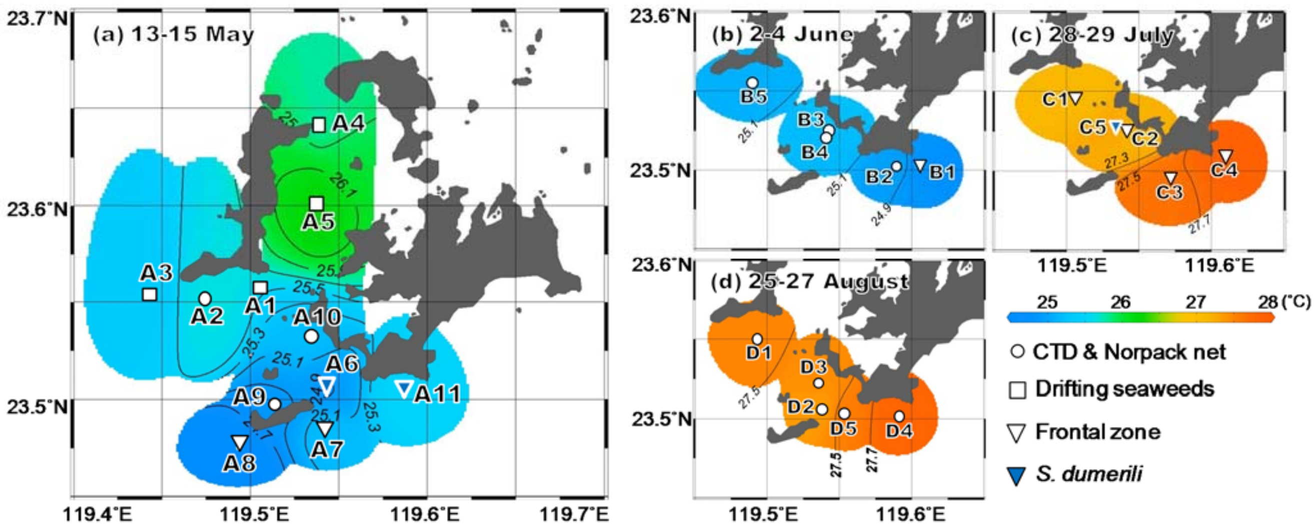
Fig. 2 The estimated sea surface temperature (SST) in (a) 13-15 May, (b) 2-4 June, (c) 28-29 July and (d) 25-27 August 2015 around the Penghu Islands, Taiwan. *Open triangle, square* and *circles* are the stations where frontal zone, drifting seaweeds were found and CTD casting, respectively. *Filled triangles* denote the stations where *S. dumerili* were collected

Fig. 3 Relationship between (a) age (days) and otolith radius (μm), (b) total length (mm) and otolith radius, (c) age and otolith increments and (d) age and total length. Panel (a)-(b) show reared-fish and panel (d) shows reared- and wild-fish. *Break line* in the panel (c) shows the line of Y=X. In the panel (d), *open circles*, *filled circles* and *solid line* indicate reared-fish, wild-fish and growth curve of reared-fish, respectively. The ages of reared-fish are days after hatching, and the ages of wild fish are determined by otolith analysis

Hasegawa et al. Figure 1



Hasegawa et al. Figure 2



Hasegawa et al. Figure 3