

Age and growth of larval Pacific flagfin mojarra (*Eucinostomus currani*) in coastal Ecuador based on otolith analysis

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ABSTRACT. The flagfin mojarra (*Eucinostomus currani*) is an important subsistence fishery resource in local Ecuadorian markets; however, very little is known about the early life history and reproductive biology of this species. In 2016 and 2017, *E. currani* larvae were collected at 3 sandy beaches in the Gulf of Guayaquil, Ecuador, and identified using DNA barcoding. Standard lengths ranged from 4.00–15.78 mm. We also collected otoliths to better understand the early life history of *E. currani*. Otoliths were used to estimate the age, population growth rate (\pm SE), hatch dates, and reproductive period. On average, the fish were 16.5 ± 4.5 days old, while the average growth rate was 0.70 ± 0.05 and 0.22 ± 0.16 mm per day in March and May, respectively. In the Gulf of Guayaquil, most hatch dates and the presumed reproductive activity of *E. currani* occurred during the wet season (December–April) when warmer water prevailed. To our knowledge, this is the first study to estimate the hatch dates and larval growth rate for a species of the family Gerreidae in the Pacific Ocean.

Key words: otolith, Gerreidae, Ecuador, larva, reproduction, surf zone.

INTRODUCTION

The Pacific flagfin mojarra (*Eucinostomus currani*) ranges from southern California in the United States (Moser 1996) to Peru, including the Galapagos Islands and Cocos Island (Froese and Pauly 2022). Adult *E. currani* in the Gulf of California and along the western coast of Mexico are found on the shallow continental shelf and in coastal lagoons from 0–30 m depth (López-Martínez et al. 2011). In Ecuador, fishery catches indicate that *E. currani* is distributed along the open coast and in the Gulf of Guayaquil, with no catches recorded in the Guayaquil River Estuary (Herrera et al. 2017). In Ecuador, *E. currani* is the target of an unregulated fishery

employing various gear types (gillnets, throw-nets, and beach seines) with catch destined for local consumption and fish meal production (Herrera et al. 2017).

The estimated reproductive period of *E. currani* in the Gulf of California occurs from March to August (López-Martínez et al. 2011) when adults release planktonic eggs (Johnson 1984). For *E. currani* in Baja California, the larval hatch length is <2.1 mm, with flexion occurring from 4.0–5.0 mm, postflexion occurring from 5.6–12.45 mm, and prejuvenile to juvenile lengths ranging from 12.9 to 30 mm (Jiménez-Rosenberg et al. 2006). Larvae of this species are pelagic, whereas juveniles are found in a variety of habitats, including rivers, lagoons, mangroves, river deltas, sandy

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beach surf zones, and in the open ocean up to 20 km offshore (Lyons and Schneider 1990, Aceves-Medina et al. 2004, López-Martínez et al. 2011). Lagoons and estuaries appear to serve as nursery and rearing environments (Ramos-Lozano et al. 2015), while reproduction occurs in the ocean.

Here, we report the distribution and life history characteristics of *E. currani* larvae in the sandy beach surf zone within and around the Gulf of Guayaquil, Ecuador. This study aimed to estimate the age and growth of larval *E. currani* and explore their reproductive period in coastal sites. We collected fish larvae from 3 beaches in 2016 and 2017, employed DNA barcoding to identify species of larvae, and analyzed otoliths to estimate age (d), hatch date, and growth rates (mm/d). Assessing larval fish age and growth is important because these factors can elucidate how the environment influences growth and survival while providing vital information for management decisions such as establishing fishery closures (Stevenson and Campana 1992).

MATERIALS AND METHODS

This research aimed to study the larval fish assemblages of sandy beach surf zones along the southern coast of Ecuador and catalog the distribution patterns of the northern anchovy (*Engraulis mordax*) and corvina (*Cynoscion* spp.), which have been documented in these areas (Marin Jarrin et al. 2015, 2017). However, few individuals of the target species were found. Nonetheless, one larval morphotype was prevalent in the samples and later identified as *E. currani*, a member of the family Gerreidae.

Study site

The Gulf of Guayaquil spans 13,000 km² and is bounded in the north by the Peninsula Santa Elena, in the south by the Tumbes region of Peru, and in the west by the continental shelf break. The Guayas River empties into the gulf, forming the largest estuary on the Pacific coast of South America. Near-shore waters in the Gulf of Guayaquil are influenced by the Guayas Estuary plume and by larger-scale, seasonal oceanographic processes, including latitudinal stratification and the cold waters of the Humboldt Current (Marin Jarrin and Lippmann 2019).

Three sites on the north side of the Gulf of Guayaquil (Reynaud et al. 2018) were surveyed to characterize the physical habitat and the presence of larval fish: (1) Data de Posorja (Data), (2) General Villamil Playas (Playas), and (3) Chipipe/Salinas (Chipipe) (Fig. 1). Surveys occurred once per site every 3 months from November 2016 to November 2017, except the survey scheduled for February, which was conducted in March. This schedule encompassed the dry/cool (June–October) and rainy/warm (December–April) seasons and transitional months. This study took place during El Niño (2016) and neutral (2017) El Niño Southern Oscillation

conditions (NOAA Physical Sciences Laboratory 2022). All procedures involving animals complied with the Ecuadorean Ministry of Environment regulations regarding the ethical treatment of animals.

Sampling and data collection

At each location, we recorded water temperature (°C), dissolved oxygen (mg/L and % saturation), salinity (practical salinity scale [PSS]), conductivity (ms/cm), pH, and total dissolved solids (mg/L) at a depth of 0.5 m with an HI 2898 multi-parameter meter (Hanna Instruments, Smithfield, USA), which was calibrated before each field trip (Supplementary Material Table S1). Larval fish were collected at a depth of 1 m with a hyperbenthic sled with a 1-mm mesh conical net (50 cm × 70 cm × 4 m), which was pulled by hand for 100 m parallel to the coastline and back again (total of 200 m). The bottom of the net was positioned 5 cm above the seafloor to avoid collecting large amounts of sand. Three tows were taken per beach per sampling day for a total of 45 samples, which were preserved in 96% EtOH (Table 1). Fish catches are reported as individuals per 100 m².

Larvae identification

Fish larvae were initially identified in the laboratory based on morphological characteristics following the methods of Moser (1996) and Jimenez-Rosenberg et al. (2006). Individuals were photographed at 4× magnification using a DS-Fi3 camera (Nikon, Minato City, Japan) mounted on a Nikon SMZ-745 dissecting scope. Standard length (SL, mm) was

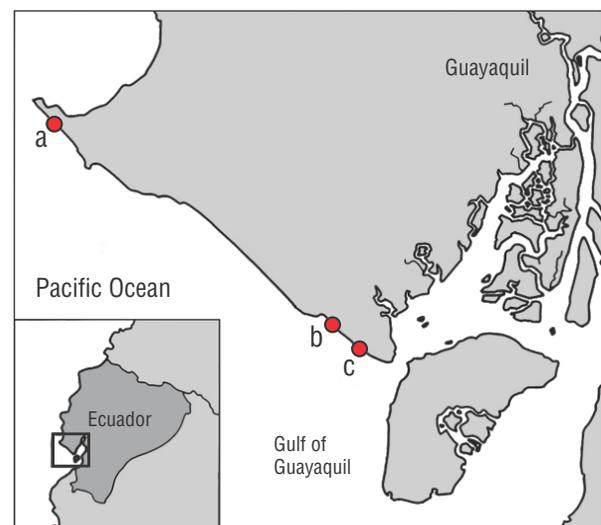


Figure 1. Map of survey locations: (a) Chipipe/Salinas (2°14' S, 80° 57' W), (b) General Villamil Playas (Playas) (2°38' S, 80°23' W), and (c) Data de Posorja (Data) (2°42' S, 80°19' W).

Table 1. Catch (ind per 100 m², \pm SD), number of otoliths analyzed, and average standard length (SL) of *Eucinostomus currani* and water temperature for the sites of Chipipe/Salinas (Chipipe), General Villamil Playas (Playas), and Data de Posorja (Data) for each sampling date.

Date	Catch of <i>E. currani</i> morphotype			Samples with identified otoliths		Average SL		Water temperature (°)		
	Data	Playas	Chipipe	Data	Playas	Data	Playas	Data	Playas	Chipipe
November 2016	5.7 \pm 6.1	1.9 \pm 1.1	0	0	0	--	--	26.19	26.98	25.79
March 2017	14.5 \pm 16.6	3.8 \pm 3.9	0	30	12	13.9	6.72	30.02	29.65	28.81
May 2017	0	4.8 \pm 0.8	0	0	18	--	8.39	25.49	26.29	24.46
August 2017	0	0.5 \pm 0.8	0	0	1	--	8.57	26.23	24.86	23.26
November 2017	0	0	0	0	1	--	9.25	25.54	26.59	24.57

determined from the photographs using NIS-ELEMENTS v. 5.02 (Nikon).

The available larval identification guide that contains eastern tropical Pacific species (Moser 1996) provides no differentiating characteristics for the 3 members of the genus *Eucinostomus* and notes that larval gerreid species in the California Current cannot be differentiated due to overlapping morphological characteristics (Jimenez-Rosenberg et al. 2006). Information on the larval stages of the 5 species of *Eucinostomus* and other gerreids present in Ecuador is even more limited.

The initial morphological identification of the larvae was supplemented with DNA barcoding using a 572-bp fragment of the mitochondrial 16S ribosomal RNA gene. The mtDNA was extracted from fin tissue using chelex methods (Walsh et al. 1991) and amplified via PCR using the following reactant concentrations and amounts: 1 μ L forward primer (400 nM) (16Sar) (Palumbi 1996), 1 μ L of reverse primer (400 nM) (16sbr) (Palumbi, 1996), 8.5 μ L nuclease-free water, 12.5 μ L PCR M7505 Master Mix (Promega, Madison, USA), and 2 μ L DNA template. The thermocycling profile consisted of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 52 °C for 40 s, and 72 °C for 10 min. Amplified products were Sanger sequenced at MCLAB (San Francisco, USA), and NCBI BLASTn was used to determine their identity.

Otolith analysis

Lapilli otoliths were extracted from larvae under a dissecting scope, and the left one was mounted on a slide when available. Lapilli otoliths have been used to estimate the age of other juvenile fish (Morales-Nin et al. 1999). In the present study, lapilli otoliths were selected over sagittae otoliths because lapilli otoliths exhibited clearly visible increments from the core to the edge and did not require polishing, which would have been particularly difficult due to their size. The otoliths were photographed using a CX31 microscope at 100 \times magnification (Olympus, Shinjuku City, Japan), and

NIS-ELEMENTS v. 5.02 (Nikon) was used to measure otolith diameter (OL). Daily rings were visible and read twice by one person from the nucleus to the edge (Stevenson and Campana 1992).

Chronological growth increments have not been evaluated for this species but were assumed to have a daily periodicity or rings (opaque + transparent zone) (Stevenson and Campana 1992). However, otolith growth increment analysis is based on the assumption that larger fish are older than smaller fish and that larger fish have larger otoliths, which is the basis of otolith analysis. To test this assumption, we examined the residuals of the relationships between larval SL and OL and larval SL and the number of rings. The coefficient of variation (CV) and average percent error (APE) (Beamish and Fourier 1981) between the 2 readings were calculated to evaluate bias and error.

Hatch dates were calculated by subtracting the number of otolith rings from the capture date. The available information indicated that the period from fertilization to hatching is short in Gerreidae (*Eugerres lineatus*, 3 days [Ortiz-Galindo et al. 2008]; *Eugerres mexicanus*, 18 h [Hernández et al. 2012]). Reproduction was assumed to occur approximately 1–5 days before the hatching date.

Statistical analysis

Catch (ind/100 m²) was statistically compared among months and sites using a two-way ANOVA that included an interaction term. Parametric assumptions were tested using quantile-quantile plots to evaluate data normality, and homogeneity of variances was evaluated with Levene's test after the data were transformed ($\log_{10} [x + 0.1]$). Post-hoc pair-wise comparisons were conducted using Tukey's Honest Significant Differences test. Growth rates were calculated as the slopes of the linear regressions of an analysis of covariance (ANCOVA) to account for water temperature variations among seasons. The ANCOVA included age (days) as the explanatory variable. Standard

length (quantitative [mm]), season (categorical), and their interaction were included as response variables (Peebles and Tolley 1988). Due to small sample sizes, only data from March and May 2017 were included. The standard error of the slopes was calculated as the average distance that the observed values deviated from the regression line. Other models (e.g., exponential) were explored visually but did not provide better fits than the linear models. Parametric assumptions were tested as described above. Flexion class was determined by length following the methods of Jiménez-Rosenberg et al. (2006), who used similar preservation methods.

RESULTS

Of the 374 fish larvae collected, 119 were identified as *E. curranii*, of which 77 larvae were collected from Data and 46 larvae were collected from Playas (Table 1). No larvae of this species were collected at Chipipe. Standard lengths ranged from 4.00–15.78 mm. Catch varied from 0–33.6 ind·100 m⁻² (average of 3.0 ind·100 m⁻²) and was positively correlated with water temperature ($n = 10$, $r = 0.73$, $P = 0.01$), which varied from 24.86–30.02 °C. No other environmental variable was significantly correlated with larvae catch per date (Supplementary Material Table S1). A significant interaction between month and site was found ($F_{(3,21)} = 3.73$, $P = 0.03$), with catch values at Data being significantly higher in March in the middle of the wet/warm season than in the transitional month of May or the dry/cool month of August ($P = 0.02$). No differences were observed at Playas ($P > 0.05$).

Lapilli otoliths of 62 mojarra were examined (30 from Data on a single date and 32 from Playas over 4 dates) (Table 1). Of these, 15 fish (all from Data) were identified as *E. curranii* using DNA barcoding (100% identity). The remaining 47 fish were classified as *E. curranii* due to similar external features, otolith morphology, and growth patterns (Fig. 2). The 2 otolith reads by the same reader yielded a CV of 7.75 ($SD = 7.2$) and an APE of 5.40 ($SD = 4.96$). Standard length was significantly and positively correlated to OL ($R^2 = 0.97$, $P < 0.0001$) and age (average number of rings, $R^2 = 0.83$, $P < 0.0001$) (Fig. 2).

The ages of the 62 fish ranged from 6 to 24 days. The average age of fish from Data in March 2017 ($n = 30$) was 19.72 days. In Playas, the average age of fish in March was 11.5 days, whereas the average age of fish in May was 14.2 days (Fig. 2). Aged fish were in the flexion ($n = 6$), post-flexion ($n = 28$), and juvenile ($n = 28$) stages. Postflexion and juvenile larvae were caught at Data. Larvae in flexion, post-flexion, and juvenile stages were caught in Playas in March 2017, while only postflexion larvae were caught in May. The ANCOVA model exhibited a significant interaction ($F_{(1, 56)} = -2.952$, $P = 0.005$), suggesting that the growth rates were different between the 2 months (average \pm SE: 0.70 ± 0.05 in March and 0.22 ± 0.16 mm in May).

DISCUSSION

The distribution of adult *E. curranii* in the Gulf of California and along the western coast of Mexico is influenced by the movements of tropical and subtropical water masses, with *E. curranii* showing a preference for tropical waters (López-Martínez et al. 2009). Reproduction of *E. curranii* in the Gulf of California occurs from March to August, based on the recruitment peaks recorded by the commercial fishery and a study of female gonads (López-Martínez et al. 2011). A similar pattern emerged in this study, with the associated hatch date data indicating that peak reproduction occurred in February and March ($n = 42$ larvae). These months correspond to the warm, wet season when the area is influenced by tropical water masses (Marin Jarrin and Lippmann 2019). Reproduction also occurred during the dry season when the cold Humboldt Current influenced Ecuadorian waters. However, reproduction in the dry season and

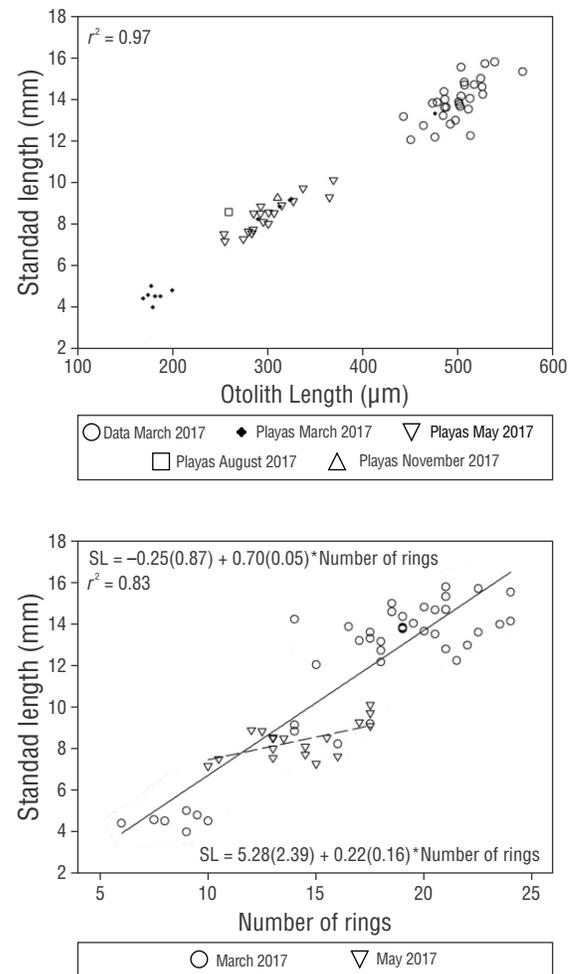


Figure 2. Standard length (mm) by otolith length (μm) and by number of rings, which is assumed to be the age of the fish.

transitional months was much lower than in the wet season (transitional months of April and May and colder water months of July and November; $n = 20$).

Temperature was measured at depths of less than 1 m and was warmest in March. The water temperature in Data and Playas when *E. currani* larvae were captured ranged from 24.8 °C to 30.0 °C, with the highest catch recorded when temperatures were between 26.3 °C to 30.0 °C. The collection of *E. currani* larvae was positively correlated with water temperature and was significantly higher during the wet/warm season at one of the 2 beaches. Water temperature was always at least 1 °C lower at Chipipe than at Data or Playas (which may be under the heavier influence of Guayas River outflow) during the same sampling cycle. This difference may explain the lack of larvae captured at Chipipe, which is located near the tip of the Santa Elena Peninsula and closer to the continental shelf. Additionally, the ocean currents around Santa Elena, the outermost point of land in western South America, may not promote larval retention.

An estimation of age (d) and growth rates using otoliths has not been previously conducted for *E. currani*. The evaluations of SL, OL, and age residuals indicated that the assumptions of otolith analysis were met. The growth rates of the larvae recorded in this study (0.7 and 0.22 mm per day) were similar to those of the only other study of Gerridae larvae. In that study, Herrera-Reveles et al. (2012) reported growth rates of 0.18–0.42 mm per day in juvenile (21–73 days) *Eucinostomus argenteus* in coastal Venezuela. However, these rates are relatively fast compared to other surf-zone Eupercarids (*Cynoscion nebulosus*: 0.40–0.42 mm per day [Peebles and Tolley 1988]; *Cynoscion* spp.: 0.02 mm per day [Andrade Vera et al. 2017]), indicating that nearshore and sandy beach environments adequately support larval and juvenile growth of mojarra species.

To our knowledge, this is the first study to estimate the hatch dates and larval growth rate of a species of the Family Gerreidae on a Pacific coast. In the Gulf of Guayaquil, *E. currani* exhibited a reproductive peak during the wet season when warmer water prevailed. Finally, our results confirm sandy beach surf zones as rearing sites for larval fish. Future research should explore juvenile and adult use of estuarine and gulf habitats and reproduction to provide valuable knowledge of this species with little available information.

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SUPPLEMENTARY MATERIAL

The supplementary material for this work can be downloaded from: <https://www.cienciasmarinas.com.mx/index.php/cmarinas/article/view/3448/420421027>.

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