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Early-stage growth and survival of *Centropomus nigrescens* larvae (Actinopterygii, Carangaria incertae sedis, Centropomidae) reared under pilot-scale hatchery conditions

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Abstract

This study reports pilot-scale larviculture trials of black snook, *Centropomus nigrescens* Günther, 1864, a candidate species for aquaculture diversification in the Eastern Tropical Pacific. Fertilized eggs (0.723–0.740 mm diameter) were obtained from spawning induced in broodstock captured from the wild as juveniles and reared in captivity for four years, exhibiting a spherical shape, transparency, buoyancy, and a single oil globule. Two larval culture trials (ER1 and ER2) conducted under hatchery conditions at 24.29 ± 0.13°C and 26.85 ± 0.18°C, respectively, employed a pseudo-green water technique and live feed protocols including enriched rotifers (20–40 ind · mL^{-1}) and *Artemia* (0.5–4 ind · mL^{-1}). Larvae were initially stocked at 10 larvae L^{-1} (ER1) and 60 larvae · L^{-1} (ER2). Growth followed an exponential model, with faster growth rate in ER2. Mean survival rates were 7.42% (ER1) and 6.84% (ER2) at the end of the trials. A total of 17 898 black snook juveniles were produced across both trials (60 and 45 days post hatch, respectively). Cannibalism, parasitic infections (*Amyloodinium* sp.), and skeletal anomalies were identified as the main bottlenecks affecting survival and juvenile quality. These findings provide preliminary operational insights and represent an initial step towards the development of larviculture protocols for this species.

Keywords

black snook, cannibalism, diversification, husbandry, larval growth, pilot-scale

Introduction

The development of marine fish aquaculture in South America remains limited, with production (1 563 055 tonnes in 2022) concentrated in a small number of species, primarily anadromous salmonids (*Salmo salar*; *Oncorhynchus kisutch*; FAO 2024). Expanding species diversity is essential to strengthen regional food security, optimise infrastructure use, and mitigate risks associated with monoculture. In this context, *Centropomus nigrescens* Günther, 1864, commonly known as black snook, a euryhaline carnivorous species native to the Eastern Tropical Pacific, has emerged as a promising candidate for aquaculture diversification due to its fast growth, high flesh quality, and local market acceptance (Resley et al. 2014; Zambrano et al. 2022).

While aspects of the reproductive biology of *C. nigrescens* have been addressed in previous studies including hormonal induction of spawning and limited descriptions of larval development (Carvajal 1997; Resley et al. 2014; Yanes-Roca et al. 2018), comprehensive and standardized larviculture protocols are still lacking.

To bolster aquaculture diversification efforts within South America, experimental initiatives are presently underway at the National Centre for Aquaculture and Marine Research (CENAIM-ESPOL), with a focus on broodstock management and larval rearing of black snook. Small-scale larviculture trials represent a critical step towards species domestication and the formulation of effective rearing strategies. This approach allows for the identification of key biological constraints such as cannibalism, deformities, and mortality patterns under manageable experimental conditions. However, limited data are currently available regarding larval performance of *C. nigrescens* at this stage, and survival rates remain low and variable. To address these gaps, this study provides the first reports of pilot-scale larval rearing of *C. nigrescens*, including baseline data on growth, survival, and mortality factors, and identifying key biological and operational constraints that could guide the development of effective larviculture protocols for this species.

Materials and methods

Source of fish and egg collection. Wild juveniles of black snook, *Centropomus nigrescens* (body weight, BW = 0.13–0.49 kg; total length, TL = 23.0–40.0 cm), were collected from a shrimp farm reservoir located in the Palmar Estuary of Santa Elena Province, Ecuador (2°00′51.40′′S, 080°43′21.17′′W), using seine nets. The fish were held in captivity at the National Centre for Aquaculture and Marine Research (CENAIM–ESPOL) under natural photothermal conditions (22–28°C; photoperiod 12L:12D) for 4 years until reaching reproductive maturity. Fish were fed three times per week ad libitum with raw frozen local herring, mackerel, and squid, supplemented with commercially available vitamin and mineral premixes (Nutravit fish®; Farmavit Shrimp®). Further cannulations for sexing and evaluations of maturity stage were made to all groups of captured fish. Fertilized eggs were obtained from four mature females and four males (mean BW = 8.43 ± 0.28 kg and TL: 97.48 ± 1.02 cm) via intramuscular injection of human chorionic gonadotropin (HCG), following institutional broodstock management protocols. In first event (ER1) both females and males received 1000 UI· kg^{-1}, whereas in the second event (ER2) females received 880 UI· kg^{-1} and males 370 UI· kg^{-1}. The latency period between injection and spawning is reported in Table 1. Two spawning events were used to initiate the larval rearing trials. The same broodstock were used in both trials. Egg quantity was estimated by counting three randomly selected sub-samples (3–5 mL), and viable eggs were incubated in 500 L black conical-bottom tanks (~2000 eggs· L^{-1}) with constant aeration and ambient seawater (temperature 24–28°C; salinity 33–34 g· L^{-1}; dissolved oxygen concentration > 5 mg· L^{-1}) flow until hatching.

Small-scale larval rearing. Two larval rearing trials, ER1 and ER2 (one for each spawning event), were conducted, each in duplicate (n = 2 tanks) using blue circular tanks of 1000 L (ER1) and 2000 L (ER2) capacity. All tanks were supplied with UV-sterilized ambient seawater and maintained under natural photoperiod conditions. At 2 days post hatch (DPH), larvae were counted and transferred from the incubation tanks to the larval rearing units at a density of 10 larvae· L^{-1} (ER1) and 60 larvae· L^{-1} (ER2). Water temperature and dissolved oxygen were monitored daily. ER1 was maintained at 24.29 ± 0.13°C, 34 g· L^{-1} salinity and 6.73 ± 0.04 mg L^{-1} dissolved oxygen (DO), while ER2 averaged 26.85 ± 0.18°C, 34 g· L^{-1} and 6.52 ± 0.04 mg· L^{-1} DO. A pseudo-green water method (Papandroulakis et al. 2001), characterized by the daily addition of green algae, *Tetraselmis maculata* Butcher, 1959 (1 × 10^5 cells· mL^{-1}) and live zooplankton, was maintained from 1 to 40 DPH. The rearing system operated as an open flow-through system with an initial semi-static phase, with 5%–10% daily water exchange until 20 DPH, after which the exchange rate was gradually increased. Each tank was equipped with surface skimmers to remove debris and oil films, and continuous aeration was provided to maintain water quality.

The timing of live prey administration differed between trials, although prey densities were similar. In both ER1 and ER2, enriched rotifers, *Brachionus rotundiformis* Tschugunoff, 1921, were supplied and adjusted at 20–40 ind · mL^{-1}, *Artemia* nauplii at 0.5–1 ind · mL^{-1}, and enriched *Artemia* metanauplii at 1–4 ind · mL^{-1}. In ER1, rotifers were supplied from 2 to 37 DPH, *Artemia* nauplii from 28 to 44 DPH, and enriched *Artemia* metanauplii from 31 to 64 DPH. In ER2, rotifers were provided from 2 to 25 DPH, *Artemia* nauplii from 19 to 30 DPH and enriched *Artemia* metanauplii from 27 to 39 DPH. All live prey were enriched with ALGAMAC 3050® prior to administration. From 25 DPH onward, larvae were co-fed a commercial microdiet (Otohime B1®), with weaning achieved at 42 DPH in ER1 and 32 DPH in ER2. Complete weaning was reached at 60 DPH (ER1) and 40 DPH (ER2). Larval growth was monitored weekly from hatching to 52 DPH by measuring the TL of 4–10 randomly sampled individuals. Daily microscopic examinations were conducted to confirm feed ingestion and detect protozoan infestations. Infections by *Vorticella* sp. were treated with a single application of 100 mg· L^{-1} formalin, whereas *Amyloodinium* sp. required repeated treatments with

0.3 mg· L^{-1} copper sulphate for up to seven consecutive days. At 60 DPH (ER1) and 45 DPH (ER2), juveniles were counted and transferred to 5000 L oval blue tanks. In both trials, juveniles were separated into two size classes based on macroscopic differences in body size. In ER1, 1544 juveniles were allocated to two tanks (1383 and 161 per tank); in ER2, 16 354 juveniles were distributed into three tanks at densities of 4662, 6084, and 5608 per tank. Juveniles were fed Otohime® B2, C1 or Zeigler Shrimp Starter 1 or 2 (Zeigler®) depending on developmental stage.

Data analysis. Larval survival (S) was calculated as a percentage using the formula

$$S = 100 \times \frac{N_f}{N_i}$$

where N_f is the number of surviving larvae and N_i is the initial number of larvae stocked. Best fit growth curves for total length (TL) were estimated using an exponential model

$$Y = ae^{bX}$$

where Y is the predicted TL [mm]. The parameter a represents the initial TL estimate [mm], b is the growth coefficient [mm· day^{-1}], X denotes DPH, and e is Euler's number. To explore differences in larval survival and growth between ER1 and ER2, a t-test ($P < 0.05$) was performed using XLSTAT® 2017.1.1 software (Addinsoft, Paris, France). Results are reported as mean ± standard error (SE). Due to the limited number of replicates ($n = 2$ per trial), statistical comparisons were performed to explore general trends rather than to infer robust conclusions. Results should be interpreted cautiously and considered as descriptive.

Results

Egg characteristics and larval hatching. The fertilized eggs of black snook, *Centropomus nigrescens*, were pelagic, transparent, and spherical, containing a single oil globule. The mean egg diameter was 0.732 ± 0.012 mm. Hatching occurred approximately 24–25 h post-fertilization under ambient temperature conditions (ER1 = 24.4°C; ER2 = 27.9°C) with the highest hatching rate (36.4%) recorded in ER2 (Table 1). A visual sequence of oocyte maturation and key embryonic and larval development stages is presented in Fig. 1.

Small-scale larval rearing. Newly hatched larvae exhibited a mean TL of 1.59 ± 0.02 mm in ER1 and 1.64 ± 0.01 mm in ER2. The growth patterns followed an exponential relationship with age (Fig. 2). Statistically significant differences ($P < 0.05$) were found between the two trials in both initial TL (parameter a) and the growth rate (parameter b), with ER2 exhibiting a 42.9% higher growth rate compared with ER1. The exponential model demonstrated a strong fit, with coefficients of determination of $R^2 = 0.93$ for ER1 and $R^2 = 0.98$ for ER2. Notochord flexion was first observed at 15 DPH in ER1 (TL = 4.40 ± 0.09 mm) and 11 DPH in ER2 (TL = 4.53 ± 0.11 mm), while the post-flexion stage, characterized by fin formation, was reached at 42 DPH in ER1 (TL = 8.18 ± 0.74 mm) and 25 DPH in ER2 (TL = 7.91 ± 0.11 mm). During and following the weaning phase, juveniles exhibited a more elongated body shape and reduced pigmentation compared to adults.

Cannibalism accounted for a significant portion of mortality, evidenced by distended digestive tracts in larger individuals, along with fin damage and signs of emaciation in others (Fig. 3a). Affected groups were subsequently size-graded and transferred to 5000 L oval tanks. In addition to cannibalism-related losses, epizootics caused by ectoparasitic dinoflagellates were observed during the larval rearing period. In particular, infections by *Amyloodinium* sp. were confirmed through microscopic examination, with parasites clearly detected on the gill epithelium, skin, and fin surfaces of affected juveniles, as illustrated in Fig. 3b. These outbreaks were managed using copper sulphate treatments as described in the methodology. Survival was assessed at 60 DPH in ER1 and 45 DPH in ER2 (Table 1). Mean survival rates were 7.42 ± 4.07% in ER1 (1544 juveniles) and 6.84 ± 1.15% in ER2 (16 354 juveniles), with no statistically significant difference observed ($P > 0.05$), resulting in a total of 17 898 juveniles produced across both trials. Skeletal abnormalities were observed in older juveniles from both spawns. Abnormalities were not estimated in the ER1. In ER2, at 110 DPH (TL = 92.8 ± 1.7 mm), a considerable proportion (51.9 ± 3.9%) exhibited skeletal abnormalities. The most prevalent types of abnormalities were primarily spinal (lordosis), and, to a lesser extent, jaw abnormalities (Fig. 4).

Discussion

This study contributes insights into the larval performance of black snook, *Centropomus nigrescens*, under pilot-scale rearing conditions, expanding our

Table 1. Comparison of spawning quality of black snook, *Centropomus nigrescens*.

Spawning code	Latency period HPI*	Total eggs $\times 10^6$	Egg diameter [mm]	Oil globule [mm]	Spawning water [°C]	Total larvae	Hatching [%]	Juvenile survival [%]
ER1	50	2.02	0.740 ± 0.010	0.200 ± 0.005	24.4 °C	20 821	1.24	7.42 ± 4.07
ER2	34	1.93	0.723 ± 0.003	0.203 ± 0.004	27.9 °C	700 639	36.36	6.84 ± 1.15

* Hour post hormonal induction until first spawn.

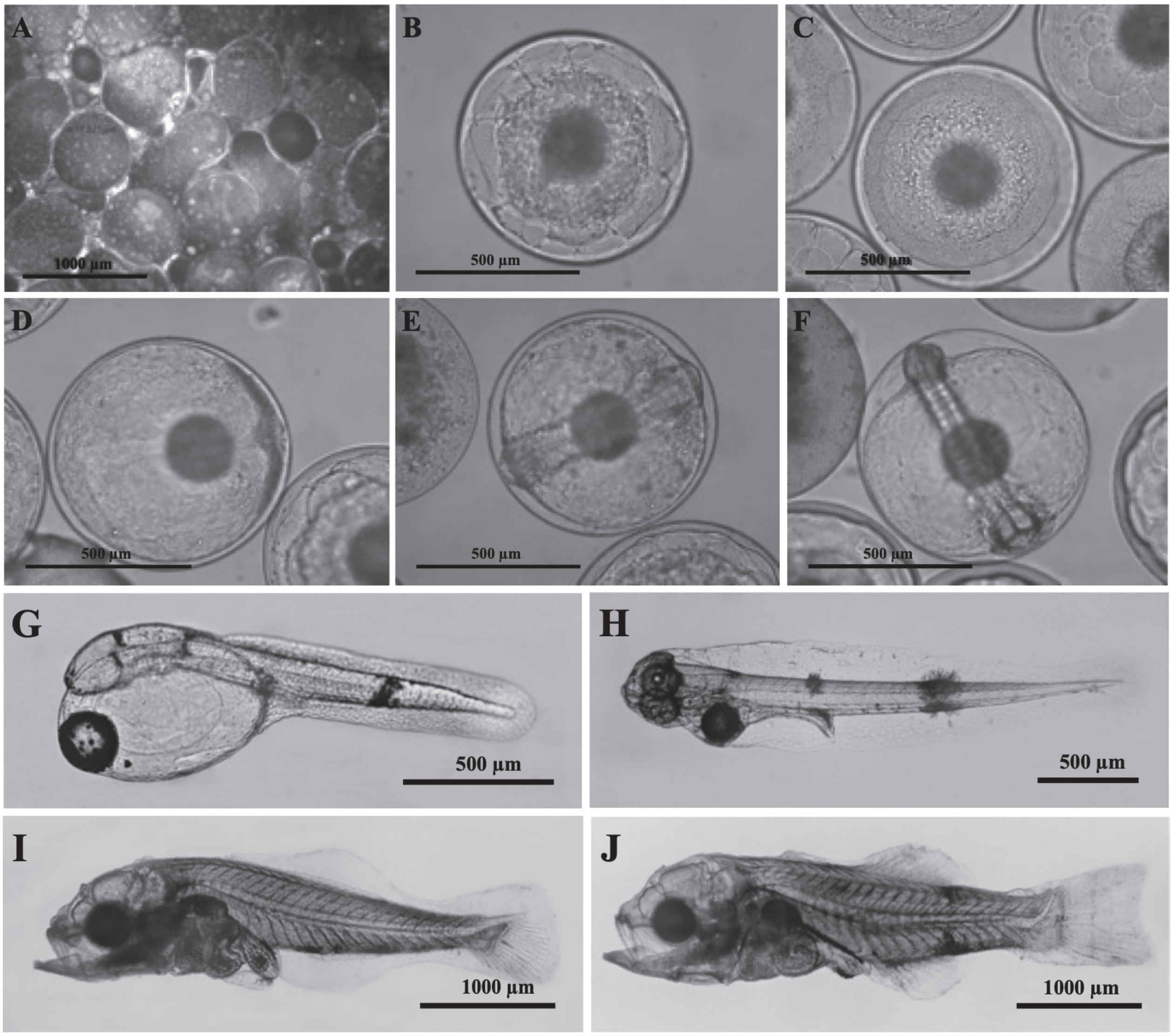

Figure 1. Key embryonic and larval developmental stages of black snook, *Centropomus nigrescens*. Microscope magnification (4×) for embryonic development. **A**) mature oocyte; **B**) early blastula; **C**) early gastrula; **D**) epiboly; **E**) elongating embryo with axis; **F**) late embryo; **G**) newly hatched larva (TL = 1.61mm); **H**) larva with pigmented eyes and absorbed yolk-sac (TL = 2.63mm); **I**) completed notochord flexion (TL = 3.96 mm); **J**) post-flexion larva (TL = 4.41 mm).

foundational knowledge for larviculture protocol development. While earlier investigations have focused primarily on aspects of reproductive biology (Resley et al. 2014; Yanes-Roca et al. 2018) standardized protocols remain unavailable. The induction of spawning in *C. nigrescens* using HCG provided evidence of its effectiveness in promoting final oocyte maturation, ovulation, and gamete release in teleost broodstock, as widely recognized for marine and estuarine species (Cerqueira and Tsuzuki 2009). In the presently reported study, HCG dosages of 880–1000 IU· kg^{-1} BW for females possessing oocytes > 450 µm were sufficient to induce ovulation and fertilization. However, further studies are needed to optimize hormonal induction protocols in *C. nigrescens*.

Larval rearing techniques were based on established methodologies previously applied to other marine fish species at CENAIM but adapted to *C. nigrescens* with modifications in prey availability and enrichment strategies. A pseudo-green water method enhanced environmental conditions (Papandroulakis et al. 2001) through the combined addition of *Tetraselmis maculata* and live prey, promoting better visual contrast for feeding. The administration period and prey densities were tailored per experiment, with ER2 receiving a slightly earlier and shorter schedule than ER1. While the density of rotifers was not systematically evaluated, the protocol aligned with previous reports in snook larvae, where improved survival was linked to increased prey availability. The prey items were: *Centropomus parallelus* Poey, 1860 (see Álvarez-Lajonchère et al. 2002) and *Centropomus undecimalis* (Bloch, 1792) (see Ibarra-Castro et al. 2011). Despite the presence of various influential factors that inevitably led to reduced survival

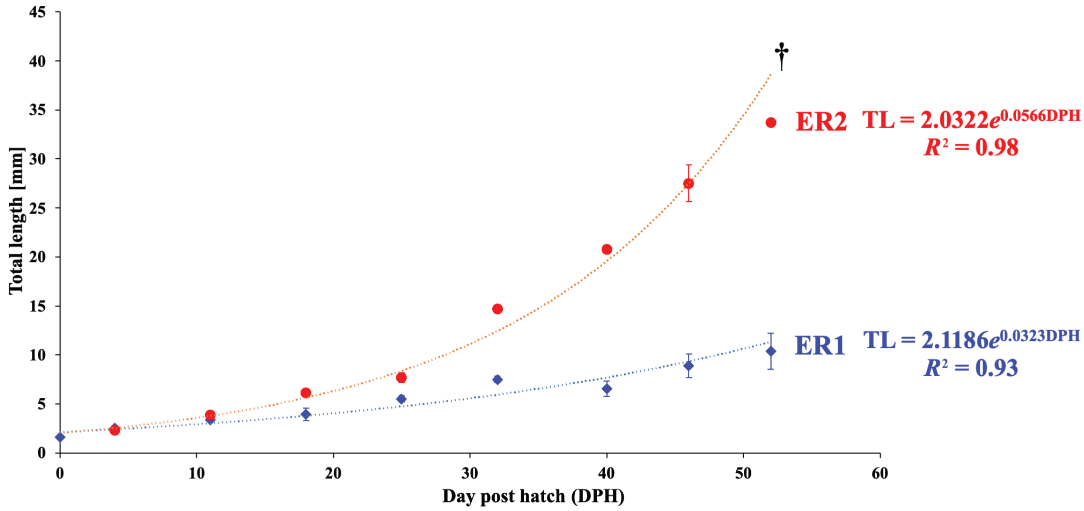

Figure 2. Larval and juvenile total length (TL) of black snook, *Centropomus nigrescens*, up to 52 DPH. Symbol (†) shows significant differences ($P < 0.05$) between ER1 (24.29 ± 0.13°C) and ER2 (26.85 ± 0.18°C).

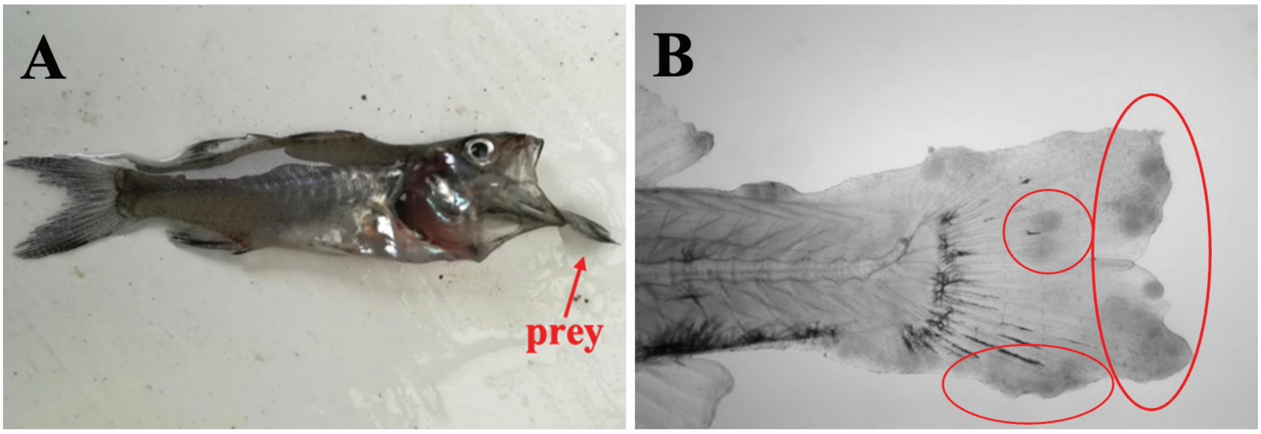

Figure 3. Causative factors for mortality in black snook larval culture. **A**) Intracohort cannibalism type "head-first ingest of the prey"; **B**) *Amyloodinium* sp. presence in tail.

rates in the current study, the obtained results (approximately 7%) are consistent with the range reported for other centropomids cultured under experimental conditions. For instance, Ibarra-Castro et al. (2011) reported total survival rates (from hatching to over 40 DPH) in *C. undecimalis* ranging from 1% to 7%, while *C. parallelus* exhibited survival rates of 4.9% to 6.5%. Notably, exceptional survival rates of 17.7% for *C. undecimalis* (see Ibarra-Castro et al. 2011) and 25.5% for *C. parallelus* (see Álvarez-Lajonchère et al. 2002) were also documented. Observed mortalities during the larval and juvenile stages were associated with intracohort cannibalism, evidenced by predation marks and obstructed digestive tracts (due to head-first ingestion of prey). This behavior, reported in other larviculture studies, may be linked to the establishment of feeding hierarchies or the presence of heterogeneous size distributions (asynchronous growth) within the population, as suggested by previous studies (Baras and Jobling 2002). However, additional investigations focusing on the behavioral and bioenergetic aspects of cannibalism are warranted to gain a comprehensive understanding of this phenomenon. From a management perspective, regular size grading and adjustments of stocking density are recommended to mitigate cannibalism-related losses. Although less prevalent, parasitic infections, particularly *Amyloodinium* sp. were detected microscopically and likely contributed to reduced vitality in some individuals. Effective management of protozoan infections through water quality control and improved prophylactic treatments is imperative to reduce the impact of parasitic outbreaks during larviculture.

The larval growth performance observed in this study exhibited similarities to previous findings reported by Yanes-Roca et al. (2018) in *C. nigrescens*, as well as in other species such as *Lota lota* (Linnaeus, 1758) (see Kupren et al. 2014) and *Seriolella violacea* Guichenot, 1848 (see Argüello-Guevara et al. 2017). This growth pattern is characterized by an initial phase of slow growth during the early days post hatch, particularly during the period of rotifer feeding, followed by a subsequent rapid growth acceleration upon the inclusion

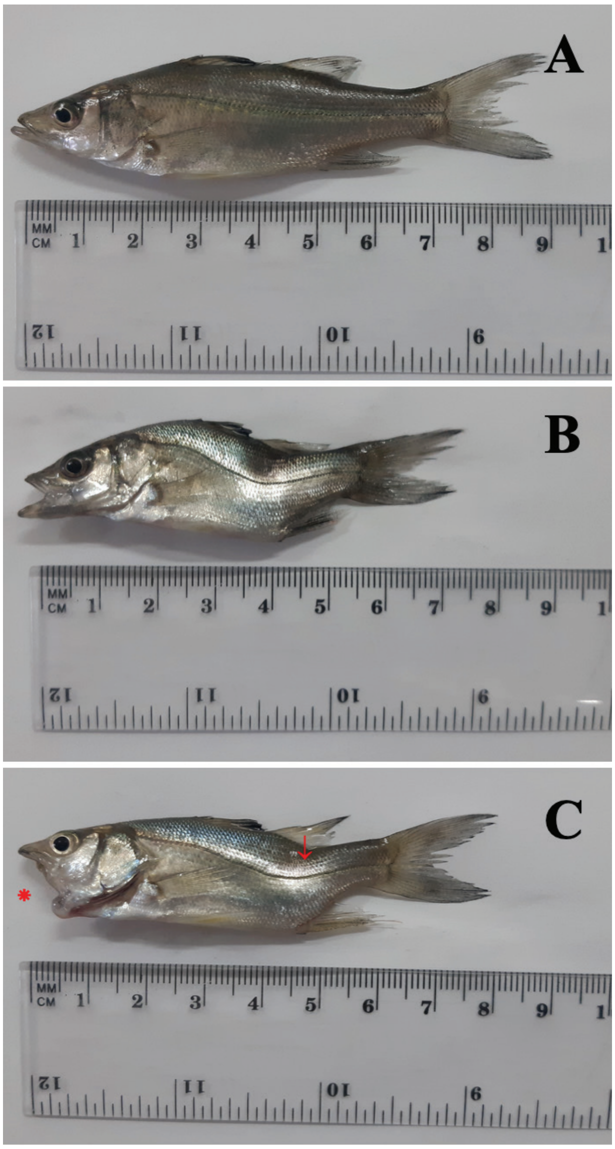

Figure 4. Normal and abnormal juveniles of *Centropomus nigrescens*. **A**) normal phenotype; **B**) lordosis; **C**) marked anomalies in the splanchnocranium (*) and concurrent lordosis (↓).

of *Artemia* in the diet. This transition in growth coincides with an increased feeding activity exhibited by the larvae. Based on previous studies by Gamito (1998) and Santos et al. (2008), the exponential model has been widely accepted as an appropriate framework for describing growth performance in fish. In this model, the *b* parameter represents the specific growth rate and remains constant. The growth analysis of the larvae in ER1 suggested a slower rate of growth [mm· day^{-1}] compared to the larvae in ER2. The nearly twofold growth rate at 27°C compared to 24°C highlights the importance of thermal management in hatchery protocols for black snook. Nevertheless, this interpretation should be made with caution, as additional variables may also have influenced the observed differences. Furthermore, the higher temperature in ER2 expedited key ontogenetic processes, including notochord flexion, fins formation, and metamorphosis. Optimal water temperature ranges for fish are typically narrow, reinforcing the importance of precise temperature control for optimal growth outcomes.

The occurrence of skeletal abnormalities in cultured fish poses significant challenges to commercial aquaculture, giving rise to concerns related to economic viability, biological implications, and animal welfare considerations (Boglione et al. 2013a). The prevalence of spinal axis anomalies, specifically lordosis, was observed to be higher in the current study. In previous studies, a lower occurrence of similar skeletal anomalies was reported in *C. undecimalis*, with percentages ranging from less than 2% (Ibarra-Castro et al. 2011) to 18% (Sanches et al. 2013). Identifying the potential causative factors responsible for these skeletal anomalies was not within the scope of this study. However, existing literature suggests that unfavorable abiotic conditions, inadequate nutrition, pollutants, pathogens, and genetic factors are commonly associated with the development of skeletal abnormalities in cultured fish (Boglione et al. 2013b). Lordosis has been frequently associated with the absence or impaired function of the swim bladder (Summerfelt 2013). In *Polyprion americanus* (Bloch et Schneider, 1801) a 50% of lordotic juvenile was related when 40% of the larvae at 25 DPH did not show inflated swim bladder (in *P. americanus*, inflation of the swim bladder occurs at 7–10 DPH; Abadi Farahani et al. 2023). Accordingly, monitoring swim bladder inflation during early ontogeny, together with dietary optimization and strict control of environmental conditions, should be prioritized to reduce the incidence of skeletal deformities. In line with our own observations, Kihara et al. (2002) proposed an alternative explanation suggesting that excessive tail beating during swimming, which generates increased muscle activity, may induce lordosis by subjecting the vertebrae to excessive stress.

This study provides preliminary evidence that black snook can be reared from induced spawning to the juvenile stage under hatchery conditions. However, low survival (~7%) and a high incidence of skeletal deformities remain major bottlenecks. Progress in domestication will depend on optimized feeding and weaning strategies, routine size grading and density management, early monitoring of swim bladder inflation, and improved prophylaxis against parasitic infections. With these refinements, *Centropomus nigrescens* holds strong potential as a candidate for aquaculture diversification in the Eastern Tropical Pacific.

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