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Research Article

Early gonadal development phases leading to sexual maturity in the Pacific white snook (*Centropomus viridis* Lockington, 1877) under captive conditions

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ABSTRACT. The advancement of biotechnology in cultivating the Pacific white snook (*Centropomus viridis*) is noteworthy. This study aimed to elucidate the reproductive biology of *C. viridis* during the early stages leading up to first sexual maturity, with particular focus on spermatogenesis. We evaluated several factors, including 1) weight and growth, with physiological indices such as the condition factor (K) and the gonadosomatic index (GSI); 2) gonadal development (GD); and 3) environmental influences, including water temperature and photoperiod. We identified four distinct phases of GD: phase I: immature, phase II: developing, phase III: spawning capable, and phase IV: regressing. The spermatogenesis process involved various cell types: 1) spermatogonia (diameter 5.01 ± 0.80 μm), 2) primary spermatocytes (3.29 ± 0.36 μm), 3) secondary spermatocytes (2.76 ± 0.30 μm), 4) spermatids (2.38 ± 0.48 μm), and 5) spermatozoa (1.48 ± 0.44 μm). Phase III, indicating sexual maturity, was first observed in July 2020 (approximately 3 years old). Almost a year later, in May 2021, K peaked (>1.0), and by July 2021, with the water temperature on the rise, GSI reached a value greater than 0.8. These findings provide the first documented description of early gonadal development and sexual maturation in captive-born male *C. viridis*.

Keywords: *Centropomus viridis* captive-born fish; gonadal phases; histological characterization; protandric hermaphrodite; male sexual maturity; spermatogenesis

INTRODUCTION

Sexual maturity in vertebrates is referred to as puberty, a term originally used to describe the onset of sexual development in humans. It is generally defined as the acquisition of reproductive capacity by the organism (Rousseau & Dufour 2007). In teleost fish, puberty represents a transitional state in which a sexually immature juvenile develops into an adult fully capable of sexual reproduction for the first time. This transition

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involves the complete activation of the brain-pituitary-gonad neuroendocrine axis (Carrillo et al. 2009, Taranger et al. 2010, Zohar et al. 2010).

Regarding gonadal development (GD), puberty commences after sexual differentiation. It involves the maturation of germ cells, coupled with the complete differentiation and activation of supporting somatic cells, specifically Leydig and Sertoli cells in males, and theca and granulosa cells in females (Okuzawa 2002, Espigares et al. 2015). In males, germ cell maturation progresses through defined stages: primary and secondary spermatocytes develop into spermatids, culminating in the formation of spermatozoa (Nagahama 1994, Brown-Peterson et al. 2011). Functional spermiation (the release of spermatozoa into the ducts) occurs following their accumulation within the reproductive tract (Schulz & Miura 2002). The process culminates in the first occurrence of spermiation in males or ovulation in females (Okuzawa 2002, Espigares et al. 2015).

The timing of puberty onset in teleosts exhibits significant phenotypic plasticity and is strongly influenced by both genetic factors and environmental conditions, primarily temperature and photoperiod (Carrillo et al. 2009, Taranger et al. 2010). For example, Atlantic croaker (*Micropogonias undulatus*) achieves maturity at 2-3 years under specific temperature conditions (20-25°C) (Lowerre-Barbieri et al. 2019). In contrast, the maturation of gilthead seabream (*Sparus aurata*) primarily correlates with body size (Alós et al. 2020). Plasticity in maturity timing represents an adaptive response to selective pressures (Lowerre-Barbieri et al. 2019), with factors such as food availability accelerating maturation (Molés et al. 2021). Climate change poses a significant threat by disrupting these regulatory processes, impacting steroidogenesis and gametogenesis (Pankhurst & Munday 2011).

Puberty profoundly influences fish populations by altering key life cycle traits, including metabolism, somatic growth, and behavioral plasticity. Additionally, it influences social dynamics and reproductive success, ultimately determining the long-term viability of a population (Schultz et al. 2010, Taranger et al. 2010, Almeida et al. 2015). Contemporary molecular approaches now enable precise monitoring of maturation stages (Escribano-Prófumo et al. 2020).

The Pacific white snook (*Centropomus viridis*), a protandric hermaphrodite (Taylor et al. 1998, Sadovy & Liu 2008), plays a vital role in fishery production and aquaculture. However, a notable lack of information exists regarding the reproductive biology, particularly in terms of GD and sexual maturation. The distribution range of the Pacific white snook spans the coasts of the central-eastern Pacific Ocean, from Baja California Sur, Mexico, to southern Ecuador, including the Galapagos Islands (Bussing 1995). This species inhabits coastal areas, primarily in bays, estuaries, and the lower parts of rivers, and it tolerates a wide range of salinity (Rivas 1986, Bussing 1995, Castro-Aguirre et al. 1999). The International Union for Conservation of Nature (IUCN) lists it as "least concern" (LC) (Lyons et al. 2020). However, some studies indicate a decline in its population due to various factors, particularly heavy fishing pressure (Labastida-Che et al. 2013).

The white snook belongs to the family Centropomidae, which includes the genus *Centropomus*, comprising 12 species (Nelson et al. 2016). A key reproductive trait of *Centropomus* species is sequential hermaphroditism, specifically protandry, where individuals initially mature as functional males and later transform into functional females. (Taylor et al. 2000, Sadovy & Liu 2008). Research on the Mexican snook (*C. poeyi*) indicates that early gonadal differentiation is directed exclusively toward the production of males (Vidal-López et al. 2018).

The current understanding of reproductive physiology in the genus *Centropomus* remains limited, with only two targeted investigations: *C. poeyi* (Vidal-López et al. 2018) and *C. undecimalis* (Passini et al. 2019). These foundational studies examined GD, seasonal fluctuations in steroid hormones, and environmental modulation of sexual maturation during puberty in wild-caught specimens.

Critically, no equivalent research exists for the commercially significant Pacific white snook (*C. viridis*), leaving its reproductive biology under captivity largely uncharacterized. Given this knowledge gap and the species' economic value, the present study establishes a comprehensive framework for analyzing sexual maturation in captive-born *C. viridis*. We hypothesize that males achieve sexual maturity through photoperiod- and temperature-regulated GD, where specific environmental conditions initiate histological progression during puberty. To test this, we characterize the histological sequence of male GD and evaluate its responsiveness to photoperiod and thermal regimes.

MATERIALS AND METHODS

Management and sampling procedures

For this study, we cultured 400 juvenile Pacific white snook (*C. viridis*), all of which were born in captivity and subsequently purchased in June 2017. These

juveniles were raised in two 200 m^3 ponds with a daily water exchange rate of 200% while maintaining natural temperature and photoperiod conditions. The research was conducted at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in La Paz, Baja California Sur, Mexico, at coordinates 24°08'N, 110°25'W.

Throughout the study, from May 2020 to October 2021, we measured water temperature daily at noon using a multiparameter device (YSI PRO20, YSI Inc/Xylem Inc, OH, USA) to compute monthly average temperatures. In addition, we monitored the number of daylight and darkness hours using the Weatherspark mobile application (https://weatherspark.com/y/2800/Average-Weather-in-La-Paz-Mexico-Year- Round).

At nearly 3 years of age (May 2020), the organisms measured 38.60 ± 2.27 cm total length (TL) and weighed 366.36 ± 123.98 g. Their diet consisted of frozen sardines, administered daily at a rate of 3% of their body weight per day.

Monthly samplings commenced from that point, with 5-8 ind randomly selected each month. Adhering to the AVMA Euthanasia Guidelines (2020) (Leary et al. 2020), sampled fish were anesthetized using eugenol (1 mL per 10 L). Once anesthetized, each fish was measured (TL, in mm) and weighed (W, body weight, in g), which were used to calculate the condition factor (K).

$$K = \left(\frac{W_{body}}{TL^3}\right) \times 100$$

Subsequently, euthanasia was performed via hypothermic shock. Gonads were dissected and weighed (g) for gonadosomatic index (GSI) calculation:

$$GSI = \left(\frac{W_{gonad}}{W_{body}}\right) \times 100$$

Finally, the extracted gonads were preserved in Davidson's AFA solution (a mixture of acetic acid, formalin, and ethanol) until they underwent histological processing.

Histological processing and analysis

To describe the various cell types and the annual changes in the phases of GD and to identify the moment when *C. viridis* reaches sexual maturity in captivity, the preserved gonad samples were processed using standard histological techniques as outlined by Muñetón-Gómez et al. (2000), following standard procedures at CIBNOR's histology laboratory. Each entire gonad was cross-sectioned into three anatomical regions (anterior, medial, and posterior), which were then placed in histological cassettes for tissue processing. Samples underwent progressive dehydration through graded ethanol concentrations, followed by clearing in xylene. Tissues were subsequently infiltrated and embedded in paraffin using Leica HistoEmbedder systems (Leica EG 1150H and EG1150C®).

Serial sections of 4 µm thickness were obtained using a Leica RM 2155® rotary microtome and individually mounted on glass slides. Histological staining was performed with Harris hematoxylin-eosin, and permanent preparations were mounted with Entellan resin and cover-slipped.

Microscopic examination and image analysis

Gonadal histology slides were examined using an Olympus BX41 light microscope equipped with a Nikon Digital Sd-Ri1® camera. Images were captured at magnifications ranging from 10x (for gross orientation) to 100x (for detailed cellular analysis). Quantitative analysis was performed using Image Pro Premier software (version 9.0, Media Cybernetics, USA).

For each slide, a systematic sampling approach was employed: nine non-overlapping fields of view (each 1.24 mm^2) were randomly selected, stratified across the anterior, medial, and posterior anatomical regions of the gonad (three fields per region). This sampling encompassed both gonadal lobes in bilaterally symmetric species.

Germ cell developmental stages within these fields were identified and classified according to established morphological criteria defined by Grier & Taylor (1998) and Brown-Peterson et al. (2011). To ensure reliability, key measurements and staging classifications were evaluated for inter-observer concordance.

Relative abundance of germ cells

To determine the relative abundance of each type of sperm cell from the identified phases of GD, we estimated the coverage area of these cells within the gonad. This assessment followed the methodology described by Rodríguez-Jaramillo et al. (2017), which relies on the intensity of specific tissue coloration and the spatial distribution of the cells within the germinal epithelium. For each histological section, we captured four photographs at a magnification of 10x, encompassing a total area of 2.2 mm^2. We then performed automatic pixel counting using the Image Pro Plus v.6.0 image processing software. The coverage area (CA) was quantified in μm^2 and multiplied by 100 to derive

the abundance, thereby enabling the construction of a relative frequency histogram:

$$\mathrm{CA} = \left(\frac{\text{gonad occupationa area}}{\text{total area}}\right) \times 100$$

Statistical analysis

The results of the GSI and K were expressed as percentages and normalized using the arcsine of the square root of the obtained data. Subsequently, we conducted a one-way analysis of variance (ANOVA) to compare means, followed by Duncan's tests to identify significant differences ($P < 0.05$) between the sampling months. The statistical analysis of these results and accompanying graphs was performed using SigmaPlot software for Windows (version 11.0, Systat Software Inc., 2008).

RESULTS

First phases of male gonadal development

Four gonadal phases of development were identified during the early stages of sexual maturity of the Pacific white snook (*C. viridis*), in which all organisms initially develop as males. Their key characteristics and histological interpretation are detailed in Table 1 and illustrated in Figure 1.

Changes in the relative abundance of germ cells, condition factor (K), and gonadosomatic index (GSI) in relation to environmental variations in temperature and photoperiod during gonadal developmental phases of sexual maturity

The timeframes associated with each phase of GD, as identified in the previous section, alongside environmental variations in water temperature and photoperiod (Fig. 2), as well as the evolution of the GSI and K, are presented (Fig. 3).

At the beginning of the study in May 2020, all the sampled gonads were classified as phase I (immature) (refer to Table 1), characterized by nearly maximal photoperiod conditions, approximately 13 h of light, and a rising water temperature of about 23°C. The sampled fish had a mean weight of 366.3 ± 124 g and a standard length (SL) of 38.6 ± 2.2 cm.

By June 2020, the sampled organisms progressed to phase II (developing), which contained abundant primary (st1) and secondary (st2) spermatocytes. Sampled fish averaged 584.7 ± 137.7 g in weight and 40.2 ± 2.9 cm in length. First reproductive maturity was attained in July 2020 as water temperatures stabilized at 26°C and the photoperiod began to decline. This shift was accompanied by sperm cells advancing to later developmental stages (spermatids and spermatozoa), characterized by increasing phase III (spawning-capable) prevalence, decreasing phase II representation, and the emergence of phase IV. Sampled fish averaged 643.4 ± 123.3 g in weight and 45.3 ± 2.2 cm in length.

During August 2020, phase I gonads were not observed. The following month (September), the organisms reached peak phase III abundance (60% at temperatures greater than 28°C), demonstrating their spawning capability. Spermatids and spermatozoa occupied 60% of the area, with the testicular lumen fully populated by mature spermatozoa. This phase was transient, as evidenced by the decreased coverage of spermatocytes and spermatids, which dropped to approximately 20% by October 2020 in phase IV.

From November 2020 to June 2021, phase II maintained a slight predominance. Later on, phase I occurred in December 2020 and January 2021, following the initial temperature decrease in November 2020. Throughout this period, phase III consistently demonstrated the second-highest relative abundance, except during the unsampled December 2020 interval. Phase IV remained the least abundant, first detected in May and June 2021. Although initially observed in January 2021, phase III reached peak expression from February to May 2021, covering approximately 60% of the gonadal area. Concomitant monthly increases in both photoperiod and temperature coincided with the maximum recorded K.

As photoperiod reached its annual maximum during May-June 2021, phase IV emerged with initial coverage <20% of the area, subsequently becoming predominant in July, September, and October 2021. This phase achieved complete gonadal coverage by September, with peak GSI (0.8%) recorded in July.

Phase IV predominated in September 2021, coinciding with peak water temperatures (>30°C). During the sampled months (July and September-October 2021), phase III showed minimal representation in July, while phase I exhibited a limited presence in October. Phase II was absent throughout this period. Gonad sampling was not conducted in August 2021. Despite fluctuations in phase abundance over the 17-month study, the variation in GSI remained minimal (<0.2%). The K reached its nadir (~0.55) in May 2020, peaked in May 2021 (>1.0), and generally remained below 0.8 during interim periods (Fig. 3).

Table 1. Histological characterization of the four gonadal phases during the first spermatogenesis of the white snook (*Centropomus viridis*) includes phase I: immature, phase II: developing, phase III: spawning-capable, and phase IV: regressing. SE: standard error.

Gonadal phases	Histological characterization of gonadal phases during the first spermatogenesis of captive-born white snook.	Spermatogenic cell diameter (µm ± SE)
Phase I: immature	Phase I is characterized by gonadal tissues exhibiting pure spermatogonia and primary spermatocytes within the spermatocysts, with no lumen observed in the lobules. The testis consists of abundant connective tissue.	Spermatogonia 5.01 ± 0.80
Phase II: developing	Phase II is characterized by the active development of germ cells into a limited number of sperm, resulting in the formation of lobules containing spermatocysts with spermatogenic cells at various stages of cellular growth. Each spermatocyst is delineated by a basal membrane attached to the surrounding connective tissue. Primary and secondary spermatocytes are present, and lumen formation occurs. Spermatids and spermatozoa may also be found within the spermatocyst, although not released into the lumen. Continuous germinal epithelium is observed during this phase.	Primary spermatocytes 3.29 ± 0.36 Secondary spermatocytes 2.76 ± 0.30 Spermatids 2.38 ± 0.48
Phase III: spawning capable	Phase III is characterized by transforming spermatids into a greater abundance of spermatozoa in the lumen of the lobules. Continuous germinal epithelium is present, and all cell types, from spermatogonia, spermatocytes, spermatids, and spermatozoa, are observed.	Spermatozoa 1.48 ± 0.44
Phase IV: regressing	Phase IV is marked by a reduction in gonadal activity, characterized by a discontinuous germinal epithelium in nearly all lobules. Residual spermatozoa are found within the lobular lumen, and active spermatogenesis is no longer occurring.	

DISCUSSION

The study aimed to determine the timing of puberty in juvenile Pacific white snook (*C. viridis*) through histological analysis. The specimens, born and raised in captivity, originated from spawnings in June 2017 using wild broodstock that had been acclimated and matured. The juveniles were cultured at the CIBNOR facilities in La Paz, Baja California Sur, Mexico. At the start of the study in May 2020, the fish were nearly 3 years old. This timing was strategically chosen based on prior findings related to *C. undecimalis*, which typically reaches sexual maturity between 3 and 5 years of age (Keith et al. 2000).

Several studies have published findings that describe the different developmental stages of GD in teleost fish. Regarding the reproductive cycle and GD in species of the genus *Centropomus*, notable studies include those by Maldonado-García et al. (2005), who focused on blackfin snook (*C. medius*); Vidal-López et al. (2018), who studied Mexican snook (*C. poeyi*); and Passini et al. (2019), who examined common snook (*C. undecimalis*). When addressing testicular development, Grier & Taylor (1998) established foundational knowledge for studying sexual maturity in male *C. undecimalis* and proposed five developmental classes: regression I, early maturation, medium maturation, late maturation, and regression II.

To standardize the terminology for describing the full sequence of GD events during the fish reproductive cycle, Brown-Peterson et al. (2011) proposed a scheme consisting of five developmental phases: phase I: immature, phase II: developing, phase III: spawning capable, phase IV: regressing, and phase V: regenerating. This scheme has gained widespread use in recent years due to its applicability for describing GD in both sexes across teleosts and elasmobranchs, regardless of reproductive strategy.

However, it is essential to note that the temporal sequence of these events can vary between species and sexes. Therefore, the scale used in this work to describe the histological changes in the testicular development of *C. viridis* during puberty is based on the previously described terminology (see Table 1 for a description of characteristics).

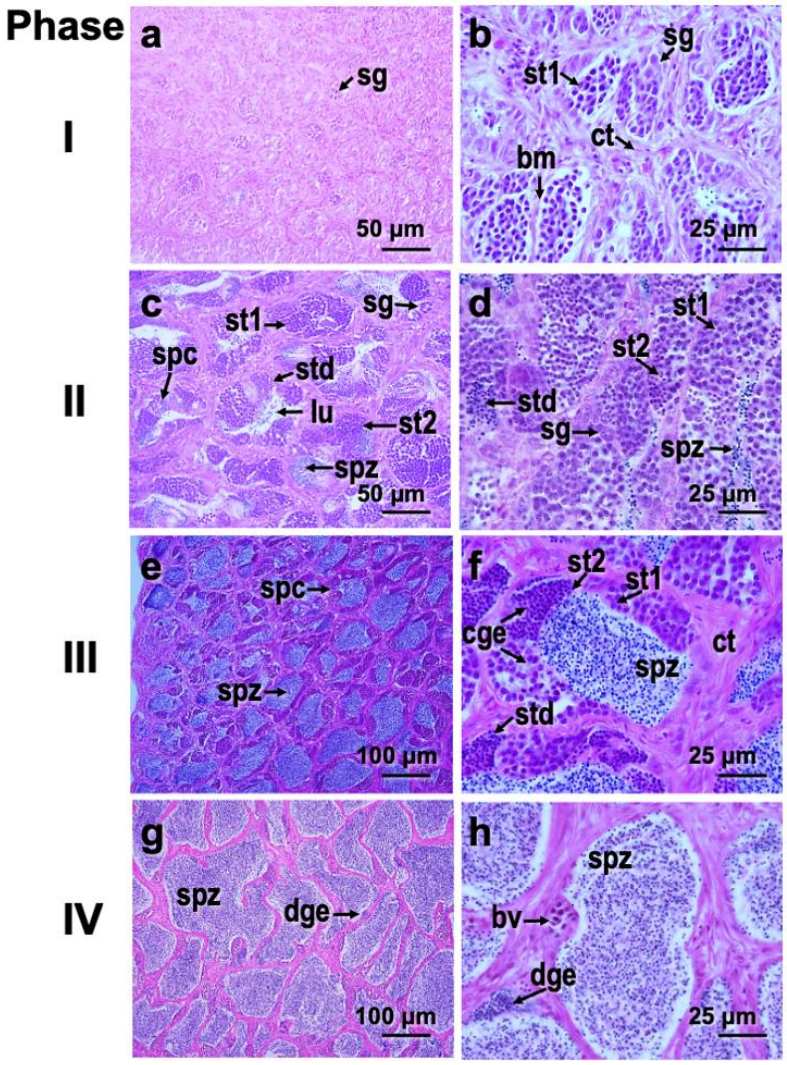

Figure 1. Photomicrographs of hematoxylin and eosin-stained histological sections of different gonadal development phases in male captive-born white snook (*Centropomus viridis*). Phase I (immature), a-b): tissue contains abundant spermatogonia (sg) and primary spermatocytes (st1), with the basement membrane (bm) and connective tissue (ct) also visible; Phase II (developing), c-d): characterized by lobule (lu) formation containing spermatocysts (spc) with spermatogenic cells, including spermatogonia (sg), primary (st1) and secondary (st2) spermatocytes, spermatids (std), and scarce spermatozoa (spz); Phase III (spawning capable), e-f): exhibits a continuous germinal epithelium (cge) with all spermatogenic cell types, from spermatogonia (sg) and spermatocytes (st1, st2) to spermatids (std) and spermatozoa (spz); Phase IV (regressing), g-h): features a discontinuous germinal epithelium (dge) in most lobules, with residual spermatozoa (spz) in the lobular lumen; blood vessels (bv) are also discernible.

Phase I: immature

Phase I, also known as the immature phase, occurs only once in an organism's life (Brown-Peterson et al. 2011). Several authors have noted that this phase is characterized by the mitotic proliferation of various cell types, including stem cells, undifferentiated spermatogonial cells, and differentiated spermatogonia, specifically type A, intermediate, and type B. This proliferation leads to the emergence of primary spermatocytes alongside inactive testicular somatic cells (Leydig cells and Sertoli cells), indicating the completion of the gonadal differentiation process (De Rooij & Russell 2000, Schulz et al. 2010).

In the Mexican snook (*C. poeyi*), researchers observed that gonadal differentiation is completed around the first year of life (355-367 days after hatching), which corresponds with the onset of spermatogenic activity, indicating the transition to

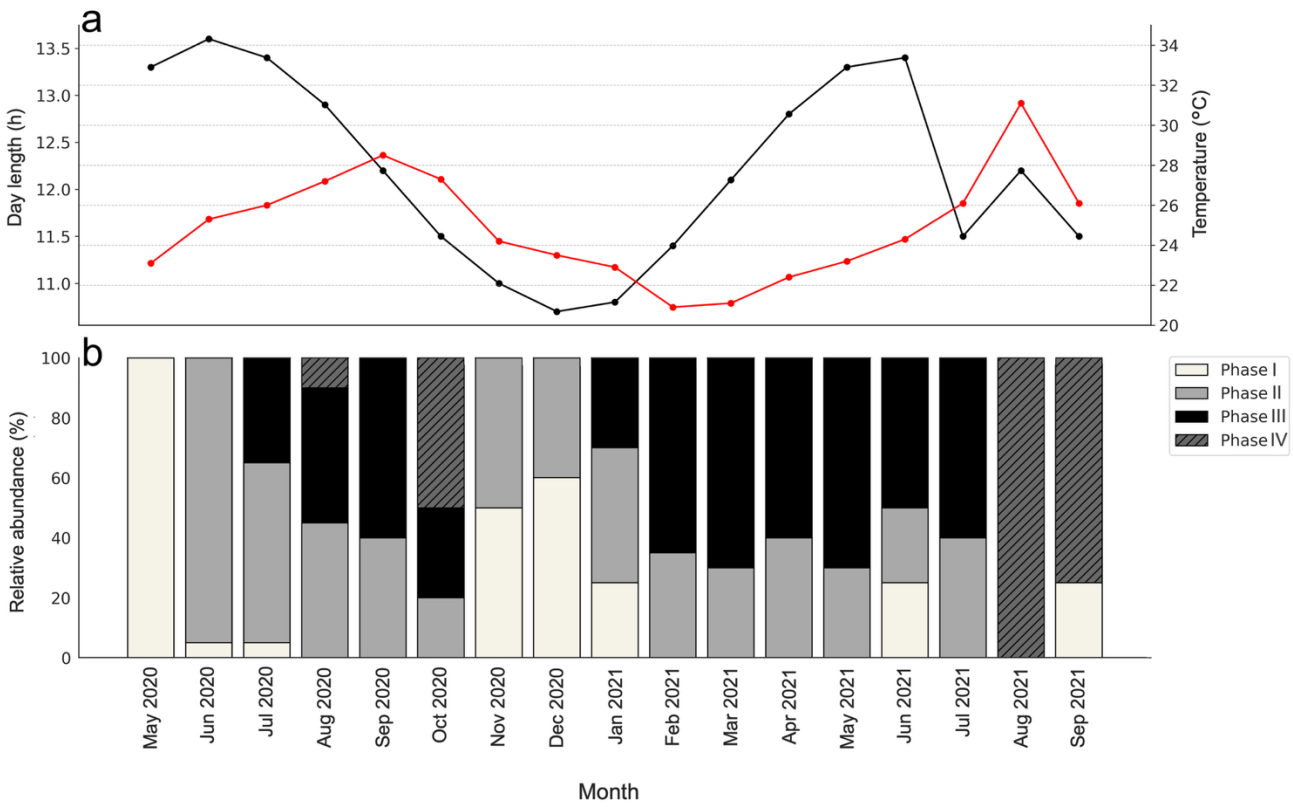

Figure 2. Monthly variation from May 2020 to October 2021 in a) water temperature (red line) and daily photoperiod in hours (black line), and b) relative abundance of each gonadal development phase in captive-born white snook (*C. viridis*).

phase I as described by Vidal-López et al. (2018). In contrast, this study found that Pacific white snook (*C. viridis*) raised in captivity completed gonadal differentiation significantly later, around 3 years of age. Despite this temporal difference, both species exhibited similar macroscopic gonadal morphology during phase I, characterized by fragile, transparent filaments attached to the abdominal wall, reflected in a low GSI of approximately 0.2% for *C. viridis*. Notably, this GSI was significantly higher than values reported for immature common snook (*C. undecimalis*), which ranged from 0.004 to 0.013% (Passini et al. 2019).

The observed variations in maturation timing and GSI among *Centropomus* species reflect evolutionary adaptations shaped by genetic divergence and environmental factors. *C. poeyi* smaller maximum size (~90 cm) *vs*. *C. viridis* (~130 cm) correlates with its earlier maturation (Rivas 1986, Vidal-López et al. 2018). For captive *C. viridis*, delayed differentiation likely stems from dietary limitations in culture, notably reduced nutrient diversity and quality compared to natural environments, which confirms that macroscopic appearance and GSI alone inadequately assess maturation; comprehensive evaluation requires integrated histological and endocrine analyses (Brown-Peterson et al. 2011). Although both species progress through identical histological phases, captive *C. viridis* exhibited abbreviated spermiation duration, potentially attributable to culture-induced oxidative stress (Watanabe et al. 2015).

These findings demonstrate partial congruence with congeneric maturation patterns. *C. undecimalis* attains sexual maturity in natural environments at 2.5-4 years (45-60 cm SL; Taylor et al. 2000), whereas *C. parallelus* in cooler habitats (22-24°C) delays maturation until 3.5-4 years (Gracia-López et al. 2006). Controlled aquaculture conditions appear to accelerate GD relative to cooler wild environments, underscoring the genus's reproductive plasticity in response to thermal regimes, photoperiodic cues, and rearing protocols.

Notably, *C. poeyi* requires substantially larger sizes (50-55 cm; Vega-Cendejas et al. 2013) to reach maturity than observed in *C. viridis* or *C. undecimalis*.

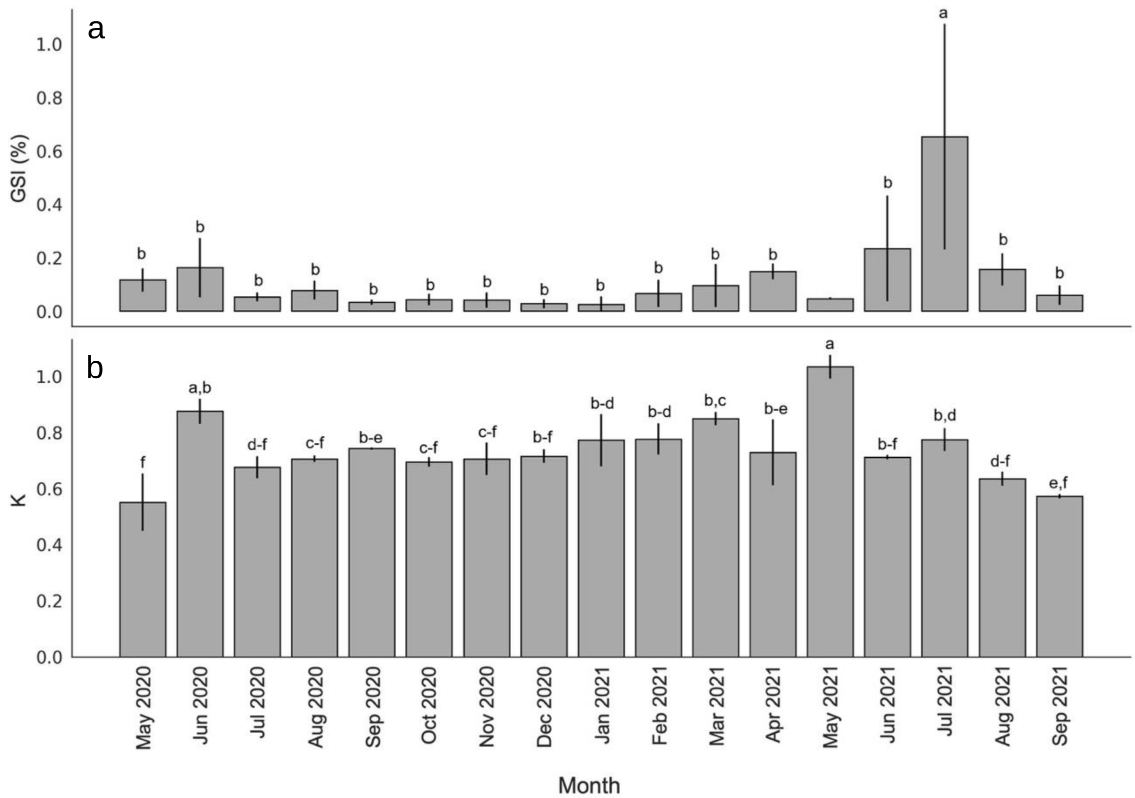

Figure 3. Monthly variation from May 2020 to October 2021 in a) gonadosomatic index (GSI) and b) condition factor (K) in captive-born white snook (*Centropomus viridis*). Data are presented as mean ± standard deviation. Different lowercase letters in panel a and b indicate statistically significant differences among months ($P < 0.05$, Tukey's HSD test).

This profound interspecific heterogeneity in maturation thresholds confirms that captive breeding programs require species-specific validation, as generalized *Centropomus* maturation models fail to capture the divergent life-history strategies of these species.

Phase II: developing

Phase II serves as a preparatory phase for the organism before the onset of the reproductive season, during which numerous cellular and molecular events occur, culminating in the formation of spermatozoa (Brown-Peterson et al. 2011, Wotton & Smith 2014). Grier & Taylor's (1998) testicular maturation scale includes the early and medium maturation classes.

Multiple studies have documented that differentiated spermatogonial cells divide through mitosis during this phase. Following this, the meiotic stage begins, transforming primary spermatocytes (Sc1) into secondary spermatocytes (Sc2), initiating the process known as spermatogenesis. In this process, the DNA contained in Sc1 is replicated, leading to genetic recombination during meiosis I. Meanwhile, Sc2 undergoes division without prior DNA replication, resulting in haploid spermatids emerging during meiosis II (Shulz & Miura 2002, Shulz et al. 2010).

Following meiosis, spermiogenesis rapidly transforms spermatids into spermatozoa. This final maturation process involves nuclear condensation, cytoplasmic reduction, organelle reorganization, and flagellum formation, as documented in zebrafish (*Danio rerio*) and Nile tilapia (*Oreochromis niloticus*) (Schulz et al. 2010). In contrast, continuous spermatogenesis, regulated by Sertoli cells, occurs in model species such as medaka (*Oryzias latipes*) and guppy (*Poecilia reticulata*) (Schulz et al. 2010, Wootton & Smith 2014).

While germ cells at various stages and sequential progression through phases II and III were observed in *C. viridis*, our results do not support the concept of continuous spermatogenesis. The lobular organization and maturation pattern align more closely with cyclic spermatogenesis typical of seasonally reproducing fish. We found no direct morphological evidence implicating

Sertoli cells in a continuous spermatogenic system similar to that of *P. reticulata* or *O. latipes*. Instead, the pattern in *C. viridis* suggests photoperiod-driven cyclical regulation, similar to that in European seabass (*Dicentrarchus labrax*) (Zanuy et al. 2014), which contrasts fundamentally with the continuous spermatogenesis of guppies. The former suggests that *Centropomus* reproductive plasticity is adapted to seasonal ecological pressures. Confirming Sertoli cell functionality, however, requires future studies using immunohistochemical or transcriptomic markers.

In other species of teleost fish, such as the Amazonian catfish (*Auchenipterichthys longimanus*) (Freitas et al. 2011), the Caspian brown trout (*Salmo trutta caspius*) (Hajirezaee et al. 2012), and the African catfish (*Clarias gariepinus*) (Eze et al. 2022), researchers have observed that during the maturation stage, equivalent to phase II, the gonads in both sexes become more pronounced and more straightforward to identify within the abdominal cavity. This change is reflected in a slight increase in the GSI, as the specimens examined were already adults that had reached sexual maturity and had previously reproduced.

However, in juvenile organisms undergoing puberty, as reported by Passini et al. (2019) in *C. undecimalis* and *C. viridis*, this study did not observe an increase in the GSI, as seen in the species mentioned above.

On the other hand, it is noteworthy that during the two years covered by this study, the organisms entered phase II of development, coinciding with an increase in daylight hours. This correlation was also observed in *C. medius* (Maldonado-García et al. 2005) and *Mycteroperca rosacea* (Estrada-Godinez et al. 2011). Carrillo et al. (2009) assert that the photoperiod, which exhibits consistent seasonal variations at a given latitude, serves as a "proximate factor" that triggers a series of neuroendocrine events leading to the onset of sexual maturity during puberty or the resumption of the reproductive cycle. These events occur following a slight increase in the number of light hours, which fish detect and process through the pineal gland, thereby activating the brain-pituitary-gonad neuroendocrine axis (Falcón et al. 2010, Falcón & Muñoz-Cueto 2024).

Phase III: spawning capable

Phase III, which indicates the ability to spawn, corresponds to the late maturation stage, as defined by Grier & Taylor (1998). Once a group of spermatozoa completes its formation within the respective spermatocyst, the connection between the spermatocyst and the supporting Sertoli cells breaks, allowing the spermatozoa to be released into the lobular lumen and the efferent spermatic ducts. At this phase, however, the spermatozoa still lack motility, remaining inactive due to the ionic balance of the seminal plasma until the organism receives the appropriate external stimulus to expel the spermatozoa (Shulz et al. 2010, Wotton & Smith 2014, Dadras et al. 2017).

Observations in other teleost fish species indicate that the testicles significantly enlarge during this phase, occupying considerable space within the coelomic cavity (Grier & Uribe-Aranzabal 2009, Brown-Peterson et al. 2011, Uribe et al. 2014). In the first year of sampling, no increase in the GSI was observed in *C. viridis*. However, the following year, when the organisms reached this developmental phase again in July 2021, we recorded a highly significant increase, reaching ~0.8%. Furthermore, by applying slight abdominal pressure to the fish, we obtained flowing spermatozoa, indicating that this represented the first sexual maturity of the Pacific white snook.

In the study conducted by Vidal-López et al. (2018) on *C. poeyi*, the authors describe gonadal differentiation in this species during its early life stages. They report that once the organisms achieve testicular maturity, the fish become sexually competent, as the spermatozoa they produce are viable and successfully used in fertilization tests, resulting in fertilized eggs and viable larvae. However, research on other teleost fish species, such as common carp (*Cyprinus carpio*), silverside (*Odontestes bonariensis*), and bighead carp (*Hypophtalmichthys nobilis*), demonstrates that several factors, including the age of the breeders, affect gamete quality. Specifically, they observe that the viability of gametes in organisms experiencing their first reproductive cycle is relatively low but improves as the organisms age (Aliniya et al. 2013, Chalde et al. 2014, Dadras et al. 2017). Therefore, it is essential to conduct fertilization tests using sperm from *C. viridis* specimens that have reached their first sexual maturity to assess sperm viability and fertilization capacity.

Phase IV: regressing

Phase IV, also designated as regressing in the classification of Grier & Taylor (1998), represents a stage where gonadal size decreases, resulting in a reduction in the number of spermatocysts within the germinal epithelium. This reduction leads to a significant decline in spermatozoa production and limits spermatogonial proliferation to the periphery of the testicles in several teleost fish species. Consequently, researchers note that organisms entering this

developmental phase experience a cessation of all reproductive activity, although this interruption is not as profound as observed in organisms during phase (Brown-Peterson et al. 2011, Uribe et al. 2014).

The histological characteristics of the gonads in *C. viridis* at this phase of development closely resemble those observed in other snook species, such as *C. undecimalis* (Grier & Taylor 1998, Passini et al. 2019) and *C. medius* (Maldonado-García et al. 2005).

As introduced earlier, members of the genus *Centropomus* spp. are protandric hermaphrodites, characterized by maturing sexually as males for the first time in their lives before gradually transitioning to females (Sadovy & Liu 2008). Research indicates that this sex change occurs between 5 and 7 years of age in the Atlantic white sea bass (*C. undecimalis*), with males reaching their first maturity at a minimum TL of 200 mm (Taylor et al. 2000). In the case of *C. viridis*, there is no available information regarding the age at which this change occurs. However, Tapia-Varela et al. (2020) reported the presence of mature males ranging from 230 to 1,310 mm TL in their study conducted along the coasts of Nayarit, Mexico. They concluded that the size at which sex change occurs in this species may be influenced by fishing pressure, although they did not provide further details.

Sexual maturation in captive-born male *C. viridis*

This study provides the first documentation of captive-born male *C. viridis* attaining first sexual maturity at 3 years of age (July 2020), with a mean weight of 643.42 ± 137.7 g and SL 45.3 ± 2.3 cm, coinciding with the appearance of phase III: spawning capable. These size metrics align with teleost size-dependent maturity thresholds (Brown-Peterson et al. 2011), mirroring patterns in *Dicentrarchus labrax* (Zanuy et al. 2014), yet diverging due to strong thermophotoperiodic regulation. Crucially, maturation was synchronized with temperatures exceeding 28°C and extended photoperiods (13.6 h of light), confirming environmental dominance over chronological age as demonstrated in *Sparus aurata* (Migaud et al. 2013).

While histological maturity (phase III) was achieved at 3 years, functional reproductive readiness was not achieved until additional energy accumulation had occurred. The subsequent peak in condition factor (K >1.0, May 2021) signaled prioritized somatic energy storage, a prerequisite for gonadal investment (Lowerre-Barbieri et al. 2017). By July 2021, rising temperatures triggered a GSI elevation of more than 0.8%, indicating the onset of active spermatogenesis. This 13-month lag may indicate that spermatozoa quality in July 2020 was deficient compared to that in July 2021, consistent with asynchrony between morphological and functional maturity reported in cultured teleosts (Schulz et al. 2010).

Thermophotoperiodic triggering aligns with the optimal window of 25-28°C for snook spermatogenesis (Zohar et al. 2010), where heat stimulates GnRH/kisspeptin pathways (Okuzawa 2002). Nevertheless, GSI > 0.8% -confirming entry into the spawning-capable phase (Brown-Peterson et al. 2011)- requires cautious interpretation, as temperatures >30°C may compromise sperm cell integrity despite high GSI (Pankhurst & Munday 2011, Sundin et al. 2022).

Gonadal progression followed the four-phase teleost model (Brown-Peterson et al. 2011). Still, phase IV regression was markedly abbreviated compared to *O. niloticus* (Böhne et al. 2023), suggesting that culture conditions limit the energy available for gonadal reversion. Early GSI stability (<0.2%) resembled that of *Atractoscion nobilis* (Lowerre-Barbieri et al. 2017), indicating a prioritization of energy homeostasis- a conservative adaptation to captive constraints. The absence of K-GSI correlation further implies that resource allocation is modulated by oxidative stress or lipid availability (*Paralichthys olivaceus*: Watanabe et al. 2015).

These findings necessitate integrating quality molecular biomarkers (e.g. hormonal profiles; Tapia-Varela et al. 2020) with morphometrics to understand physiological asynchronies. Future research must quantify semen quality under several experimental conditions as predictive biomarkers, particularly given the delayed functional maturity observed despite early histological readiness.

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Credit the author's contribution

V. Laurencez-Reyes: writing-original draft; M. Maldonado-García: conceptualization, funding acquisition, project administration; J.A. Estrada-Godínez: conceptualization, writing-original draft; C. Rodríguez-Jaramillo: methodology, review and editing; F. Ascencio-Valle: funding acquisition, project administration; M.A. Hernández-de Dios: methodology, review and editing; M. Aguilar-Juárez: supervision; M. Pacheco-Marges: supervision; D. Maldonado-García: conceptualization, review and editing. All authors have read and accepted the published version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Data availability statement

The authors can provide the data supporting the tables and graphics of this study upon request.

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