UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA
FACULTAD DE CIENCIAS MARINAS
INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS

DOCTORADO EN CIENCIAS EN OCEANOGRAFÍA COSTERA

MODULACIÓN FISIOLÓGICA, METABÓLICA Y ESTRUCTURAL DEL HOLOBIONTE *Totoaba macdonaldi* EN DIFERENTES CONDICIONES TÉRMICAS Y NUTRICIONALES DURANTE SU DESARROLLO LARVARIO

TESIS

QUE PARA CUBRIR PARCIALMENTE LOS REQUISITOS NECESARIOS
PARA OBTENER EL GRADO DE DOCTORADO

PRESENTA

ÁLVARO HERNÁN HERNÁNDEZ MONTIEL

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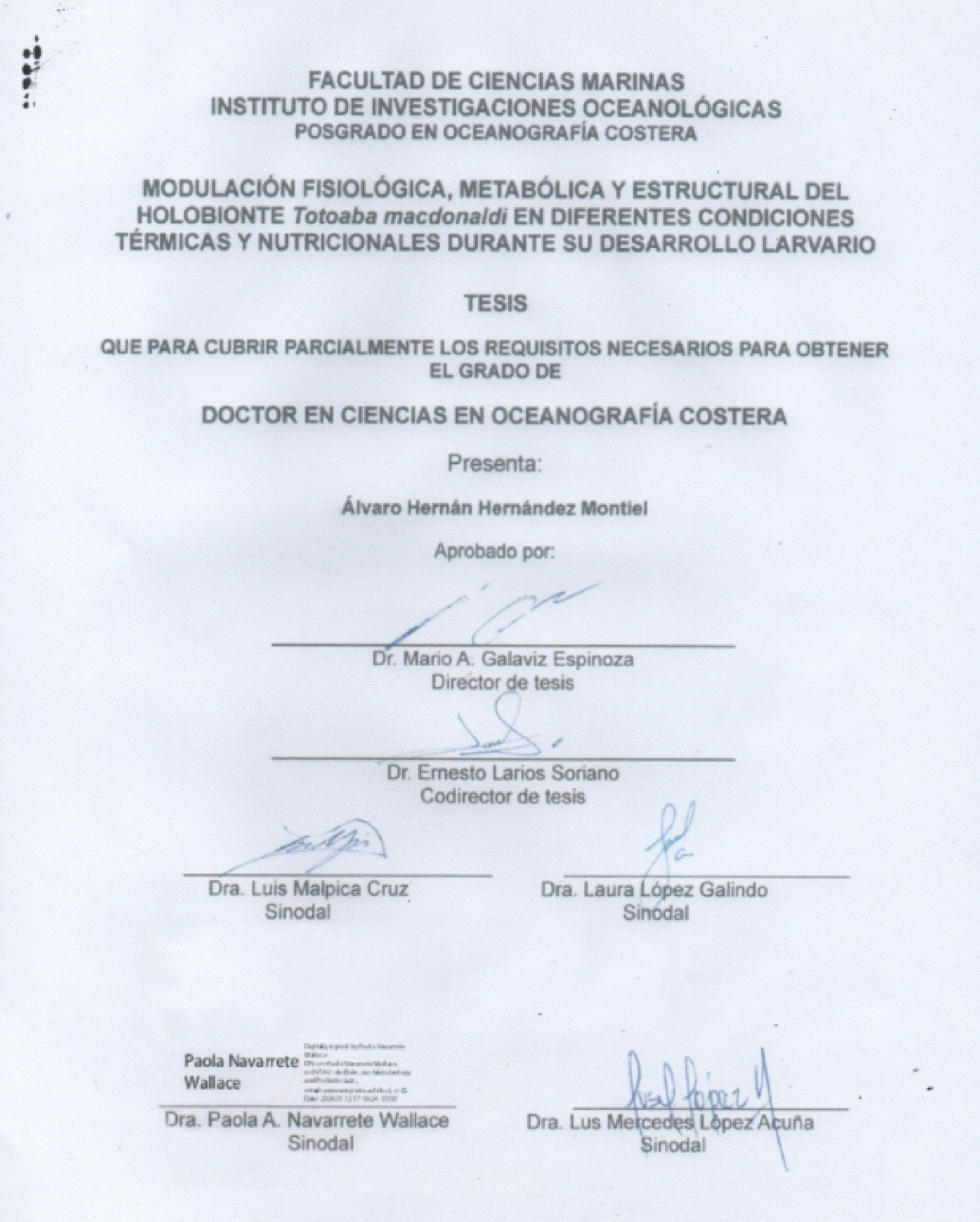

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Resumen

Para lograr la seguridad alimentaria y reducir el impacto de las pesquerías en los ecosistemas acuáticos, es fundamental implementar una acuicultura sustentable que optimice la zootecnia del cultivo de las diferentes especies. La totoaba (*Totoaba macdonaldi*) ha adquirido una relevancia creciente en la acuicultura mexicana; sin embargo, al igual que otros peces marinos carnívoros, presenta elevadas tasas de mortalidad durante la etapa larvaria. En la larvicultura marina se reconocen dos fases críticas: el inicio de la alimentación exógena con presas vivas y el periodo de destete, cuando las larvas transitan hacia dietas formuladas y ocurre la transición larva–juvenil.

Además de los desafíos nutricionales, la temperatura ambiental es un factor determinante que modula el metabolismo, el comportamiento y el crecimiento de los peces. En este contexto, la acuicultura contemporánea ha incorporado el concepto de holobionte, que integra al hospedero y su microbiota asociada, la cual desempeña funciones clave en la nutrición, la inmunidad y el desarrollo.

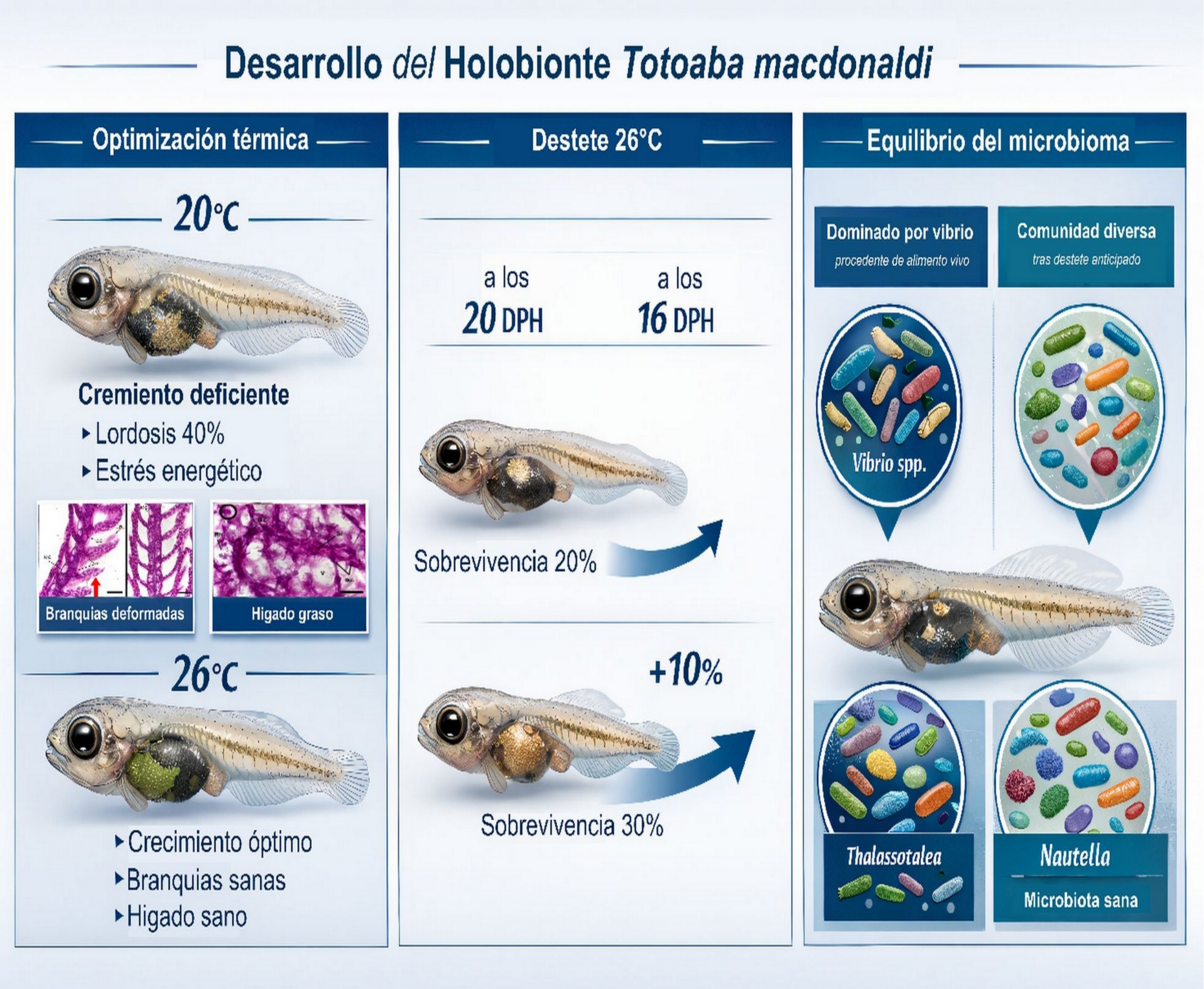
El presente estudio evaluó el efecto de la temperatura y del momento de destete sobre el crecimiento, la fisiología, la morfometría y la estructura de la microbiota asociada al holobionte de larvas de *Totoaba macdonaldi* durante las primeras etapas de desarrollo. Las larvas fueron cultivadas durante los primeros 24 días post-eclosión (DPE) bajo cuatro temperaturas ecológicamente relevantes (20, 24, 26 y 28°C). Se aplicaron dos protocolos de destete: un protocolo estándar, iniciado a los 20 DPE, y un protocolo temprano, iniciado a los 16 DPE. Se evaluaron indicadores de desempeño de crecimiento, incluyendo longitud y peso finales, tasa específica de crecimiento y factor de condición de Fulton, así como la morfometría de branquias y hepatocitos, la alometría peso–longitud y la tasa respiratoria como indicador de la tasa metabólica de rutina (MRM). Asimismo, se analizó la composición de la microbiota asociada a lo largo del desarrollo bajo las distintas combinaciones de temperatura y régimen de alimentación.

La temperatura tuvo un efecto significativo sobre el crecimiento larvario y la morfometría de órganos asociados al metabolismo energético. A 26°C se observó un mayor crecimiento y una mejor condición morfológica de branquias e hígado en comparación con las demás temperaturas evaluadas. La temperatura no afectó la diversidad beta de la microbiota a nivel de filo, la cual estuvo dominada por Pseudomonadota (96 %). En contraste, la diversidad alfa mostró diferencias significativas durante la etapa post-flexión en función de la temperatura; sin embargo, estas diferencias dejaron de ser significativas cuando el destete se anticipó entre los 16 y 20 DPE. El género Vibrio fue dominante durante la etapa larvaria, pero disminuyó de manera notable en la etapa juvenil (24 DPE) a 26°C y 28°C. El destete temprano a 26°C incrementó la supervivencia en un 10% y se asoció con una mayor abundancia de los géneros *Thalassotalea* y *Nautella*. A 28°C no se observaron cambios en la supervivencia, aunque la microbiota se caracterizó por una mayor contribución de *Alteromonas* y *Vibrio*.

En conjunto, estos resultados demuestran que la temperatura modula el crecimiento y el desarrollo larvario de *Totoaba macdonaldi* mediante cambios estructurales y metabólicos integrados en el holobionte, estrechamente relacionados con la transición larva–juvenil y el inicio del destete. Mantener temperaturas cercanas a 26°C se perfila como una condición óptima para maximizar el desempeño productivo y la supervivencia durante las primeras etapas de cultivo.

Palabras claves: *Totoaba macdonaldi*, temperatura, microbiota, destete

Resumen gráfico



Este estudio evaluó el efecto de la temperatura (20, 24, 26 y 28°C) y el momento del destete (habitual vs. anticipado) en larvas de totoaba (Totoaba macdonaldi). Los resultados identificaron que 26°C es óptima para el crecimiento, la histología branquial y hepática, y la homeostasis energética. El filo Pseudomonadota dominó la microbiota en todos los tratamientos. La diversidad microbiana se vio significativamente afectada por la temperatura durante la postflexión, efecto que se mitigó con el destete anticipado. Este protocolo a 26°C incrementó la supervivencia en un 10% y promovió géneros bacterianos beneficiosos como Thalassotalea y Nautella, mientras redujo la abundancia de Vibrio en etapas juveniles. En conclusión, combinar una temperatura de 26°C con un destete anticipado optimiza el desarrollo del holobionte de la totoaba, mejorando su supervivencia y equilibrio microbiano para una acuicultura más sustentable.

Abstract

To achieve food security and reduce the impact of fisheries on aquatic ecosystems, it is essential to implement sustainable aquaculture that optimizes the husbandry of different species. The totoaba (*Totoaba macdonaldi*) has become increasingly important in Mexican aquaculture; however, like other carnivorous marine fish, it has high mortality rates during the larval stage. Two critical phases are recognized in marine larviculture: the start of exogenous feeding with live prey and the weaning period, when larvae transition to formulated diets and the larva-juvenile transition occurs. In addition to nutritional challenges, ambient temperature is a determining factor that modulates fish metabolism, behavior, and growth. In this context, contemporary aquaculture has incorporated the concept of holobiont, which integrates the host and its associated microbiota, which plays key roles in nutrition, immunity, and development. This study evaluated the effect of temperature and weaning time on the growth, physiology, morphometry, and structure of the microbiota associated with the holobiont of Totoaba macdonaldi larvae during the early stages of development. The larvae were reared for the first 24 days post-hatch (DPH) under four ecologically relevant temperatures (20, 24, 26, and 28°C). Two weaning protocols were applied: a standard protocol, initiated at 20 DPH, and an early protocol, initiated at 16 DPH. Growth performance indicators were evaluated, including final length and weight, specific growth rate, and Fulton condition factor, as well as gill and hepatocyte morphometry, weight-length allometry, and respiratory rate as an indicator of routine metabolic rate (RMR).

The composition of the associated microbiota was also analyzed throughout development under different combinations of temperature and feeding regimes.

Temperature had a significant effect on larval growth and the morphometry of organs associated with energy metabolism. At 26°C, greater growth and better morphological condition of gills and liver were observed compared to the other temperatures evaluated. Temperature did not affect the beta diversity of the microbiota at the phylum level, which was dominated by Pseudomonadota (96%). In contrast, alpha diversity showed significant differences during the post-flexure stage depending on temperature; however, these differences ceased to be significant when weaning was brought forward to between 16 and 20 DPH. The genus Vibrio was dominant during the larval stage but decreased significantly in the juvenile stage (24 DPH) at 26°C and 28°C. Early weaning at 26°C increased survival by 10% and was associated with a greater abundance of the genera Thalassotalea and Nautella. At 28°C, no changes in survival were observed, although the microbiota was characterized by a greater contribution of Alteromonas and Vibrio.

In summary, these results demonstrate that temperature modulates the growth and development of *Totoaba macdonaldi* larvae through integrated structural and metabolic changes in the holobiont, closely related to the larval-juvenile transition and the onset of weaning. Maintaining temperatures close to 26°C appears to be an optimal condition for maximizing productive performance and survival during the early stages of cultivation.

Keywords: *Totoaba macdonaldi*, temperature, microbiota, weaning.

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Dedicatoria

Para mi familia y amigos.

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I.1. Introducción general

El cultivo de Totoaba (*Totoaba macdonaldi*) se perfila como una actividad productiva y económica atractiva debido a que la especie tiene diferentes características peculiares. Por ejemplo, la totoaba presenta una tasa de crecimiento de 2-3 kg/año, se considera una especie resistente a la temperatura, la zootecnia del cultivo está siendo estudiada en diferentes laboratorios de México, además de ser una especie endémica del Golfo de California donde existen diferentes empresas con la infraestructura para su cultivo (Machuca et al., 2025). De tal manera, la especie se considera como uno de los prospectos más importantes del sector acuícola en México, sin embargo, como varias de las especies de peces marinos carnívoros, durante su etapa larval llega a tener mortalidades de hasta el 85%.

La alta mortalidad de larvas de peces marinos puede estar asociada a factores ambientales, nutricionales y de manejo, pero principalmente, en esta etapa de desarrollo (primeros 24 días post eclosión) se debe a la falta de protocolos óptimos de alimentación. Por ejemplo, se realizan cambios de alimentación cuando existe una baja capacidad digestiva durante el cambio de alimento vivo a formulado conocido como destete (Mata-Sotres et al., 2015; Córdova-Montejo et al., 2019). Pese a ello, hoy en día perdura la tendencia buscar anticipar el proceso de destete para reducir los costos de producción, sin embargo, esto representa un desafío al coincidir con la etapa final del desarrollo morfológico-funcional del sistema digestivo, donde pasa de un catabolismo alcalino a uno ácido (Galaviz et al.,2015).

El cambio del catabolismo ocurre durante la transición larva-juvenil, una etapa crítica del crecimiento para organismos ectotermos donde temperaturas fuera del rango óptimo puede afectar diferentes procesos metabólicos en larvas y juveniles de totoaba (Larios-Soriano et al. 2023). La temperatura ambiental es un factor determinante de la fisiología de los poiquilotermos, ésta modula el comportamiento, metabolismo energético y sobrevivencia (Ritchie et al., 2022). En ambientes naturales, la temperatura está asociada con migraciones verticales y horizontales en el cuerpo de agua como mecanismo de termorregulación de los peces. Como se observa en totoabas adultas que permanece en un rango de profundidad entre 25 y 35 m, llegando a los 70 m en verano entre temperaturas de 21 a 23ºC (Machuca et al., 2025). En acuacultura, se busca determinar los rangos térmicos más adecuados para el desarrollo en cautiverio de las especies acuáticas. La elección de este rango de temperaturas debe considerar, por ejemplo, los procesos catabólicos y anabólicos de la especie en cuestión. Que en peces carnívoros de aguas subtropicales a templadas como la totoaba, la temperatura para análisis ecofisiológicos ronda los 25°C (Gisbert et al., 2018). Además, se sabe que la temperatura puede regular tanto el crecimiento larvario como de su microbiota (visualizado como un solo organismo bajo el concepto de holobionte). Por lo tanto, la temperatura puede afectar de manera directa al holobionte y generar cambios en su fenotipo. Con el fin de mantener la relación simbiótica que se adapte los retos metabólicos (Réalis-Doyelle et al., 2016) o, por el contrario, inducir cambios que afecten el mantenimiento de la homeostasis.

Los peces marinos presentan diversas estrategias de desarrollo, crecimiento y sobrevivencia, por lo que, su domesticación implica retos importantes para la acuacultura. Particularmente, especies endémicas como la *T. macdonaldi* ha requerido años de estudios e investigación para lograr su desarrollo en cautiverio. Donde el estudio de las respuestas intrínsecas de los peces a factores ambientales, como la temperatura que limita la ingesta de alimento han sido evaluadas (Kaushik 1986). Además, en estudios recientes donde se evalúa a los peces como un sistema biológico denominado holobionte, se observó que la temperatura afecta la homeostasis energética que influye en el crecimiento y desarrollo (Jin et al., 2023).

Ya que se considera a la microbiota como un componente esencial de los seres vivos, su inclusión en la investigación resulta fundamental para comprender y optimizar la plasticidad metabólica de los peces (Lindsay et al., 2020). En este sentido surge la pregunta de cómo una variable abiótica como la temperatura, puede afectar al crecimiento, desarrollo, metabolismo y composición taxonómica de un holobionte en peligro de extinción como la totoaba.

Con el fin de mejorar las prácticas de cultivo y desarrollar una acuicultura sustentable en beneficio de la población, la FAO impulsó el concepto de la transformación azul. Con este concepto se espera lograr seguridad alimentaria y protección de los ecosistemas marinos para el 2030 (FAO 2020). Con el fin de contribuir con la transformación azul y apoyar el programa de repoblamiento de la totoaba (especie en peligro de extinción; NOM-059-SEMARNAT-2010), este trabajo aborda la adaptación de protocolos que consideren la temperatura y deshabituación alimentaria (destete) para la etapa larvaria del cultivo del holobionte totoaba.

I. 2. Hipótesis

a) La exposición de las larvas de totoaba a una temperatura ambiental optima proporcionará un equilibrio en el presupuesto energético que genera, en esta etapa del ciclo de vida, un aporte significativo de esta energía al desarrollo y crecimiento. Este equilibrio energético podrá acelerar o mantener las condiciones idóneas para el cambio de metabolismo alcalino a uno ácido y eficientizar los protocolos de alimentación.

b) Al ser la totoaba un organismo ectotermo, la condición fisiológica y metabólica están determinadas por la temperatura ambiental, por tanto, los rangos fuera de un intervalo optimo tendrán efectos adversos en el holobionte que podrían incluir, una pérdida de la homeostasis energética, afectaciones morfométricas, daños tisulares y cambios en la estructura de la microbiota asociada.

c) El desarrollo de la totoaba cultivada en temperaturas optimas permitirá que las interacciones ecológicas entre la microbiota y su hospedero, mantengan la simbiosis en el holobionte. Ayudando a disminuir la posibilidad de modificaciones en la comunidad microbiana por la presencia de patógenos oportunistas, relacionados con infecciones y mortalidad.

I. 3. Objetivos

I. 3.1 Capítulo II. Objetivo general

Evaluar el efecto de la temperatura ambiental sobre la fisiología, metabolismo respiratorio y sobrevivencia del holobionte totoaba durante el desarrollo larvario-juvenil.

I.3.1.1 Objetivos particulares

a) Evaluar el crecimiento (peso/talla y alométrico/isométrico), la sobrevivencia y el desarrollo histológico (branquias e hígado) de las larvas de totoaba durante los primeros 24 días post eclosión (DPH) cultivadas a diferentes temperaturas de 20, 24, 26 y 28°C.
b) Evaluar el presupuesto energético disponible para mantener la tasa metabólica de rutina de las larvas de totoaba durante los primeros 24 días post eclosión (DPH) cultivadas a diferentes temperaturas de 20, 24, 26 y 28°C.
c) Analizar los rangos óptimos para el desarrollo y crecimiento de larvas de totoaba, así como identificar las etapas del desarrollo más susceptibles a las temperaturas subóptimas de cultivo.

I.3.2 Capítulo III. Objetivo general

Evaluar los efectos de la temperatura ambiental en la modulación de la microbiota asociada a la larva de totoaba y examinar la relación de los principales OTUs con la condición fisiológico y metabólico.

I.3.2.1 Objetivos particulares

a) Evaluar el efecto de las diferentes temperaturas de cultivo (20, 24, 26 y 28°C) en la estructura de la comunidad bacteriana asociada a los primeros 24 DPH de totoaba.

b) Analizar el efecto de un destete habitual y un destete anticipado en la estructura de la comunidad bacteriana asociada a larvas expuestas a 26 y 28°C.

c) Vincular los efectos positivos y negativos en el desempeño fisiológico de las larvas expuestas a las diferentes temperaturas con las Unidades Taxonómicas Operativas representativas en los estados óptimos y subóptimos.

Capítulo II

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RESEARCH

The effects of temperature changes on *Totoaba macdonaldi* larval development, growth, and respiratory rates

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Abstract Understanding the effects of environmental temperature on marine fish larvae is crucial for optimizing feeding regimes and promoting optimal development and growth from a zootechnical perspective in aquaculture practices. The present study investigated the impact of different rearing temperatures on development, growth, and oxygen consumption rates of *Totoaba macdonaldi* larvae. An open-flow seawater recirculating system was used in which larvae (150 specimens/L) were exposed in triplicate to four rearing temperatures (20, 24, 26, and 28 °C) for 24 days under standard larval rearing conditions. Larvae were sampled at 7, 12, 16, 20, and 24 days post hatching (DPH) to assess growth performance, survival, histological condition, and respirometry. At the end of the trial, the highest total length (TL) and body weight (BW) values were observed at 26 °C, with larvae displaying negative allometric growth in terms of TL and BW, similar to those reared at 24 and 28 °C. In contrast, larvae reared at 20 °C showed lower growth and an isometric growth pattern. No significant differences in Fulton's condition factor (K) were detected among treatments. Survival rates were the highest at 28 °C, while increased cannibalism at 26 °C was associated with elevated specific growth rates and thermal growth coefficients. Histological analysis revealed better structural organization in the gills and liver of larvae reared at 26 °C, whereas other treatments showed signs of inflammation. Respirometry results indicated that larvae reared at 20 °C had significantly higher metabolic rates. However, these differences diminished by DPH 20 and 24, likely reflecting developmental changes and phenotypic plasticity during the larval-to-juvenile transition. Based on physiological and metabolic indicators, a rearing temperature of 26 °C is recommended for *T. macdonaldi* larviculture.

Keywords Totoaba · Larvae · Temperature · Thermal Stress · Growth · Development

Introduction

Totoaba (*Totoaba macdonaldi*) is a carnivorous marine fish species with indirect ontogenetic development characterized by progressive physiological adaptations

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Effect of temperature on the growth, development and respiratory rate in larval - juvenile *Totoaba macdonaldi*

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Abbreviated running headline: Temperature affects fish larval physiology and development

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Abstract

Understanding the effects of environmental temperature on marine fish larvae is crucial for optimizing feeding regimes and promoting optimal development and growth from a zootechnical perspective in aquaculture practices. The present study investigated the impact of different rearing temperatures on development, growth, and oxygen consumption rates of *Totoaba macdonaldi* larvae. An open-flow seawater recirculating system was used in which larvae (150 specimens/L) were exposed in triplicate to four rearing temperatures (20, 24, 26, and 28 °C) for 24 days under standard larval rearing conditions. Larvae were sampled at 7, 12, 16, 20, and 24 days post hatching (DPH) to assess growth performance, survival, histological condition, and respirometry. At the end of the trial, the highest total length (TL) and body weight (BW) values were observed at 26 °C, with larvae displaying negative allometric growth in terms of TL and BW, similar to those reared at 24 and 28 °C. In contrast, larvae reared at 20 °C showed lower growth and an isometric growth pattern. No significant differences in Fulton's condition factor (K) were detected among treatments. Survival rates were the highest at 28 °C, while increased cannibalism at 26 °C was associated with elevated specific growth rates and thermal growth coefficients. Histological analysis revealed better structural organization in the gills and liver of larvae reared at 26 °C, whereas other treatments showed signs of inflammation. Respirometry results indicated that larvae reared at 20 °C had significantly higher metabolic rates. However, these differences diminished by DPH 20 and 24, likely reflecting developmental changes and phenotypic plasticity during the larval-to-juvenile transition. Based on physiological and metabolic indicators, a

rearing temperature of 26 °C is recommended for *T. macdonaldi* larviculture.

Keywords: Totoaba, larvae, temperature, thermal stress, growth, development.

II.1. Introduction

Totoaba (*Totoaba macdonaldi*) is a carnivorous marine fish species with indirect ontogenetic development characterized by progressive physiological adaptations to biotic and abiotic factors (Copp and Kováč 1996). For example, early development of the gill respiratory system supports and eventually replaces gas exchange through the skin during larval development, shifting from passive skin to active branchial respiration (Rombough 1999). This respiratory transition enhances aerobic capacity, increasing energy availability for feeding and evading predators during the larval-to-juvenile transition. Moreover, the increase in energy supply and demand is associated with anaerobic metabolism development and acid digestion, which, may be associated with the transition from larval to a juvenile phenotype (Galaviz et al. 2012; Xue et al. 2012; Srichanun et al. 2013; Canada et al. 2017; Khoa et al. 2019; Gamberoni et al. 2021; Larios-Soriano et al. 2023).

Totoaba as a poikilothermic organism, environmental temperature affects fish physiology by modifying metabolic rates and energy homeostasis, which directly affects the organism's development. These effects have been associated with changes in water viscosity, ion concentration and gas solubility due to water temperature variations (Volkoff and Rønnestad 2020).

Marine fish raised at suboptimal temperatures struggle to regulate body temperature due to their poikilothermal condition, which maintains metabolic and energy processes (Pepe-Victoriano et al. 2021; Roman et al. 2019). For example, in a confined environment under low temperatures, a reduction in feed intake was observed, resulting in slower growth rates and immunological and metabolic disorders (Richard et al. 2016). Conversely, increasing temperature inversely affects concentration and dissolved oxygen availability, resulting in a reduced somatic growth as observed in some fish species and other poikilothermic organisms (Burke et al. 2022; Lavin et al. 2022). Furthermore, high rearing temperatures increase oxidative stress damage, decrease mitochondrial respiratory activity, and compromise the overall fitness of the organism (Keil et al. 2015). Thus, temperature is considered the most important abiotic factor that modulates development and growth throughout the life cycle of poikilothermic organisms (Réalis-Doyelle et al. 2016).

In aquaculture, suboptimal water temperatures may prolong larval rearing periods, thereby increasing production costs associated to infrastructure use, manpower, and feeding. These conditions may also lead to malnutrition caused by decreasing appetite, reducing feed conversion, and ultimately, limiting available energy that larvae need for proper growth, morphogenesis, and development (Lema et al. 2019; Dahlke et al. 2020). As previously mentioned, a decrease in skin respiration—coupled with an increase in gill gas exchange—is observed during the transition from larval-to-juvenile stages (Kupren et al. 2014). This shift in respiratory mechanism improves the efficiency in CO_2 and O_2 exchange, which could enhance energy metabolism, increase available energy for movement, promoting foraging behaviour (Luiza et al. 2020). This situation is translated into body allometric changes in the head region and tail, which are linked to critical ontogenetic development milestones, such as the that of the gill apparatus, as well as the notochord and caudal fin development (Gibb et al. 2006; Hu et al. 2018; Downie et al. 2020). Higher temperatures have been observed to accelerate growth and development, but the thermal range that promotes totoaba growth without compromising physiological and metabolic performances is still unknown. Furthermore, temperature-induced somatic growth rate acceleration may lead to adverse outcomes, such as increased cannibalism or misalignment between rearing practices and larval morphogenesis. In summary, suboptimal temperatures, combined with changes in body mass, activity levels, and feeding result in morphological changes, which in turn cause physiological and energy demand alterations to maintain metabolic rates leading to various developmental issues (Steffensen 2005). Evaluating fish larval morphological, physiological, and metabolic responses at different temperatures is essential to identify energy expenditure demands, which could allow a feeding protocol development designed to meet energy requirements at different environmental temperatures, improving larval physiological performance.

Farming *Tototaba macdonaldi* began at UABC (Universidad Autónoma de Baja California) in 1993 for conservation and restocking purposes (True et al. 1997). According to the IUCN, *T. macdonaldi* conservation status is listed as vulnerable, primarily due to intense and unregulated fishing pressure on wild populations driven by the high value of the species’ swim bladder in the Asian market. Today, the species is cultured in various breeding centers and commercial farms, making it an important commercial aquaculture product for Northwestern Mexico (López et al. 2015; Maldonado-Othón et al. 2022). However, high mortality rates during larval stage remain a challenge probably associated with biotic and abiotic factors, such as weaning practices and/or inadequate maintenance of physicochemical water quality parameters (Mata-Sotres et al. 2015; García-Ortega and Lazo 2004; Giffard-Mena et al. 2020). The present study hypothesizes that establishing optimal or near-optimal water temperature regime for *T. macdonaldi* larvae may improve current rearing practices. The main objective is to evaluate the effect of different rearing temperatures (20, 24, 26, and 28 °C) on transition from larval-to-juvenile stages in *T. macdonaldi*. Therefore, water temperature for totoaba larval rearing should be determined by comparing biological and physiological variables, such as survival, somatic specific growth rates, Fulton’s condition factor, and changes in the histological organization in gills and liver, as well as in their respiratory metabolism. Considering the vulnerable conservation status of this species, the significance of the present study goes beyond improving aquaculture production techniques, since by focusing on a species of conservation concern, the present research contributes valuable knowledge that may support both sustainable aquaculture practices and conservation efforts for a threatened species.

II.2. Materials and methods

II.2.1 Larval rearing and sampling

Fertilized totoaba eggs were obtained from a captive broodstock kept in two separate groups (30 fish per group; 25–30 kg in weight, sex ratio male to female of 2:1) held at the Faculty of Marine Sciences at UBP (Unidad de Biotecnología en Piscicultura) at UABC (Universidad Autónoma de Baja California), Mexico. Gonadal maturation in adult totoaba was induced using photothermal control to mimic natural seasonal cycles. Ovulation and spermiation were stimulated using [des-Gly10, D-Ala6]-LHRH ethylamine acetate salt hydrate (SIGMA^{R}). Fertilized, buoyant eggs were collected 28–36 h after spawning, treated with 100 mg L^{-1} formalin for 1 h, rinsed and stocked at a density of 100 eggs L^{-1} in 2200-L cone bottom tanks with 24 °C seawater recirculated at 1.5–2.0 L min^{-1} through a fluidized bed biofilter, ultraviolet sterilizer and foam fractionator. Eggs hatched approximately 20 h after incubation. Egg viability was estimated by quantifying several 1-ml samples under a stereoscopic microscope as described by Kjørsvik et al. (1990).

After hatching, totoaba larvae were stocked at a density of 150 individuals L^{-1} in a system consisting of 12 conical fibreglass tanks, each with a 100-L water capacity. The tanks were connected to a recirculating water system equipped with biological and mechanical filters, a ½ horsepower (hp) pump and ultraviolet (UV) sterilization to maintain water quality. Each tank was equipped with a titanium immersion heater connected to a temperature regulation system with digital thermostat (± 0.3 °C accuracy). Larval rearing water temperatures were 20, 24, 26 and 28 °C, with each temperature treatment tested in triplicate. Feeding protocols followed those described by Galaviz et al. (2015) and were performed three times daily (08:00, 12:00, and 18:00 h). Exogenous feeding began once the yolk sac and oil droplet were fully consumed, which occurred between 3 and 4 days post hatching (DPH). During larval rearing, water flow increased to 4-L min^{-1} when feeding began with rotifers (*Brachionus plicatilis*) enriched with a commercial emulsion (Selco-S.presso-INVE Aquaculture, Belgium) and *Artemia* metanauplii. The feeding schema consisted of larvae with enriched rotifers from 4 to 12 DPH, using a combination of enriched rotifers and *Artemia* metanauplii from 10 to 16 DPH, whereas larvae from 16 to 24 DPH were co-fed with *Artemia* metanauplii and a micro-diet (Otohime B 250–360 mm, Japan); proximate composition: protein 52.1%, lipid 16.3%, ash 11.2%). For sampling purposes, larvae ($n = 15$) from each tank were euthanized by placing them in water saturated with 1:1 ethanol and 70% of eugenol (E51791, Sigma-Aldrich, USA) and then rinsed with distilled water for further analyses. Larval samples were collected at 7, 12, 16, and 20 DPH and juveniles at 24 DPH.

II.2.2 Survival and growth measurements

Survival (S) was calculated at the end of the experimental study for each of the experimental tanks by averaging over the three replicates of each treatment according to the following equation:

$S\% = (Nf / Ni) * 100$

where Nf and Ni are the final and initial number of fish, respectively.

At the end of the trial, the percentage of early juvenile cases with severe lordosis was calculated according to the following formula:

$sl\% = (fl / N) * 100$

where fl was the number of fish with lordosis and N the total number of fish examined.

For each sampling day (7, 12, 16, 20, and 24 DPH), larvae were collected from each tank to measure total length (snout tip to longest caudal lobe) and wet weight. A total of 15 larvae per tank were sampled at 08:00 h under 14 h fasting conditions. Larvae were weighed using a 0.0001 g (± 0.0001 g) precision balance (Sartorius AG CP224S, Germany) and measured for body length under a microscope (MEIJI EMZ-13TRH, Japan), which were superimposed on a Neubauer camera. Images were captured using ImageJ v1.53j software, which was used to measure the exact length of each larva to calculate the following parameters:

Specific growth rate in length (SGR % day^{-1}): $SGR = 100 (\ln TLf - \ln TLi)/t$, where TLf is the final total length (mm), TLi is the total initial length (mm) and *t* is the time in days.

Allometric growth (body weight in relation to larval size in length): $BW = aL^b$, where BW is the body weight (g), *L* is the total length (cm), *a* is the intercept of the linear regression, and *b* is the slope of the line.

To determine the type of allometric growth: log (Wti) = log (a) + blog (Lti) + Ei, where log (Wti) = *y*, *b* is the slope, log (a) is the intercept, and Ei is the multiplied error of the i-th fish.

With this regression growth type was assessed: isometric when H0: $b = 3$, while H1: $b \neq 3$ indicates allometric growth. The maximum length of the lowest growing treatments was then compared to the highest growing treatment using linear regression with log-transformed length data.

Fulton condition factor (*K*) was obtained using the formula: $K = 100 \times (BW/TL^3)$, where BW is the wet body weight (g) and TL is the total length (cm).

The thermal growth coefficient (TGC) was calculated as follows: TGC = $[(3\sqrt{BWt} — 3\sqrt{BWo})/(T \times t)] \times 1000$, where BWt is the final BW, BWo is the initial BW, *T* is the average temperature for the time interval considered, and *t* is the time.

II.2.3 Histological analyses

Larvae were collected at 7, 12, 16, 20, and 24 DPH for histological analyses (*n* = 20 per day from each tank). After being euthanized as previously described, larvae were fixed in 4% paraformaldehyde for 12 h; then, they were dehydrated in an increasing series of graded ethanol in a Leica tissue processor (model TP1020, Leica Microsystems, USA) and embedded in paraffin. After that, 4–5-µm-thick longitudinal sections were made with a Leica manual rotary microtome (model RM2125RTS, Leica Microsystems, USA) and stained with Alcian Blue and Mayer’s hematoxylin (Merck, MA, USA) to observe acidic mucins and mucopolysaccharides. A cell morphometric analysis was performed to evaluate differences between cells (hepatocytes and gill cells) from the different treatments; cell and nucleus diameter measurements were taken from 30 randomly selected organisms for thermal treatment to calculate cytoplasm diameter using ImageJ v1.53j software (Schneider et al. 2012). While for the gills, the width of primary lamellae (PL) and secondary lamellae (SL) was measured in 30 randomly selected organisms.

II.2.4 Metabolic rate determination

Oxygen consumption rate was determined by the difference between initial and final oxygen of the water contained in the respirometry chamber. This system consists of a 24-well glass plate each one with a capacity of 750 µL. A Dish Reader® oxygen sensor (PreSens, Regensburg, Germany) is below each of the wells with an accuracy of ± 1% O_2. Dissolved oxygen was measured in triplicate, placing larvae from each experimental condition (20, 24, 26, and 28 °C) at different post hatching ages [7 DPH ($N = 10$), 16 DPH ($N = 5$) and 24 DPH ($N = 1$)] following the protocol used by Larios et al. (2023). Oxygen consumption data were expressed as mg O_2 larva^{-1} h^{-1}. Temperature quotient (Q_{10}) was evaluated following the equation of Schmidt-Nielsen (1997): $Q_{10} = (\mathrm{TCO2}/\mathrm{TCO1})^{(10/\mathrm{T2}-\mathrm{T1})}$, where T is the water temperature, TCO1 is the initial oxygen consumption rate, and TCO2 is the final oxygen consumption rate.

II.2.5 Statistical analyses

The analyses were performed using the mean and standard deviation; normality and homogeneity of variances were evaluated using Shapiro–Wilk's and Levene's tests, respectively. To compare final length and weight, survival, severe lordosis, a thermal growth coefficient and one-way analysis of variance (ANOVA) were conducted, followed by Tukey's multiple comparison of means test at 95% confidence level. Additionally, normality and homogeneity of variances were evaluated to apply a mixed-effects ANOVA, for which sphericity was assessed using the Mauchly test; the Greenhouse–Geisser correction was applied when the covariance between matrices was not met for O_2 consumption for DPH 7, 16, and 24 and hepatocyte cytoplasm diameter between DPH 16 and 24. A post hoc pairwise *t*-test was then conducted, considering asymmetry of the model. Subsequently, variables that did not meet normality and homogeneity of variance criteria, such as SGR, K, primary lamella (PL), and secondary lamella (SL), were compared by assessing the sum of ranks for each group with Kruskal–Wallis' test; Dunn's post hoc test followed with *P* value adjustment by Bonferroni method (rstatix v0.7.0), using R version 4.2.1 and Rstudio version 2022.07.0 + 548. A Spearman correlation (stats v4.2.1) was also performed between O_2 consumption, feed type, and growth parameters: age in DPH, L, W, SGR, and K condition was visualized with the corrplot package v0.92, eliminating the values of variables with no significant correlation.

A least square adjusted local regression model with confidence intervals of 0.95 was performed to compare length (cm) and weight (g) at different temperatures, with span = 0.75 (stats v4.2.1) (Cleveland et al. 2017), (ggplot2 v3.3.6). A linear regression was performed with the logarithm of length (log L) and weight (log W) to assess allometric growth at different temperatures (FSA v0.9.3). Another linear regression followed with DPH and log TL to compare growth time of the different temperatures with one with the highest growth. To reduce bias in weight predictions relative to length, the log-transformed allometric values were corrected by multiplying them by a logarithmic correction factor (Sprugel 1983). Finally, a principal component analysis (PCA) was performed to determine each variable contribution per component about experimental larval rearing conditions; then, clusters were made to assess whether significant differences existed between the variables with the highest eigenvalue.

II.3. Results

Survival rates at 28 °C (23.05 ± 1.06%), followed by 26 °C (20.83 ± 0.75%) and 24 °C (18 ± 3.47%) showed no statistically significant differences ($p > 0.05$). However, significantly lower survival ($p < 0.05$) was observed from the 20 °C treatment (10.4 ± 2.5%) to the remaining temperature groups. Regarding severe lordosis, a higher number of cases were identified at 20 °C (40.0 ± 4.3%), followed by 24 °C (33.33 ± 3.0%), 28 °C (15.3 ± 4.33%), and 26 °C (11.56 ± 6.23%), where significant differences were observed from 20 to 24 °C treatments with 26 and 28 °C ($p < 0.05$). A negative correlation was observed between survival rates and vertebral column deformities of − 62% ($p < 0.05$). While with respect to temperature they showed a correlation of 78% and − 87%, respectively. In addition, only the 26 °C treatment was identified with thermal growth coefficient (TGC), showing significant differences with the rest of the groups ($p < 0.05$).

As expected, water temperature influenced larval growth performance under current experimental conditions (Fig. 1). By the end of the trial, the highest BW and TL average were observed in totoaba larvae reared at 26 °C (BW = 0.078 ± 0.02 g and TL = 2.03 ± 0.27 cm) (Fig. 1C), followed by those reared at 28 °C (BW = 0.047 ± 0.006 g and TL = 1.83 ± 0.07 cm) (Fig. 1D), 24 °C (BW = 0.023 ± 0.004 g and TL = 1.31 ± 0.08 cm) (Fig. 1B), and 20 °C (BW = 0.016 ± 0.001 g and TL = 1.11 ± 0.04 cm) (Fig. 1A). In contrast, the 26 and 28 °C treatments presented significant differences with regard to the 20 and 24 °C treatments in TL (ANOVA, F (3,8) = 38.62, $p < 0.05$) and BW (ANOVA, F (3,8) = 27.83, $p < 0.05$).

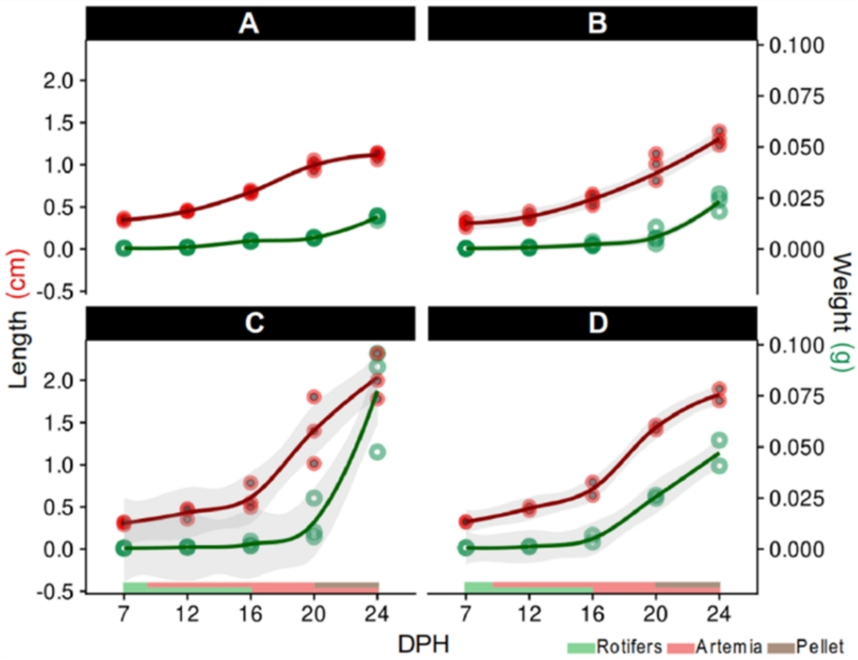

Fig.1 Growth at different temperatures in totoaba (Totoaba macdonaldi) larvae. A–D Local regression models with 95% confidence intervals (non-normalized data), to visualize the correlation among feed type, length, and weight at 20 °C (A), 24 °C (B), 26 °C (C), and 28 °C (D), respectively.

On the other hand, the linear regression model between BW and TL (Fig. S1) showed similar variability patterns among water temperature treatments, arranged from 28 °C ($R^2 = 96.17\%$), followed by 24 °C ($R^2 = 95.92\%$), 26 °C (R^2 95.54%), and 20 °C ($R^2 = 94.32\%$) based on R^2 values. Subsequently, the evaluation of the null hypothesis to determine growth patterns, whether isometric (H_0: $\beta = 3$) or allometric (H_1: $\beta \neq 3$), indicated that larvae reared at 20 °C showed isometric growth ($\beta = 2.75 \pm 0.16$), as the 95% confidence interval for the slope (CI_{95}%) ranged from 2.41 to 3.08, encompassing the value of 3. In contrast, larvae from the other temperature treatments showed slightly negative allometric growth. The degree of negative allometry increased with temperature: 24 °C ($\beta = 2.68 \pm 0.12$, $CI_{95}\% = 2.43–2.94$), 26 °C ($\beta = 2.51 \pm 0.14$, $CI_{95}\% = 2.20–2.82$), and 28 °C ($\beta = 2.49 \pm 0.16$, $CI_{95}\% = 2.11–2.88$).

To compare the final TL between treatments, a growth curve of larvae exposed to 26 °C was used as a reference (Fig. 2). In this curve, the DPH were identified where the TL in each treatment (20, 24, and 28 °C) was the same TL value reached at 24 DPH in the 26 °C treatment. The results showed that the final TL of totoaba larvae when reared at 28, 24, and 20 °C was found at 23, 20, and 19 DPH when compared to the reference standard of 26 °C, respectively (Fig. 2A), which indicated that rearing totoaba larvae at 26 °C reduced larval rearing time by 1, 4, and 5 days, respectively. Furthermore, the PCA analysis revealed that 80.4% of variance was explained by the PC1 (DPH, feed type, and oxygen consumption) and the PC2 (SGR and Tm). Four K-mean clusters on PCA (k) were identified, which discriminated among experimental water temperatures; in particular, the four cluster k4 ($n = 5$) was composed of larvae aged 24 DPH from the 26 and 28 °C treatments, while k3 ($n = 14$) was composed of larvae aged 24 DPH from the 20 and 24 °C groups. The variation of k4 was observed mostly explained by PC1 and k3 by PC2 (Fig. 2B).

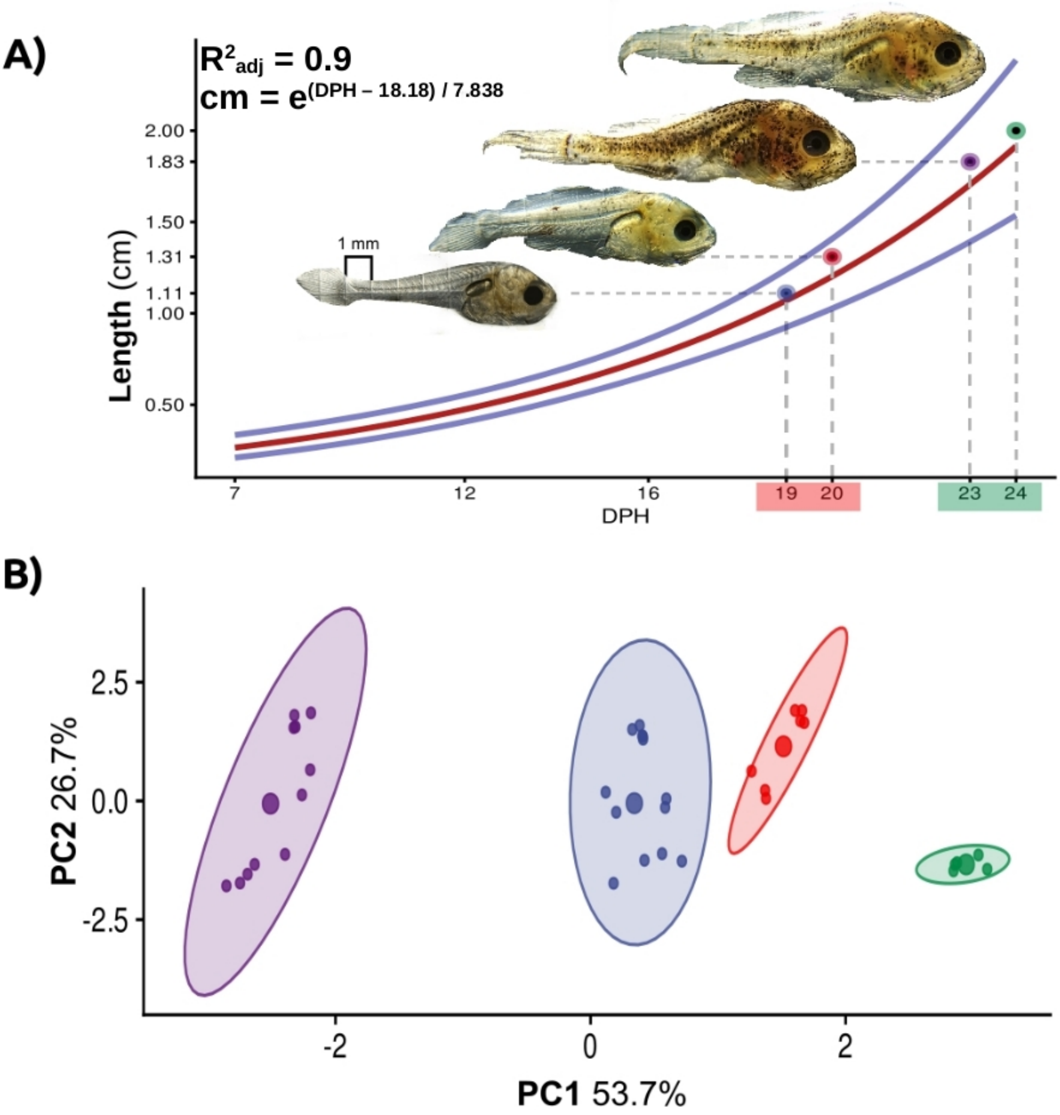

Fig. 2 Delay in final length relative to temperature and grouping of factors involved in totoaba (Totoaba macdonaldi) larval growth. A Growth curve for treatment at 26 °C, where blue, red, purple, and green dots indicate the final average size for treatment at 20, 24, 28, and 26 °C. Larval images are 24 days post hatching (DPH) (blue lines represent confidence intervals). The dotted lines indicate the DPH where each treatment reached its maximum size, with respect to 26 °C at 24 DPH variance. B Grouping the data in four clusters (k), where k1 (purple, n = 12; DPH 7, Tm: all), k2 (blue, n = 12; DPH 16, Tm: all), and k3 (red, n = 7; DPH 24, Tm: 20, 24 °C) are related to PC1 and k4 (green, n = 5; DPH 24, Tm: 26, 28 °C) to PC2.

The SGR value for larvae reared at 26 °C (11.05 ± 0.43% TL day^{-1}) was similar to that of congeners kept at 28 °C (10.17 ± 0.18% TL day^{-1}) ($p > 0.05$). However, significant differences were observed with larvae reared at 20 °C

(6.9 ± 0.35% TL day^{-1}) and 24 °C (7.79 ± 1.65% TL day^{-1}) (Fig. S2a, $p < 0.05$). In addition, *K* did not show significant differences between treatments (Fig. S2b, $p > 0.05$). However, factor values were negatively correlated with O_2 consumption rates (− 45%), age in DPH (− 44%), TL (− 54%), and feed (− 44%) (Fig. S2c). This pattern aligns with the observed positive correlation of SGR in length with temperature (82%) and, to a lesser extent, with BW (14%). These findings are consistent with the previously described negative allometric growth in BW relative to TL in larvae reared at 24, 26, and 28 °C.

For oxygen consumption rates, the post hoc analysis showed significant differences ($p < 0.05$) between average oxygen consumption rates in 7 DPH larvae reared at 20 °C (0.97 ± 0.15 mg O_2 $larva^{-1}$ h^{-1}) with regard to larvae kept at 24, 26, and 28 °C (0.82 ± 1.04, 0.27 ± 0.10 and 0.26 ± 0.08 mg O_2 $larva^{-1}$ h^{-1}, respectively). In this case, the lowest Q_{10} value was observed in the range from 24 to 26 °C (Fig. 3a). At 16 DPH, significant differences in oxygen consumption were observed from 20 °C (3.48 ± 0.81 mg O_2 $larva^{-1}$ h^{-1}) to 26 °C (2.30 ± 0.45 mg O_2 $larva^{-1}$ h^{-1}), as well as with 26 °C with 28 °C (3.37 ± 1.03 mg O_2 $larva^{-1}$ h^{-1}). Additionally, the lowest Q_{10} value at this age was recorded from 24 to 26 °C (Fig. 3b). On the other hand, at 24 DPH, no significant differences were observed in oxygen consumption among treatments ($p > 0.05$), with a higher numerical Q_{10} value found in the range from 24 to 26 °C (Fig. 3c). Oxygen consumption was statistically analysed using a mixed-effects ANOVA where significant differences ($p < 0.05$) and a larger effect size were observed for the variable age (DPH; $\eta^2 = 0.93$) compared to water temperature ($\eta^2 = 0.58$). The experimental group with the largest effect size during development was 26 °C ($\eta^2 = 0.96$), followed by 20 °C ($\eta^2 = 0.80$), 28 °C ($\eta^2 = 0.80$), and 24 °C ($\eta^2 = 0.76$).

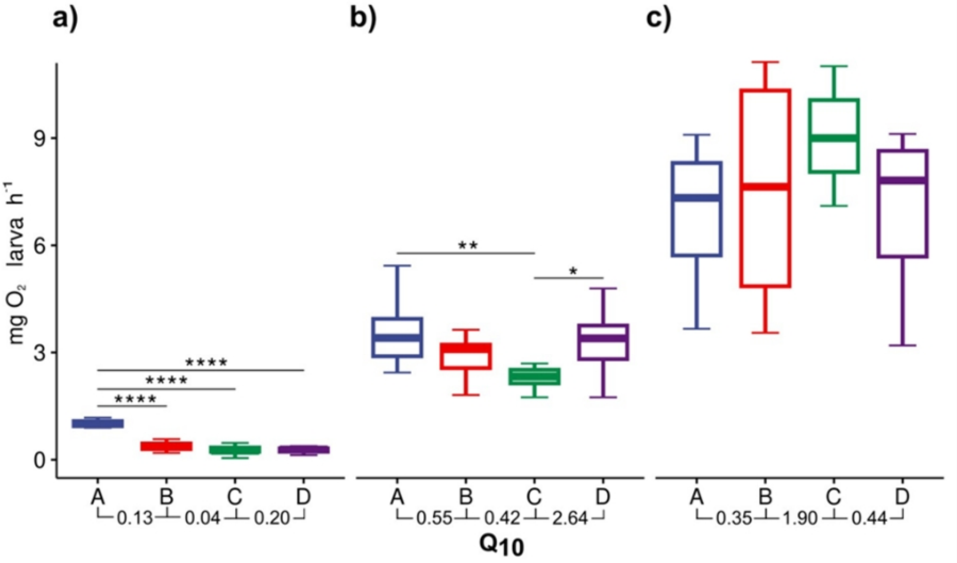

Fig. 3 Oxygen consumption (mg O_2/larvae/h^{-1}) of totoaba (Totoaba macdonaldi) larvae exposed to different temperatures for 24 days post hatching (DPH). a Post hoc analysis for DPH 7 with significant differences (p. adj < 0.05) between A–B, A–C, and A–D. b Post hoc analysis for DPH 16 with significant differences (p adj < 0.05) between A–C and C–D. c Post hoc analysis with not significant differences (A = 20 °C, B = 24 °C, C = 26 °C, and D = 28 °C). Mixed-effects ANOVA and Dunn's post hoc test with Bonferroni adjustment between 7, 16, and 24 DPH, where significant differences were observed (ANOVA, F (1.39, 12.48) = 5.42, $p < 0.05$, $\eta 2 = 0.35$) with a greater effect size of DPH on oxygen consumption ($\eta 2 = 0.94$). While by treatment, C had the largest effect size (p. adj < 0.05, $\eta 2 = 0.96$).

The histological organization of liver and gills was analysed at 16 and 24 DPH. Regarding the histological organization of the liver in larvae aged 16 DPH, a smaller diameter of hepatocytes was observed in larvae reared at 20 °C (3.47 ± 0.89 µm) when compared to their congeners reared at 28 °C (4.22 ± 1.08 um), 26 °C (5.47 ± 1.78 µm), and 24 °C (7.58 ± 1.95 µm) (Fig. S3). At 24 DPH, the smallest hepatocyte diameters were observed in larvae kept at 26 °C (3.22 ±

0.83 µm), followed by those reared at 24 °C (4.06 ± 1.36 µm), 20 °C (8.80 ± 3.17 µm), and 28 °C (12.97 ± 4.75 µm) (Fig. 4A–D). The mixed-effects ANOVA used to assess the hepatocyte cytoplasm diameter from 16 to 24 DPH showed a larger effect of water temperature ($\eta^2 = 0.332$) than age in DPH ($\eta^2 = 0.159$). In particular, 28 °C presented a larger effect size ($\eta^2 = 0.825$), followed by 24 °C ($\eta^2 = 0.691$), 20 °C ($\eta^2 = 0.677$), and 26 °C ($\eta^2 = 0.441$). The post hoc analysis at the age of 16 DPH (Fig. S4a) showed a smaller cytoplasmatic diameter with significant differences ($p < 0.05$) between larvae reared at 20 °C compared to those reared at 24, 26, and 28 °C, while at 24 DPH (Fig. S4b), significant differences were observed between 20 and 28 °C compared to 24 °C and 26 °C ($p < 0.05$).

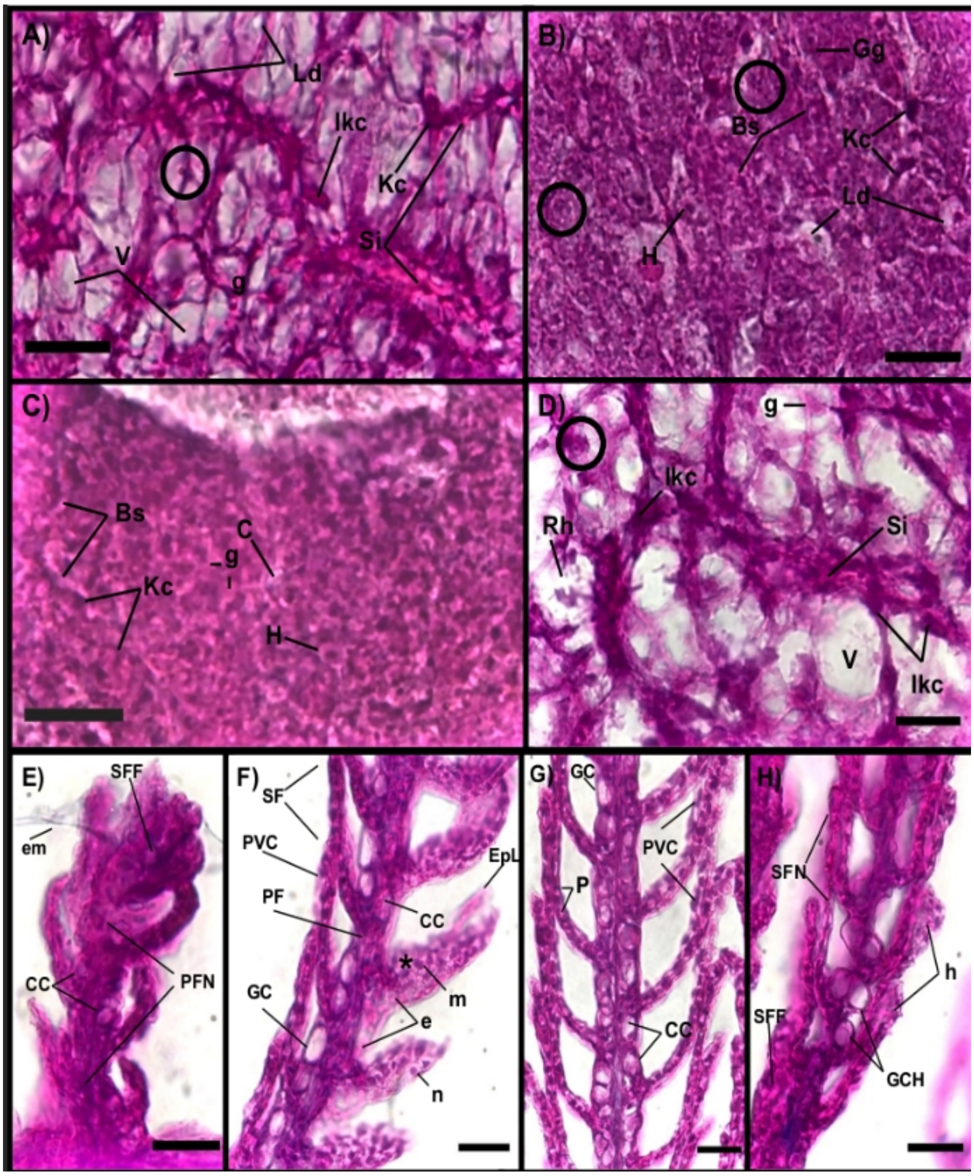

*Fig. 4 Histology of liver and gills of totoaba (Totoaba macdonaldi) larvae at 24 days after hatching (DPH). A and E Liver and gill filament of totoaba at 20 °C. B and F Liver and gill filament at 24 °C. C and G Liver and gill filament of totoaba at 26 °C. D and H Liver and gill filament at 28 °C. AB staining; magnification bar = 20 μm. Abbreviations: PVC, pavement cells; h, hyperplasia; GCH, goblet cell hypertrophy; PF, primary filament; SF, secondary filament; e, erythrocytes. *: lamellae congestion: em, excess of mucus secretion; P, pillar cells; SFF, secondary filament fusion; EpL, cell detachment; PFN, primary filament necrosis; CC, chloride cells; Bs, blood sinusoid. Circulus: pyknotic nucleus; V, vacuolization; Si, inflamed sinusoid; Kc, Kupffer cells; IKC, increased Kupffer cells; Rh, hepatocyte rupture; H, hepatocyte; C, central vein; Ld, lipid droplets; g, glycogen; Nc, nucleus compression; Cs, sinusoidal congestion*

Furthermore, variation in oxygen consumption observed in larvae aged 24 DPH at 24 °C might be related to the physical integrity of the gills with a negative correlation (− 60%) between secondary lamella (SL) width and oxygen consumption ($p < 0.05$), where treatment at 24 °C (12.45 ± 4.32 μm) differed from that at 20 °C (7.91 ± 1.03 μm), 26 °C (7.43 ± 1.68 μm), and 28 °C (8.32 ± 1.67 μm) ($p < 0.05$). On the other hand, a positive correlation (45%) was observed between primary lamella (PL) and K ($p < 0.05$) with a significantly smaller diameter ($p < 0.05$) between the treatment at 28 °C (10.07 ± 1.92 μm) and 20 °C (18 ± 1.96 μm), 24 °C (19.93 ± 4.18 μm), and 26 °C (19.03 ± 2.89 μm) (Fig. 4E–H).

II.4. Discussion

Larval growth and development are influenced by multiple biotic and abiotic factors; however, in aquaculture where the maximum survival and growth rate are expected, water temperature, monitoring of morphological development, and feed quality and quantity are among the most critical factors (Boglione et al. 2013). Thus, testing these factors in a combined manner was essential for proper tuning up rearing conditions, since correlations exist between growth rate at different temperatures, larval mouth size and prey size available during larval development (Carter et al. 2022). Additionally, the development of key organs involved in primary functions, such as feeding, respiration, and locomotion, is related to allometric growth (Osse and Van der Boogaart 1995), which may be modified by changes in robustness and larval growth rate at different acclimation temperatures or nutrition conditions (Shin et al. 2022; Xu et al.

2023). In this sense, temperature variations affect larval swimming kinetics in rearing tanks and contribute to increased energy expenditure during feeding, which may be also compounded by other suboptimal biotic rearing conditions (Osse and Van der Boogaart 1995). Although totoaba juvenile is considered a thermotolerant fish that exhibits phenotypic plasticity and can compensate for temperature-associated growth variations, thus, modifying fish kinetics (Oufiero and Whitlow 2016; Hernández-Aguilar et al. 2018), prolonged culture periods during larval stages increase costs related to live feed production, maintenance, aeration systems, water heating, manpower, and the risk of exposure to infectious diseases (Araujo et al. 2022). This situation is of special relevance since to our knowledge no studies on larval have been available on development and feeding transition during totoaba weaning; therefore, the present information is of interest for improving larviculture conditions for this fast-growing species.

II.4.1 Water temperature effect on somatic larval growth

The present study showed that maximum TL (2.03 ± 0.27 mm), SGR and TGC values were observed in totoaba larvae reared at 26 °C. Furthermore, the statistical analysis revealed significant differences in TL and TGC at 26 °C compared to the other rearing temperatures. These results indicated that temperature negatively affected larval growth at 20, 24, and 28 °C compared to 26 °C, showing a quadratic response with regards to larval growth in TL and water temperature. Besides, these results indicated that 26 °C represents an optimal thermal environment for totoaba larvae that promotes nutrient assimilation and weight gain (Jobling 2003). In this case, optimal water temperature ensures the correct water viscosity to support larval survival and development by reducing dragging forces and swimming efforts (Downie et al. 2020). On the contrary, low temperatures increase energy expenditure due to water viscous forces, which delay larval growth, since energy should derive from swimming rather than somatic growth (Osse and Van der Boogaart 2004). On the other hand, temperatures above the optimal range compromise muscle power and feeding ability, as observed in *Sparus aurata*, which showed higher swimming speed when reared at 25 °C and swimming performance declined at 28 °C (Koumoundouros et al. 2009). Previous studies have indicated that 26 °C is the optimal growth temperature for totoaba larvae and juveniles, with an appropriate thermal range from 26.4 to 27.7 °C (Talamas 2001; Yen et al. 2021; Larios-Soriano et al. 2023). The results in the present study are consistent with those observed in other studies indicating that increasing temperature from 24 to 26 °C promoted totoaba somatic larval growth, even though growth rates were compromised at water temperatures lower or higher than this thermal range (i.e. 20 or 28 °C).

It is also relevant to highlight that growth improvements were correlated with increased BW and changes in feed types during weaning at 26 °C. However, larvae exposed at 28 °C showed more elongated bodies due to a pronounced negative allometric growth in terms of BW, whereas larval growth at 20 °C was isometric in both BW and LT (associated with their lower SGR and K condition values). The negative correlation between Fulton's condition factor, feeding schedule, BW, and TL reinforced the idea that changes in somatic growth were associated with malnutrition during weaning at 20, 24, and 28 °C, which reduced the possibility of reaching the corresponding length and weight to the developmental stage (Osse and Van der Boogaart 2004).

II.4.2 Survival of totoaba larvae at different framing temperatures

The lowest survival rates were observed at temperatures from 20 to 24 °C, which may be related to metabolic rate change and assimilation efficiency mentioned above. Furthermore, the detection of severe lordosis cases decreased over time, indicating that larvae with deformities did not survive the weaning process. In fish larvae, the vertebral column plays a crucial role in inducing rapid muscle fibre development, which are characterized by strong, short-duration contractions (Blagden et al. 1997). In larvae, the vertebral column and muscle fibre development are essential for foraging behaviour and avoiding predators in the wild or cannibalism in a culture environment. On the other hand, the highest survival rate under current rearing conditions was observed at 28 °C, which was slightly higher than at 26 °C. The general larval condition indicates that cannibalism may have affected the survival rate at 26 °C. Due to the negative larval cannibalism impact on survival rate, the weaning process was anticipated in totoaba at 26 °C to minimize mortality associated with cannibalism (Sánchez-Hernández et al. 2018). To further reduce larval rearing costs, the thermal environment and feeding protocol were identified, optimizing morphological development, energy homeostasis, and growth performance for fish during early life stages. In this context, the cost–benefit of maintaining optimal culture temperatures and implementing effective weaning protocols should be evaluated during larval-juvenile transition. These analyses should include an assessment of the economic profitability index to identify the critical growth point under the most effective feeding rates (Jauralde et al. 2011).

II.4.3 Water temperature effect on liver histological organization

The liver and its hepatic parenchyma histomorphological organization may serve as a sensitive physiological biomarker caused by nutritionally imbalanced diets, feed deprivation, or energy imbalance caused by rearing conditions (Gisbert et al. 2008). The present study revealed significant differences in the hepatocyte cytoplasm diameter, as well as inflammation signs in the hepatic sinusoids in early juveniles (24 DPH) reared at 20 and 28 °C. The histological alterations found at 20 and 28 °C were not associated with larval growth. Thus, the hypothesis is that alterantions might instead be related to the inflammatory process and vacuolization, respectively, triggered by extreme culture temperatures. In the case of low temperatures, alterations in the production of superoxide radicals, lipid peroxidation levels, and antioxidant enzymes have been observed in totoaba liver (Hernández-Aguilar et al. 2018). On the other hand, an increase in vacuolization was observed in the present study at 28 °C, which may likely be related to a higher micro-diet intake due to an increase in hepatic vacuolization and observed in totoaba larvae from 20 to 28 DPH (reared at 24 °C) during the transition from live prey to the micro-diet and the different quantity and quality of dietary lipids with regards to live prey (Galaviz et al.

2015). Oxidative stress has been observed to negatively affect hepatocyte morphology in cold water fish subjected to a higher (28 °C) culture temperature (Yan et al. 2022; Han et al. 2023). Furthermore, vacuolization, nucleus displacement, and pyknotic nuclei of hybrid catfish hepatocytes increase at high temperatures (Khieokhajonkhet et al. 2022). Heat stress in farmed fish has been associated with increased lipid deposits, and lipid increase in hepatocytes, which may hinder its metabolic performance due to lipid peroxidation and the resulting harmful products that damage cell membranes, leading to adverse morpho-physiological changes that promote cell death (Ayala et al. 2014; Richard et al. 2016). Additionally, lipid peroxidation may affect cell morphology in the liver by disrupting detoxification mechanisms that decrease inflammation (Vinagre et al. 2014; Madeira et al. 2016). For example, reactive oxygen species (ROS) are known to accelerate glycogen-to-glucose conversion, impairing its storage in hepatocytes through oxidative phosphorylation, NADPH oxidase, oxidative bursts of immune cells, and inflammatory responses (Jiang et al. 2017; Ritchie and Friesen 2022; Qin et al. 2023). These findings suggest inflammatory symptoms in the liver of totoaba larvae, possibly linked to the weaning process, micro-diet ingestion, and possible damage due to oxidative stress at these temperatures. During larval transition to juveniles (20 to 28 DPH), the energy metabolism in liver cells may have provided some protection against ROS activity (Häussinger 1998; Tseng and Hwang 2008; Giffard-Mena et al. 2020); however, this transition did not improve liver condition at 20 and 28 °C as heat stress persisted. Therefore, growth results at these temperatures correlate with larval culture conditions that were evident in PC2 of PCA. This thermal stress likely contributed to malnutrition together with premature or delayed feed withdrawal at 20 and 28 °C, respectively. The result suggests that both 20 and 28 °C might represent the thermal limits for larval-juvenile transition in totoaba. Finally, although, no increase in hepatocyte vacuolization was detected at 24 °C in 24 DPH in the present study, longer exposure to formulated feed may be required to observe liver morphological changes at this temperature. Nevertheless, further research is needed to test the former hypotheses correlating oxidative stress with micro-diets and totoaba nutrition.

II.4.4 Water temperature effect on the gill histological organization

Contrary to that observed in gills of larvae kept at 26 °C, inflammatory processes were present in gills from larvae reared at 20, 24, and 28 °C as indicated by the fusion of secondary filaments and hyperplasia. In addition, the presence of mast cells and neutrophils in secondary filaments was also observed together with an increase in mucous cells at temperatures ranging from 24 to 28 °C. In gills, proinflammatory processes may be indicative of the presence of pathogens, toxicant exposure, and adverse environmental conditions (Pacorig et al. 2022). These proinflammatory processes impair the morphological state of the gills and limits gas exchange, which may compromise larval transition to juvenile stages. In the present study, larvae reared at 24 °C exhibited gills with larger variation in size and width, which negatively correlated with oxygen consumption rates. In addition, significant differences in SL at 24 °C—compared to the other experimental groups—indicated that they were in inadequate rearing conditions (temperature or salinity). In teleost fish, gills function as the main osmoregulatory organ and their euryhaline capacity depends on the number of chloride cells (Samei et al. 2021; Surendran and Ampili 2023). In totoaba larvae cultured at 22 °C, higher survival rates were observed in salinity gradients of 11 to 26 PSU before 20 DPH, while juveniles older than 28 DPH maintained high survival rates in a gradient from 5 to 40 PSU (Giffard-Mena et al. 2020). These results indicate that the transition to juvenile from 20 to 28 DPH may be related to a lower level of oxygen consumption of the larvae at 24 DPH. On the other hand, the morphological state of gills at 26 °C might have contributed to the observed decrease in mortality during the juvenile transition and weaning, whereas the damage observed at PL of 28 °C would indicate proximity to the thermal limit for the condition of gill tissue.

II.4.5 Water temperature effect on oxygen consumption rates

Determining temperature effect is certainly a challenge on metabolic rate during ontogenetic aquatic organism development. In these studies, in addition to the effect of temperature on metabolic rate, considering different variables is important, such as skin respiration, developing respiratory organs, body, and general physiological state. Nevertheless, interesting tendencies were identified in the present study in *T. macdonaldi*. Firstly, an inversely proportional effect at 7 and 16 DPH on oxygen consumption was observed with respect to the increase in water rearing temperature. In both cases (at 7 and 16 DPH), oxygen consumption rates were higher at a temperature of 20 °C and decreased with increasing temperature values up to 28 °C (at 7 DPH) and 26 °C (at 16 DPH). The same tendency was observed in totoaba larvae exposed for 5 h at different temperatures, higher oxygen consumption related to respiratory metabolism and energy expenditure, and at 21 and 24 °C than 27 °C in larvae aged 6, 8, and 14 DPH (Larios-Soriano et al. 2023). This result might indicate the dependence on oxygen diffusion through the skin in early stages of totoaba development where the gill is not yet complete, and in this particular case, the results might indicate that a delay in gill development could result in increased respiratory effort when totoaba larvae were reared at 20 °C. Since the increase in convective oxygen transport depends on larval body mass (Jacob et al. 2002; Killen et al. 2007), totoaba larvae reared at 20 and 24 °C could be at a disadvantage for both reasons, a reduced growth and poor gill development, which may result in increased stress and high oxygen consumption rated as it was observed in comparation with larvae kept at 26 and 28 °C. Therefore, oxygen consumption through the skin helped larvae to cope with gill damage/developmental delay, which was confirmed by the constant increase in oxygen consumption throughout larval development and transition to the juvenile stage. This result is highly correlated to larval age in DPH (94%), oxygen consumption (93%), and food (94%) with respect to body weight gain.

At 24 DPH, totoaba larvae showed a different trend—a lower oxygen consumption rate at 20 and 24 °C—the highest oxygen consumption rate was observed at 24 °C and a slight reduction at 28 °C. These trends resemble an Arrhenius curve where temperature influences molecule kinetics; the higher the temperature, the higher the oxygen consumption up to a point where the decrease in oxygen consumption is interpreted as loss of aerobic performance or aerobic scope (Clark et al. 2013). At this point in the development of totoaba larvae (24 DPH), evidently, temperature may start to be a determining metabolic rate factor. On the one hand, a value of 1.90 is observed in Q_{10} from 24 to 26 °C, suggesting that the metabolic rate has doubled in this temperature range, reflecting a high susceptibility. Likewise, the highest rate of oxygen consumption was observed at 26 °C, suggesting an aerobic energy activation and greater ATP availability through these metabolic pathways. These results corroborate that the optimal temperature for resting metabolic rate (RMR) is 26–27 °C.

Finally, larval age had the most evident effect on oxygen consumption rates in older age, indicating higher oxygen consumption rates. This result is consistent with PCA results, where data in PC1 are grouped by DPH and only at 24 DPH is a distinction observed between organisms exposed to 20–24 °C and 26–28 °C, indicating that temperature directly influences larval body size, therefore oxygen consumption. Furthermore, this result also suggests that temperature has a more pronounced effect on growth during the larval stage and transition to juveniles at lower culture temperatures, as indicated by PC2 and the significant correlation between temperature and SGR values. In addition to larval age, oxygen consumption variability was also influenced by temperature and its effects on gill tissue condition and organization. This result is evident from an increase in SL damage, caused by heat and oxidative stress, which was manifested as an inflammatory process, epithelial tissue sloughing, and mast cell presence. Under unsuitable rearing conditions, these processes may promote cell apoptosis and tissue damage (Sun et al. 2019). Additionally, heat stress and hypercapnia are known to reduce digestive enzyme activity (Pimentel et al. 2015); metabolic and physiological disorders may compromise larval weaning and the transition from the larval to the juvenile stage, which may result in the slight decrease in oxygen consumption found in totoaba reared at 28 °C under current experimental conditions.

Although RMR considers growth cost, it is advisable to also assess the specific dynamic action (SDA), active metabolic rate (AMR), and maximum metabolic rate (MMR) to evaluate energy expenditure during larval-juvenile fish transition (Chabot et al. 2016; Peck and Moyano 2016). This recommendation is made because totoaba larval stage is characterized by anabolic-catabolic metabolism development (Larios-Soriano et al. 2023), acid digestion (Galaviz et al. 2015), and its transition from stenohaline to euryhaline (Giffard-Mena et al. 2020), which increased weight gain and also oxygen requirements. The change from lecithotrophic to exotrophic feeding promotes and increases in food intake, resulting in an increase in aerobic metabolism that increases ATP production (Van de Pol et al. 2017). This result is reflected in the relationship between k4 and PCA1 (early juveniles aged 24 DPH reared at 26 and 28 °C), where age, weaning, and oxygen consumption were the variables that better explained the observed phenotypic variability. However, the observed trend in O_2 consumption at 24 DPH suggests lower energy availability to maintain basal metabolism at temperatures below the optimal range. These results suggest that the energetic metabolism presented outside the range of 24–26 °C may be compromised during the larval-juvenile transition, compromising the development and energetic demand of body systems, such as the respiratory, osmoregulatory, immunological, or digestive ones.

II.5. Conclusion

The present results indicate that the optimum culture temperature is close to 26 °C, based on the significant gains in body size in length and weight in totoaba larvae reared at 26 and 28 °C. Temperature-dependent variations at 20 and 24 °C resulted in the formation of two distinct clusters at 24 DPH. This clustering pattern corresponded to reduced mean final body size at 28, 24, and 20 °C compared to the optimal temperature of 26 °C, indicating growth delays of 1, 4, and 5 DPH, respectively. Temperature was also identified, finding it significantly correlated with SGR, which influenced isometric growth at 20 °C and negative allometric growth at 24, 26, and 28 °C. Temperature also had a significant effect on hepatocyte diameter at 20 and 28 °C, together with a significantly higher oxygen consumption than at 26 °C. This result was related to a lower thermal coefficient for routine metabolic rate in the range from 24 to 26 °C and up to 16 DPH.

However, oxygen consumption variability increased significantly from 24 °C to 24 DPH, which correlated with substantial inflammation of lamellar secondary structures (LS) in the gills. Conversely, at elevated temperature (28 °C), gill morphology was characterized by a significant reduction in lamellae primary (LP) diameter. Therefore, the absence of significant differences in oxygen consumption, combined with the morphological status of gills and hepatocytes at 24 DPH, suggests that metabolic adjustments and phenotypic plasticity facilitated the larval-juvenile transition under suboptimal thermal conditions. The present findings indicate that metabolic adjustments and phenotypic plasticity enabled successful larval-juvenile transition under suboptimal thermal conditions. Finally, the efficacy of early weaning protocols at 26 °C as a mortality reduction strategy warrants further investigation.

Contributions

H-M is responsible for data analysis and manuscript writing. G-E and L-S are responsible for proposing and designing the research scheme, provide funding acquisition, technical support and make significant revisions to the manuscript. S–S and T-C are responsible for developing the research scheme and providing technical support. G and L technical support, assistance with research scheme development and significant revisions to the manuscript and D. Fischer for English edition.

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Statements

The authors declare that they have no conflict of interest in relation to this research article.

Ethics approval

Fish were handled and treated following the technical specifications for the production, care, and use of laboratory animals issued in the Official Mexican Standards (NOM-062-ZOO-1999) and according to the Research Ethics Committee (CEI-UABC-GU2010) of the Autonomous University of Baja California, Mexico, based on international guidelines. In addition, all procedures and experimentation conducted with organisms produced at the marine finfish hatchery (registration number: DGVS-CR-IN-1084-B.C./09) are reported and evaluated by the DGVS Dirección General de Vida Silvestre, in Spanish (General Management for Wildlife) on an annual basis.

Competing interests

The authors declare no competing interests.

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Capítulo III

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Effect of temperature and diet on Totoaba macdonaldi larval-juvenile holobiont

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Effect of temperature and diet on *Totoaba macdonaldi* larval-juvenile holobiont

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Abbreviated running headline: Development of totoaba holobionts.

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Abstract

Temperature is a determining physiological and metabolic factor, and even a modulator of intestinal microbiota composition, influencing food intake, growth, and overall health in ectotherms. This study compared the structure of the totoaba larval microbial community across four thermal treatments (20, 24, 26, and 28°C) and evaluated the effects of weaning strategy by analyzing microbiota composition under standard weaning (20 days post-hatch, DPH) and early weaning (16 DPH) at 26 and 28°C. A core community composed of four main phyla was identified on 7, 16, and 24 DPH: Pseudomonadota (96.4%), Bacteroidota (2.1%), Actinomycetota (0.8%), and Bacillota (0.7%). The dominant genera were *Vibrio* (47.8%), *Alteromonas* (10.5%), and *Pseudoalteromonas* (6.4%). At 28°C, administration of formulated feed occurred earlier (early weaning) but showed no significant differences in richness or abundance compared to 26°C. Also, significant compositional shifts at genus level were associated with larval-juvenile transition. The genus *Vibrio* was most abundant at the end of the larval stage while members of the phylum Pseudomonadota were more prevalent overall, suggesting an important role in development and metabolic regulation. In conclusion, the interaction of temperature, diet and DPH modulates the structure and abundance of the microbiota. Therefore, temperature and feeding strategy can be managed to limit opportunistic pathogens and improve the health of the holobiont.

Keywords: *Totoaba macdonaldi*, Temperature, Weaning, microbiota, 16S rRNA gene, *Vibrio*

III.1. Introduction

The term holobiont refers to the host and its associated microbiota considered together as a single biological entity (Bordenstein and Theis, 2015; Simon et al., 2019; Diwan et al., 2023). Similarly, the concept of the hologenome (the combination of the host genome and the genomes of its microbiota) is gaining increasing relevance due to the functional implications of these associations. For example, the gut microbiota is known to produce bioactive compounds, synthesize vitamins, and solubilize dietary components (Limsuwan and Lovell, 1981; Uebanso et al., 2020; Lall and Kaushik, 2021); thereby enhancing nutrient availability and absorption in the intestinal lumen. Furthermore, the development of the immune system is closely linked to the colonization and balance of microbial communities during early life (Vestrum et al., 2021; Paralika and Makridis, 2025). Therefore, microbial communities provide the host with a broader metabolic capacity that is essential for maintaining homeostasis and health, which is why the gut microbiota is often considered an extension of the digestive system.

Aquaculture, defined as the farming of aquatic organisms, seeks to produce high-quality food sources (protein and fatty acids), in controlled aquatic systems located both on land and in coastal environments (e.g. land-based ponds, rivers and lakes cages). These production systems rely on constant physicochemical, environmental, and nutritional monitoring to achieve optimal performance. Recently, sustainable aquaculture development has increasingly focused on maintaining fish health, with particular attention to the holobiont concept (fish and their associated microbiota) to reduce disease susceptibility (Egerton et al., 2018; Tran et al., 2022). Both fish and their associated microbiota are directly affected by different factors, including temperature fluctuation and the nutritional composition of the diet (Butt and Volkoff, 2019; Hassenrück et al., 2020; Álvarez-Mercado and Plaza-Diaz, 2022; Ghosh et al., 2022). Therefore, understanding how extrinsic factors can disrupt the symbiotic relationship between fish and their microbiota is important to understand the role of these microorganisms during fish development in captive conditions.

The larval stage in fish is characterized by profound morphological and physiological changes that involve the activation of various systems, including the digestive, respiratory, visual, and locomotor systems (Liu et al., 2024). These morphological changes allow larvae to respond to biotic and abiotic fluctuations; however, during larviculture, even small physicochemical, nutritional, and environmental changes may have significant effects for survival and growth (Joly et al., 2023). For example, optimizing the transition from live to inert formulated feed (weaning or feed dishabituation) is critical to meeting the nutritional requirements of larvae in accordance with their digestive capacity during the larval-juvenile transition. Proper weaning can reduce mortality associated with malnutrition and larval-juvenile cannibalism, which often arises from size heterogeneity and aggressive interactions between smaller, less developed larvae and larger conspecifics (Sánchez-Hernández et al., 2018). In addition, temperature fluctuations in aquaculture systems affect energy consumption rate, thereby altering holobiont metabolism, growth performance, and overall fish health (Volkoff and Rønnestad, 2020).

To investigate how developing holobionts respond to environmental and nutritional variables such as temperature and diet, the present study characterized the microbiota associated with totoaba larvae and juveniles. Specifically, we compared the structure of the larval microbial community across four thermal treatments (20, 24, 26, and 28°C) and evaluated the effects of weaning strategy by analyzing microbiota composition under standard weaning (20 days post-hatch, DPH) and early weaning (16 DPH) at 26 and 28°C.

III.2. Materials and methods

III.2.1 Cultivation and sampling

Fertilized eggs of totoaba (*Totoaba macdonaldi*) were obtained from the Fish Farming Biotechnology Unit (UBP), Faculty of Marine Sciences, Universidad Autónoma de Baja California (UABC). The experiment was performed at the Aquaculture Nutrition Laboratory (UABC facilities). Approximately 15,000 eggs were stocked in triplicate into 100-L tanks (150 larvae L^{-1}) and maintained at four temperatures: 20, 24, 26, and 28°C. All tanks were maintained under identical culture conditions, with a salinity of 35 PSU and dissolved oxygen levels >4 mg L^{-1}. Water temperature in each tank was regulated using an 800-W heating element connected to a temperature sensor and control system, while dissolved oxygen levels were maintained through continuous aeration using air stones.

The standard weaning protocol from live feed was implemented as follows: from 4 to 7 DPH, larvae were fed rotifers enriched with fatty acids; from 12 to 16 DPH, they received enriched rotifers and enriched *Artemia*; from 16 to 20 DPH, they were fed with enriched *Artemia*; and from 20 to 24 DPH, the diet consisted of *Artemia* and commercial diet Otohime B, 250-360 mm diet (Nisshin Feed Co., Ltd., Japan). Early weaning protocol was performed from 16 to 20 DPH. In both feeding protocols, larvae were fed three times per day at 08:00, 12:00, and 16:00 h.

Samples were collected at 7, 16, and 24 DPH. Larvae were euthanized by immersion in water from their respective treatment tanks saturated with 0.1x eugenol (Sigma-Aldrich, Darmstadt, Germany). Then, they were rinsed with distilled water 3 larvae per sample (depending on DPH and larval size) were transferred into 1.5 mL Eppendorf tubes containing 800 µl of absolute alcohol. Samples (n = 3 per condition) were stored at -80°C in an ultrafreezer until DNA extraction.

III.2.2 Microbiota composition

Total DNA was extracted from the whole larvae (~100 mg) using CTAB and a pistil for mechanical disruption according to the protocol of Larios-Soriano et al, (2022). Larvae were weighed on an analytical balance (Thermo Scientific, Massachusetts, U.S.A) and transferred to new pre-labeled tubes. The tissue was added to 300 µL of CTAB in Eppendorf tubes, where it was homogenised with a pistil. It was then incubated with 10 µL of proteinase K (20 mg/mL) for 2 hours at 55°C in a thermal plate with vortex agitation every 10 minutes. Subsequently, 10 µL of lysozyme was added and incubated for

10 minutes at 37°C and 60 minutes more at 65°C. 500 µL of chloroform (Fermont, Nuevo León, México) was used to isolate DNA, 250 µL of absolute ethanol (Sigma, Darmstadt, Alemania) to precipitate DNA, and 750 µL of 75% ethanol (Sigma, Darmstadt, Alemania) to wash DNA. Finaly DNA pellets were resuspended in MiliQ water; and DNA integrity and concentration were assessed using agarose gel electrophoresis (1%) and a NanoDrop spectrophotometer (Thermo Scientific, Massachusetts, U.S.A), respectively.

The V3-V4 hypervariable regions of the 16S rRNA gene were amplified by PCR using ~5 ng of template DNA and 200 nM of primers 341F 5′-ACY-CCT-ACG-GGN-GGC-WGC-AG-3′ and 805R 5′-GAC-TAC-HVGGGT-ATC-TAA-TCC-3′. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 35 s, annealing at 56°C for 35 s, and extension at 72°C for 30 s; and a final extension at 72°C for 10 minutes. Amplicons were purified using Ampure XP magnetic beads (Beckman Coulter, Indiana, USA).

Illumina indices were added during a second PCR, followed by another bead-based purification step. Purified libraries were quantified, pooled equimolarly, and measured using for the Qubit dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, USA). The pooled library was adjusted to 4 nM, diluted to 2 pM, and sequenced on an Illumina Miniseq (Illumina, San Diego, CA, USA) using 300-cycles medium output flow cell.

III.2.3 Quality filtering, chimera removal, and taxonomic annotation

Quality filtering, adapters trimming, chimera removal, read assembly, and taxonomic annotation were performed using the pipeline developed by the microbial genomics laboratory at Centro de Investigación en Alimentación y Desarrollo (CIAD) Mazatlán, México (https://github.com/Genomia/metagenomica_pipeline). Adapters were removed and pair-end reads were assembled using the pair-end-cleaner v.0.9 tool, using CUTADAPT for adapter trimming and PEAR for read merging. Assembled files were screened for chimeric sequences using the chimeradetector v.0.1.1 tool. Operational taxonomic unists (OTUs) were clustered at 97% similarity, and taxonomic assignment was performed using mg_classifier v.1.7.0 with VSEARCH against the RDP_V3-V4 v11.5 database (Yarza et al., 2014; Rognes et al., 2016). Sequence data have been deposited in the NCBI Sequence Read Archive under Bioproject accession number PRJNA1266834 and BioSamples SAMN48693614 to SAMN48693655.

III.2.4 Statistical analysis

To compare the total number of observed taxa, alpha diversity indices (Chao1 and Shannon), and the most abundant phyla across temperatures, data were first tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests, respectively. For each variable, a mixed-effects analysis of variance (ANOVA) was performed including DPH (7, 16, and 24 DPH), temperatures, and weaning strategy (standard weaning, H; early weaning, A) at 26 and 28°C. Post hoc pairwise comparisons were performed using t-tests considering the model asymmetry together with a Bonferroni adjustment.

Beta diversity was assessed using PERMANOVA based on Bray-Curtis dissimilarity matrices transformed with the Hellinger method, for the variables DPH, temperature, and developmental stage. In addition, restricted principal coordinate analysis (R-PCoA) was performed using the Vegan package v2.7-1 (Oksanen et al. 2007) to analyze the extent to which OUT composition was explained by DPH, temperature, and larval-juvenile stage.

Spearman correlation analysis and principal component analysis (PCA) were also performed (package corrplot v0.92 and stats v4.2.1) integrating microbiota composition at the phylum level, temperature, and diet. These analyses further integrated physiological parameters, including oxygen consumption (mg O_2 larva^{-1} h^{-1}), branchial histomorphometry (primary and secondary lamellae), and hepatocyte size (μm) evaluated in Hernández-Montiel et al. (2025)(Chapter II); significant differences among temperatures reflected increased basal metabolism and histological deterioration of gills and liver tissues. All analyses were performed using R Statistical Software (v4.2.1; R core Team 2022). Finally, the functional potential of microbial communities at different temperatures was inferred using the iVikodak platform (Nagpal et al. 2019).

III.3. Results

The total number of observed individuals (OTUs) showed a high significant effect of temperature (generalized eta squared = ges = 0.41) and a large interaction effect with DPH (ges = 0.63; mixed-effect ANOVAs). Temperature had a significant effect at both 26 and 28°C (ges = 0.84 and 0.93, respectively), while significant effects across development time were observed at 7 and 24 DPH (ges = 0.68 and 0.94, respectively).

Post-hoc analysis revealed that at 7 DPH, the lowest number of OTUs was detected at 20°C and the highest at 26°C. A similar trend was observed at 16 DPH. In contrast, at 24 DPH lower numbers of OTUs were observed at 24 and 26°C, while 20 and 28°C showed the highest numbers of individuals ($p < 0.05$; Fig. 1a).

A significant effect of temperature (ges = 0.75) and weaning strategy (ges = 0.27) was observed under standard weaning, with significant differences between 26 and 28°C (p.adj < 0.05). Under early weaning, only temperature showed a significant overall effect on OTUs richness; however no significant pairwise differences between temperatures were detected (ges = 0.77; Fig. 1b).

For OTU richness (Chao1), temperature had a significant main effect (ges = 0.47), although no significant pairwise differences were identified between treatments across DPH or between weaning strategy at 26 and 28°C ($P > 0.05$; Fig. 1c, d). A trend was identified, with lower richness at lower temperatures (20 and 24°C) and higher richness at higher temperatures (26 and 28°C).

For the Shannon diversity index, mixed-effects ANOVA showed no significant effects of temperature, DPH, or their interaction ($P > 0.05$). However, post-hoc analysis revealed that at 24 DPH, Shannon index was significantly lower at 28°C compared with 24 and 26°C (*P* < 0.05; Fig. 1e). This difference was not observed under early weaning conditions (Fig. 1f), where a significant effect of weaning strategy on Shannon index was observed (ges = 0.56).

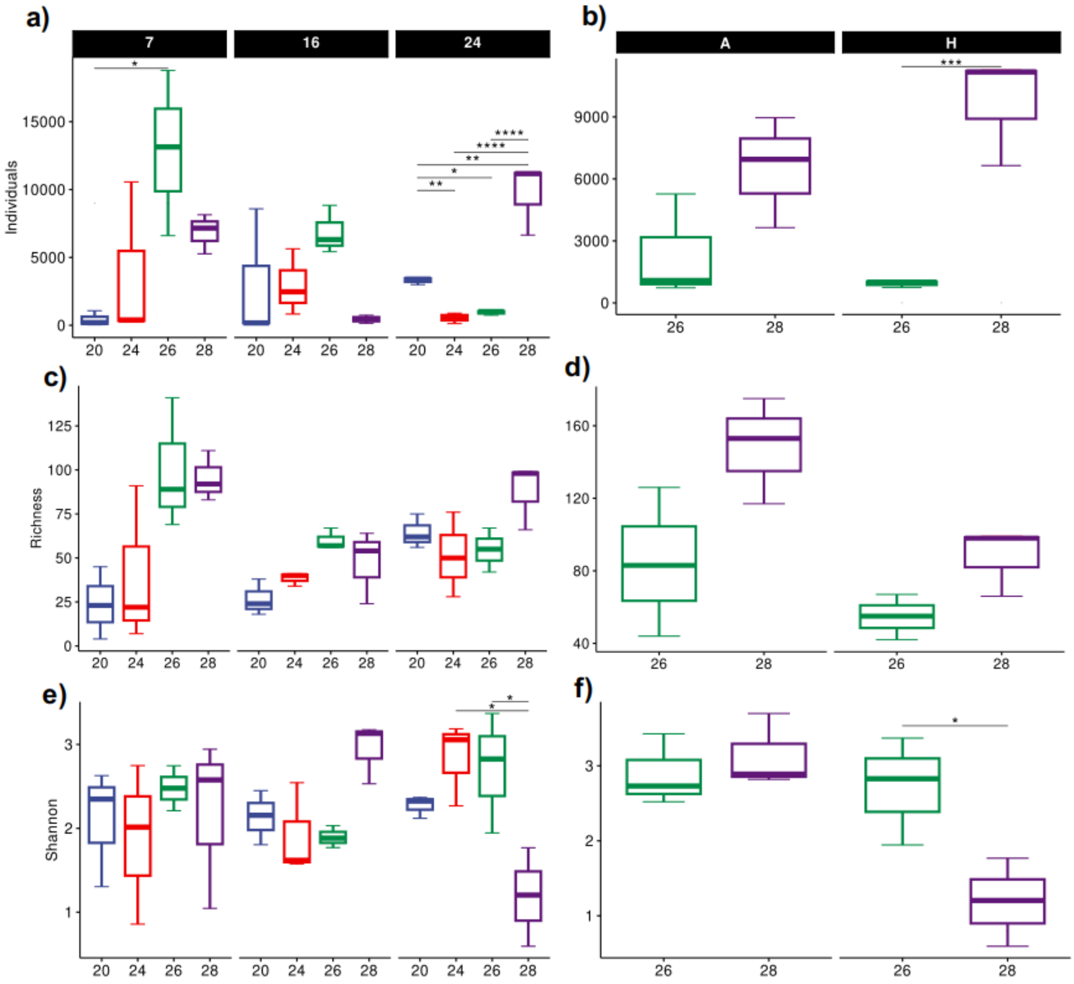

Fig 1. Mixed ANOVA effects. (a) Number of individuals (OTUs) at each temperature and day post-hatch (DPH); (b) Number of individuals (OTUs) at 26 and 28°C in early weaning (A) and standard weaning (H); (c) OTU richness at each temperature and DPH; (d) OTU richness by weaning type at 26 and 28°C; (e) Shannon index at each temperature and DPH; (f) Shannon index at 26 and 28°C for early (A) and standard (H) weaning. Asterisks indicate significant differences between treatments (p < 0.05).

Beta diversity based on Bray-Curtis dissimilarity showed no significant differences among temperatures (PERMANOVA, $R^2 = 0.08$, $p > 0.05$), although samples at 26°C exhibited tighter clustering, suggesting greater community similarity within this treatment (Fig. 2a). Likewise, no significant differences were observed across DPH (PERMANOVA, $R^2 = 0.08$, $p > 0.05$, Fig. 2b) or between larval and juvenile stages (PERMANOVA $R^2 = 0.05$, $p > 0.05$, Fig. 2c).

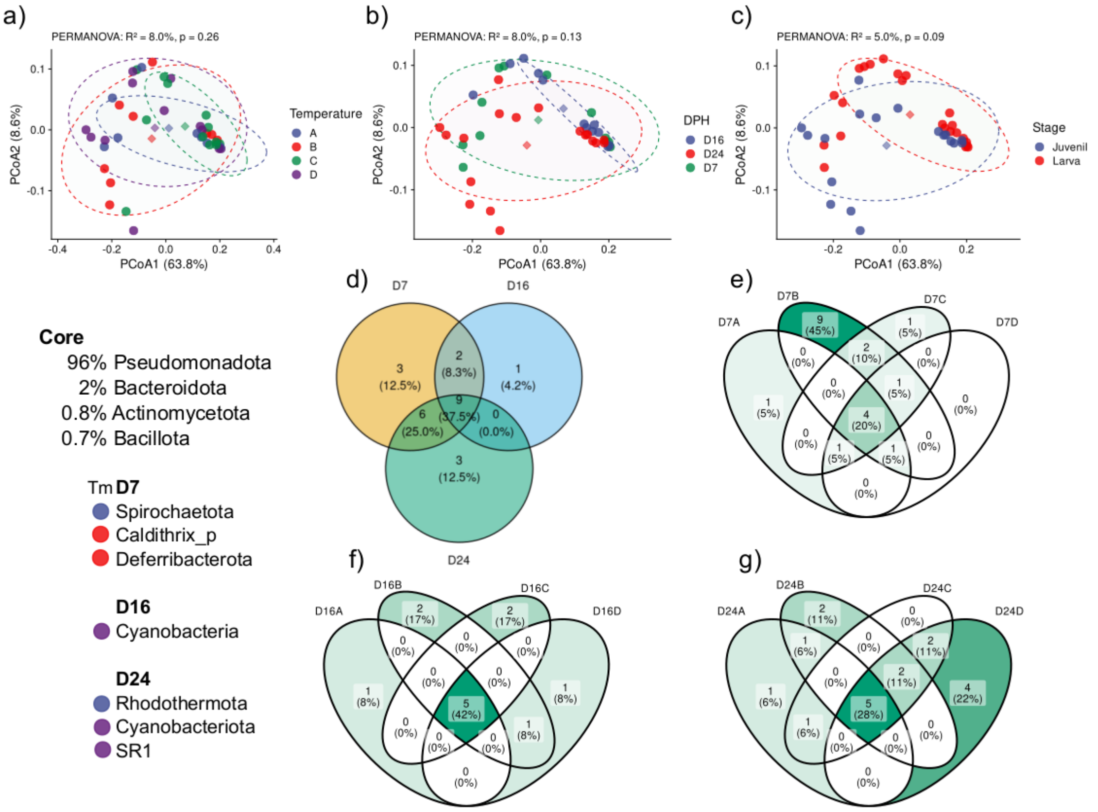

Fig. 2 PCoA analysis for beta diversity by Bray Curtis dissimilarity and PERMANOVA by a) Temperature A = 20°C, B = 24°C, C = 24°C, and D = 28°C. b) DPH. c) Development stage. Venn diagrams showing shared and unique phyla among treatments (d) Core phyla shared across developmental stage 7, 16, and 24 day post-hatch (DPH); (e) core phyla at 7 DPH; (f) core phyla at 16 DPH; (g) core phyla at 24 DPH. A conserved core composed of four dominant phyla was identified: Pseudomonadota, Actinomycetota, Bacteroidota, and Bacillota. Treatment (A = 20°C, B = 24°C, C = 26°C and D = 28°C).

When comparing phyla composition across DPH, a core of nine shared phyla (37.5%) was observed at 7, 16, and 24 DPH (Fig. 2d). When core phyla were examined by temperature whithin each DPH, a highly conserved core dominated by four phyla was identified (Fig. 2e-f), primarily Pseudomonadota (96%), followed by Bacteroidota (2%), Actinomycetota (0.84%), and Bacillota (0.7%).

When comparing the three most prevalent phyla in larvae at 7, 16, and 24 DPH, Pseudomonadota consistently dominated the bacterial community, representing 95.7%, 98.2%, and 95.6%, of the total OTUs respectively (mean OTU abundance across temperatures per DPH). A decreasing trend in the OTUs abundance of this phylum was observed at 26°C toward the end of larval development (24 DPH, ges = 0.77; Fig. 3a).

Bacteroidota showed a decrease in relative abundance from 7 to 16 DPH (3% to 0.34%), followed by an increase at 24 DPH (1.83%). According to the mixed-effect ANOVA, DPH had a significant effect on Bacteroidota abundance (ges = 0.67). At 16 DPH, relative abundance was significantly lower at 24°C compared with 20 and 28°C ($p<0.05$; Fig. 3b).

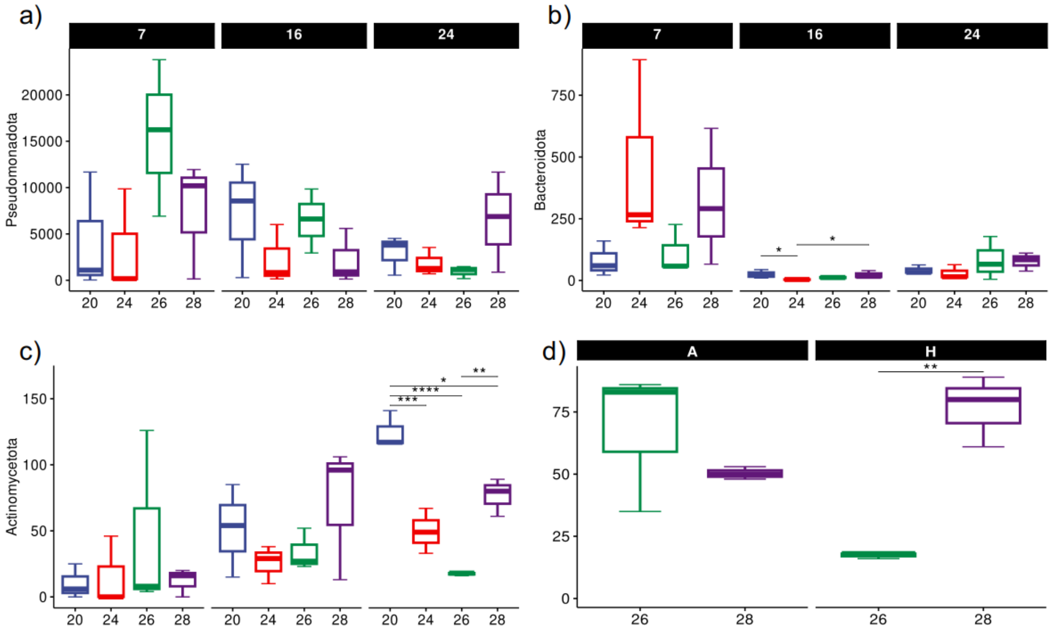

Fig. 3 Effect of mixed-effects ANOVA on the number of OTUs of dominant phyla across temperature and developmental stage. (a) Pseudomonadota by day post-hatch (DPH); (b) Bacteroidota by DPH; (c) Actinomycetota by DPH; (d) Actinomycetota across weaning. A: early weaning; H: standard weaning (U). Asterisks indicate significant differences between treatments (p < 0.05)

The relative abundance of Actinomycetota increased progressively during development from 0.26% at 7 DPH to 0.98% at 16, and 2.10% at 24 DPH. The mixed-effects ANOVA showed significant effects of both DPH and temperature (ges = 0.45), with pairwise differences detected only at 24 DPH. Post-hoc analysis showed that the number of OTUs was significantly higher at 20°C compared with 24, 26, and 28°C, and significantly lower at 26 °C compared with both 20 and 28ºC (Fig. 3c). These differences were eliminated when weaning was brought forward to 28°C (Fig. 3d).

Analysis of relative abundance revealed that the dominant genera shifted throughout development (Fig. 4a). At 7 DPH, the most abundant genera were *Vibrio* (60.4%), *Alteromonas* (19.9%), and *Pseudoalteromonas* (6.5%). At 16

DPH, *Vibrio* was also the most abundant (68.4%) followed by *Acinetobacter* (13.8%), and *Bradyrhizobium* (7.6%). At 24 DPH, there was a marked shift toward *Alteromonas* (39.6%), *Vibrio* (9.9%), and *Pseudomonas* (9.1%).

When relative abundances were compared by temperature within each DPH, distinct patterns were observed. At 7 DPH, larvae at 26°C were dominated by *Vibrio* (68%), *Pseudoalteromonas* (17.7%) and *Alteromonas* (2.1%). At 16 DPH, the most abundant genera were Vibrio (93%) and *Citrobacter* (1%), except at 20°C where *Acinetobacter* (34.7%) and *Bradyrhizobium* (19.4%) predominated. Finally, the most abundant genera at 24 DPH were *Thalassotalea* (16.4%), *Nautella* (16%) and unclassified genera (17%), except at 20 and 28 °C, where *Vibrio* (33.4%) and *Alteromonas* (75%) predominated, respectively (Fig. 4a).

PERMANOVA analysis revealed that combination of DPH, temperature and interaction have a significant effect on microbial community structure, explaining 40% of the total variance. The relative contribution analysis of OTUs showed that dominant taxa varied according to developmental stage and temperature. Across DPH, the main contributors were *Alteromona abrolhosensis* (24%), *Vibrio hyugaensis* (6.75%), and *Nautella italica* (5.78%) (Fig. 4b, blue bars). In contrast, under the temperature grouping, the dominant contributors were *Alteromonas abrolhosensis* (17%), *Vibrio chagasii* (14.64%), and the unclassified OTU EU795102 (4.87%) (Fig. 4b, red bars).

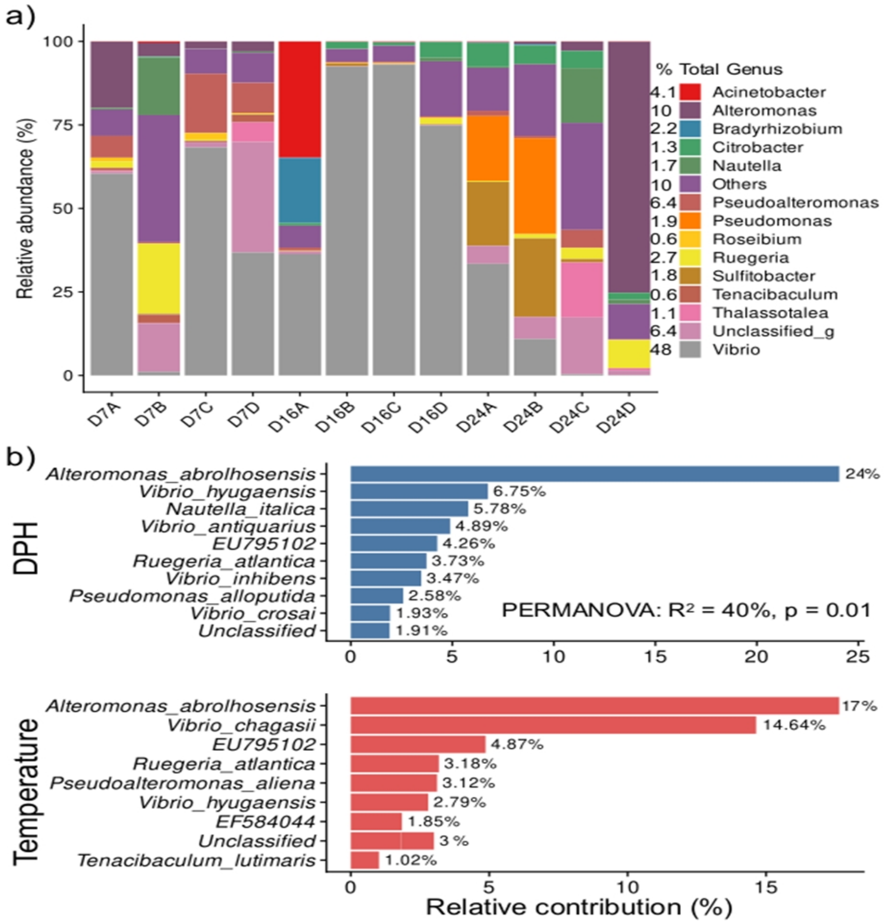

Fig. 4 Beta diversity analysis based on Bray Curtis dissimilarity and Permutation Analysis of Variance (PERMANOVA). Principal Coordinated Analysis (PCoA) colored by (a) Temperature A = 20°C; B = 24°C, C = 26°C, and D = 28°C; (b) day post-hatch (DPH); c) development stage (larvae or juvenile). (d) Dominant bacterial genera by DPH and temperature: A = 20°C; B = 24°C; C = 26°C; and D = 28°C; e) Relative contribution of Operational Taxonomic Units (OTUs) grouped by DPH (blue bars) and temperature (red bars).

As previously described, physiological variables such (oxygen consumption rates, body length, body weight, and gill and hepatocyte histometry (Hernandez-Montiel et al., 2025) were integrated with microbiota composition using principal component analysis (PCA). In the first PCA (Fig. 5a), two clusters were observed that were independent of acclimation temperature but clearly separated by larval-juvenile stage. Cluster 1 grouped all samples from 24 DPH (juveniles), while cluster 2 included the larval stages (7 and 16 DPH) across all treatments. The variables with the highest contributions to PC1 were body size, oxygen consumption, DPH, body weight, *Aminocenantes* OP8, *Chlorobiota* SR1, and *Chlamydiota*. PC2 was mainly associated with *Pseudomonadota*, *Chlorobiota* SR1, *Aminocenantes* OP8, temperature (Tm), *Bacteroidota*, *Verrucomicrobiota*, DPH, and *Peregrinibacteria*. The relative abundance of *Aminocenantes* OP8 and *Chlamydiota* were significantly higher in cluster 1 ($p < 0.05$).

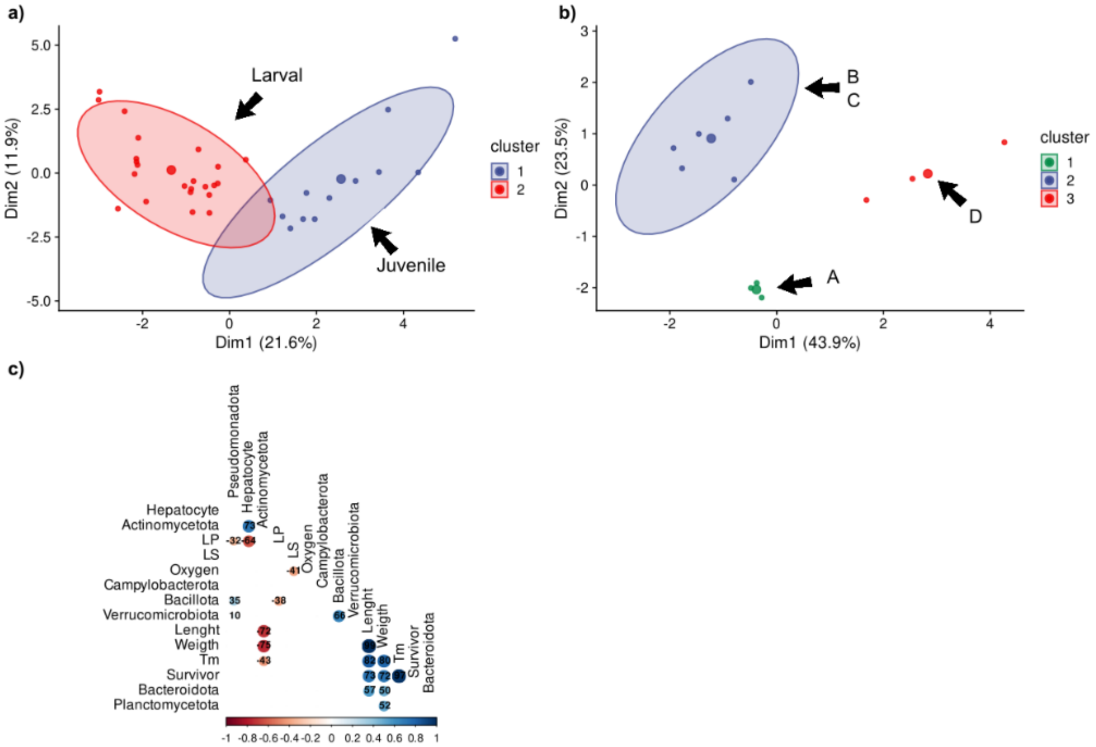

Fig. 5 Principal component analysis (PCA) integrating microbiota and physiological variables. (a) PCA based on temperature, day post-hatch (DPH), body weight, body length, and the 20 most abundant phyla; b) PCA including temperature (Tm), primary lamella length (LP), secondary lamella length (LS), hepatocyte diameter, and the four dominant phyla. Spearman correlation analysis was performed to identify significant associations among variables. A = 20°C, B = 24°C, C = 26°C, and D = 28°C.

In the second PCA, physiological variables (body size, body weight, oxygen consumption), DPH, and temperature were integrated with histological measurements of gills and hepatocytes diameter, along with the four core phyla. This analysis revealed three clusters separated by temperature (Fig. 5b): Cluster 1 grouped larvae reared at 24 and 26°C, Cluster 2 included larvae at 28°C, and Cluster 3 consisted of larvae at 20°C (Fig. 5b). PC1 explains 43.9% of the total variance and was primarily driven by contributions from primary lamella (LP), *Bacillota*, *Pseudomonadota*, and hepatocyte diameter. PC2 explains 23.5% of the variance and was mainly associated with *Actinomycetota* and temperature.

Spearman correlation analysis integrating seven dominant phyla with morphometric variables, final survival, oxygen consumption, and temperature at 24 DPH showed distinct associations (Fig. 5c). *Pseudomonadota* showed a significant negative correlation with primary lamella length (LP) (ρ =-0.32), and positive correlations with *Bacillota* (ρ = 0.35), and *Verrucomicrobiota* (ρ = 0.10). *Actinomycetota* correlated positively with hepatocyte diameter (ρ = 0.73), body lengh (ρ = 0.72), but negatively with body weight (ρ = -0.75) and temperature (ρ = -0.43). *Bacillota* correlated negatively with LP (ρ = -0.38) and positively with *Verrucomicrobiota* (ρ = 0.66). Finally, *Bacteroidota* correlated positively with body length (ρ = 0.57) and body weight (ρ = 0.50), while *Plantomycetota* correlated positively with body weight (ρ = 0.52).

Network Analysis of functional potential at the genus level showed and increased potential for quorum sensing at 20 and 24°C (Fig. 6a). When functional potential was evaluated between low (20°C) and high (28°C) temperatures during standard weaning, the lower temperature was associated with a higher predicted activity of metabolic pathways related, to energy metabolism including glycolysis/gluconeogenesis, glycine-serine and threonine metabolism, glycerophospholipid and glycerolipid metabolism, glutathione metabolism, fructose and mannose metabolism, fatty acid degradation, and fatty acid biosynthesis (Fig. 6b and c). In addition, samples at 20°C exhibited higher predicted functional potential for beta-lactam and vancomycin resistance, suggesting the presence of bacterial resistance mechanism (Fig. 6b and c). Finally, when comparing standard and early weaning, early weaning reduced the predicted potential for beta-lactam and vancomycin resistance (Fig. 6d).

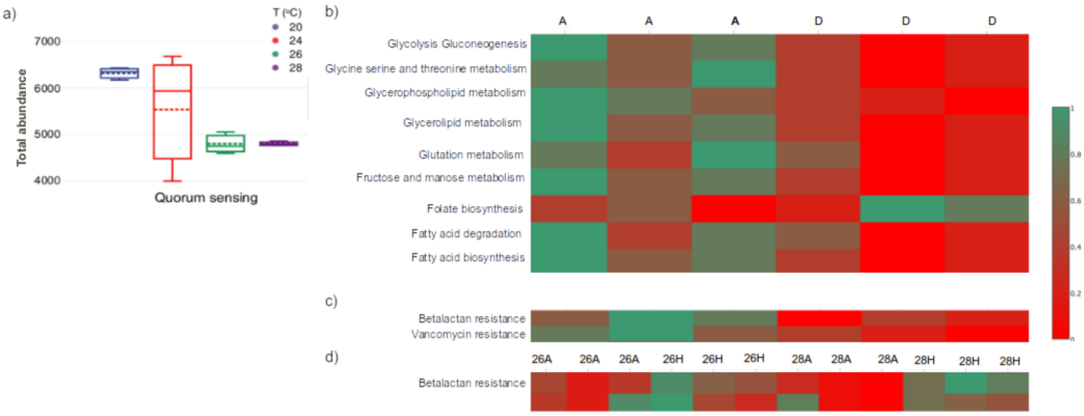

Fig. 6 Predicted functional potential of the microbiota based on iVikodak network analysis. (a) Quorum sensing pathway potential across temperature treatments; (b) metabolic pathways related to energy metabolism comparing low (A = 20 °C) and high (D = 28 °C) temperature thresholds; (c) Predicted functional potential for antimicrobial resistance (beta-lactam and vancomycin resistance) between A (20ºC) and D (28ºC); (d) Effect of weaning strategy (early vs.standard) on antimicrobial resistance functions at 26°C and 28°C. Weaning strategy: early weaning (A), standard weaning (H).

III.4. Discussion

The present study characterized the temporal dynamics of the bacterial communities associated with totoaba (*T. macdonaldi*) during the first 24 days after hatching under different thermal regimes and feedings transitions. Our results demonstrated that both temperature and feeding strategy are important modulators of the bacterial communities.

III.4.1 Alpha diversity indices

Direct proportional effects on the alpha index were observed of larval development at 7 DPH (rotifer food) and 16 DPH (Artemia food); at 20 and 24°C, lower alpha index were identified, while with the increase of water temperature from 26 to 28°C the alpha index increases. Interestingly, at 24 DPH (formulated diet), a significant increase in richness indexes and a significant decrease in Shannon index were observed at 28°C. The observed trends indicate that in the early life stages of totoaba, increasing temperature from 20 to 26°C can increase some components of the structure of microbial communities. On the other hand, at the end of larval development, temperature of 28°C generated an imbalance in microbial community composition (evident decrease in Shannon index and an increase in individual richness). The results show uniformity and balance are more feasible in the temperature range of 24 to 26°C in totoaba from 7 to 24 DPH. Because stability or balance is associated with healthy microbiota (Egerton et al., 2018), a decrease in the Shannon index at a temperature of 28°C in the larval/juvenile transition with late weaning may be associated with the negative effect.

The increasing temperature could increase the nutrient demand and energy metabolism of the holobiont (Butt and Volkoff 2019). This may have affected the results of the present study, which generated significant changes in the richness of rare OTUs and abundance of more highly represented OTUs. Temperature effects were eliminated when weaning was anticipated at 24 DPH, mainly decreasing the abundance of already dominant species (*Pseudomonads*, *Bacteroideota*, and *Actinomycetes*). This indicates that live food generates high dominance of a few OTUs, high temperatures (28°C) increase richness and, anticipating feeding with formulated diets can attenuate these effects. Previous studies have already shown that rising water temperatures can lead to an increase in the richness and abundance of certain members of the microbial communities, causing an imbalance and loss of fish health (Diwan et al., 2023; Chen et al., 2025). Also, formulated diets can improve the balance of OTUs homogeneity (Li et al., 2019; Lindsay et al., 2020). Thus, anticipating the formulation of feed under a culture regime with elevated temperatures appears to be a good strategy to mitigate potential adverse effects of an imbalance in the structure of microbial communities.

III.4.2 Taxonomic composition food effects

In regard to phylum composition, a core group was observed with a higher presence of *Pseudomonadota* (96%), followed by *Bacteroidota* (2%) and

Actinomycetota (0.8%). At genus level, a greater presence of the genera Vibrio (10-69%), *Pseudoalteromonas* (1-12%), and *Alteromonas* (5-40%) were observed, which is common in the intestines of marine fish like *T. macdonaldi* (Talwar et al., 2018; Barreto-Curiel et al., 2018; González-Feliz et al., 2018; Larios-Soriano et al., 2022).

It was identified that changes in microbiota composition increased with days and temperature, mainly during the transition to juvenile and weaning. At the beginning (7 and 16 DPH), the prevalence *Vibrio* may be related to rotifers and Artemia used as live feed since these feeds are associated with high concentrations (Gómez-Gil et al., 2003; Soto-Rodríguez et al., 2003). *Vibrio* is also abundant in seawater, culture water, and sediments (Puente et al., 1992; Vandenberghe et al., 2003; Quiroz-Guzmán et al., 2013; Roy et al., 2022). *Pseudomonas, Aeromonas*, *Flavovacterium*, and *Pseudoalteromonas* genera have also been identified, but *Vibrio* remains the dominant genus (Sánches-Fernández et al., 2022). Pseudomonadota/*Vibrio* abundances were observed to decrease, and genera as *Nautella*, *Ruegeria* and *Thalassotaela* increase at 26°C at 24 DPH which could have had a positive influence on development, growth, and survival since *Vibrio* is considered one of the most important pathogens in fish (Gómez-Gil et al., 2004). These indicate that replacing live feed with feed formulated at an appropriate temperature and development stage can reduce the bacterial load of the *Vibrio* genus and increase the uniformity of the microbiota.

Controlling the dominance of bacterial genera with a high incidence in the marine environment, such as *Vibrio*, is essential for maintaining energy homeostasis, since a high diversity of intestinal microbiota stimulates fish digestive and immune systems during the development of the adaptive immune system (Talwar et al., 2018; Morshed et al., 2023). However, their high prevalence of *Vibrio* (dominance of a single OTUs) may damage the balance and structure of the bacterial community, as well as the energy status and affect larval development (Xiong et al., 2019). Although live feed is essential for larval development, the use of probiotics, especially at these early stages of culture, has been shown to have beneficial effects on larval health and survival (Gomez-Gil et al., 2000). Therefore, the use of probiotics, as well as the administration of formulated feed, can balance microbial communities by limiting the proliferation of a few OTUs and balancing abundances before 24 DPH. Although this hypothesis was not tested in the present study, there is sufficient information to indicate that suboptimal temperatures and live feed generate bacterial dominance related to suboptimal larval health.

III.4.3 Correlation of microbiota with tissue damage

The genus *Vibrio* presents different pathogenic and non-pathogenic species, and is a heterotrophic genus with the characteristic of being ubiquitous (Sampaio et al., 2022). In this sense, in the present study a greater relative abundance of *Vibrio* at low temperatures (20 and 24°C) at 24 DPH may be related to a nutritional and thermal environment sufficient to favor these organisms. In addition, it is possibly related to the negative effect of temperature on the morphology of gills (inflammation) and hepatocytes (incipient steatosis) observed in Hernández-Montiel et al., (2025), with greater abundance of *Vibrio* during weaning at temperatures below 26°C observed in the present study. This visible damage in fish has been observed in infections by *Vibrio* and *Aeromonas*, which produce lipopolysaccharides (LPS) and pore-forming toxins that trigger strong immune responses and high mortality rates (~50%) (Cohen et al., 2020; Mabrok et al., 2023; Santos et al., 2023). Chronic heat stress played an important role in the deterioration of the holobiont as previously observed in totoaba juveniles during a temperature increase from 16 to 25.5°C, which can be linked with vibriosis with structural damage to lamellae and congestion of the hepatic portal system vessels (Trejo-Ramos et al., 2022). These results indicate that in addition to the physiological, tissue and metabolic effect that suboptimal temperatures (20 and 28°C) have on totoaba larvae, the incidence of morphological changes caused by changes in the microbiota is an evident reality and whose consequences can generate high mortality rates during the first 24 DPH.

III.4.4 Relative OTUs contribution derived from temperature and days post-hatch

In the present study, a greater relative contribution of the phylum Pseudomonadota was observed for DPH and temperature, mainly *Alteromonas abrolhosensis* (24% and 17%, respectively). Bacteria of the genus *Alteromonas* are opportunistic, persist in the ocean, and are ecologically significant (Yang et al., 2022), making them one of the dominant genera in fish egg microbiota during the early colonization stages (Hansen and Olafsen, 1989). *Alteromonas abrolhosensis* is an algicidal bacterium with the potential to control harmful algal blooms by destroying the cell wall through ROS accumulation and lipid peroxidation (Jia et al., 2023; Jia et al., 2025). Since these bacteria has been identified as an organism with an optimal growth temperature range close to 30°C (Nóbrega et al., 2018), this can be inferred that the increase in temperature (28°C) at 24 DPH generated the increase in microbial communities associated with warm climates such as *A. abrolhosensis*.

The largest contribution to the relative abundance of the microbiota by the *Vibrio* genus were *V. hyugaensis* (related to the DPH: 6.75%) and *V. chagasii* (related to the temperature: 14.64%). The increase in *V. hyugaensis* and *V. chagasii* may affect the larvae, which together with other bacteria with pathogenic potential such as *V. alginolyticus*, may limit the cellular proliferation of the larvae of the Atlantic herring *Clupea harengus* (Sánchez-Fernández et al., 2022; Urtubia et al. 2023; Franke et al., 2024). Also, these effects on microbial communities affect the interaction of the adherent (biofilm-forming) and non-adherent microbiome present in fish, feed, and the culture system (Gajardo et al., 2017; Stagaman et al., 2017; Talwar et al., 2018; Paralika and Makridis, 2025). Thus, the adverse effects identified in previous studies (Hernández-Montie, et al., 2025) may be related to the increase in the relative abundance of these genera, *Alteromonas* and *Vibrio*, however, different studies are needed to confirm this hypothesis.

In zebrafish larvae, the composition of the intestinal and environmental microbiota has been observed to diverge rapidly after the start of feeding, increasing under constant diet conditions (Wong et al., 2015). Thus, the low *Vibrio* abundance at 26 and 28°C on 24 DPH indicates that feeding with formulated diets evidently decreased the bacterial load of these two species, allowing the colonization of new individuals capable of providing different metabolic capacity.

III.4.5 Possible temperature effects on ontogeny and associated microbiota

One of the main negative morphological effects in larvae exposed to low temperatures is an increase in lordosis cases since notochord growth depends directly on temperature (Viader-Guerrero et al., 2021; Hernandez-Montiel et al., 2025). The notochord also plays an important role in mucus formation, which is released by mucosal tissue in the digestive and respiratory systems for physical protection and modulating immune signals (Brinchmann et al., 2018; Tapia-Paniagua et al., 2018). Also, chronic inflammation underlying pathological inflammation produced scoliosis during notochord infections in zebrafish larvae (Nguyen-Chi et al., 2014). The morphological, metabolic, physiological effects and changes in the structure of microbial communities caused by temperatures of 20 and 28°C (Hernandez-Montiel et al., 2025; the present study) may be an indication that the loss of homeostasis increases the incidence of malformations in the totoaba.

The increase in Actinomycetota at 26°C with food anticipation should be considered related to an increase in survival of ~10% compared to normal weaning. Thus, the best strategy is to control Pseudomonadota abundance with functional potential for quorum sensing and antimicrobial resistance, which would increase the phylum Actinomycetota and other phyla abundance with beneficial bacteria during weaning. The balance in the relative abundance of the bacterial community members allows for the digestion of a greater number of dietary components (De Marco et al., 2023). Therefore, lipids, proteins, and carbohydrates would be available for larval development and not for the generation of biofilm or virulence factors, such as LPS and resistance genes.

The relationship between bacteria of the phylum Aminocenantes OP8 and SR1 with variables of CP1 (oxygen consumption, weight) and CP2 (temperature) mainly affected juveniles at 28°C. Where temperature modified oxygen diffusion and transport, generating anoxic conditions for OP8 and SR1 (Farag et al., 2014; Campbell et al., 2013). It is possible that anoxia at 28°C is related to primary lamellae thinning with an increase in goblet cells, which limits oxygen diffusion.

In relation to optimal growing temperature for totoaba larva (26°C) determined in Hernandez-Montiel et al. (2025), a greater relative abundance of the genus *Thalassotalea* (Pseudomonadota) was observed on day 24. This genus is related to biodegradation and homeostasis in the dissolved organic matter redistribution in marine environments, degrading polysaccharides and chitin with a requirement for peptone as an energy source (Kim et al., 2020). In aquaculture, peptones are used to hydrolyze fish by-products, allowing for greater digestibility and amino acid availability (Vázquez et al., 2020), which indicates that at 26°C greater nutrient assimilation at weaning and members of the microbial community could help the nutrient solubilization processes. In addition, the genus *Thalassotalea* was isolated from the gills of farmed sole (*Paralichthys olivaceus*), where its optimal culture temperature was ~28°C (Zhang et al., 2014). Other bacteria with higher relative abundance were *Ruegeria* and *Nautella* in 26°C treatment. Some strains of the *Ruegeria* genus have been linked to the collagenase inhibition and *Vibrio* growth (Miura et al., 2019). In the case of *Nautella*, pathogenic elements related to diseases in macroalgae have been observed in common sea bass larvae alongside *Ruegeria* and *Pseudoalteromonas* (Tarnecki and Rhody, 2017). Finally, the microbiota present in farmed fish shows significant differences from wild fish (Kanika et al., 2025), thus it would be interesting to evaluate whether the abundance of bacteria observed in the present study coincides with those present in larvae and juveniles in the natural environment.

III.5. Conclusion

This study shows that the microbiota of farmed totoaba *T. macdonaldi* larvae is dominated by members of the genera *Vibrio*, *Alteromonas*, and *Pseudoalteromonas*. Although temperature and age (DPH) alone did not significantly affect alpha or beta diversity, their interaction modulates structure and relative abundance. A high abundance of *Vibrio* was observed during the initiation of exogenous feeding, which decreased with weaning. These results suggest that temperature and feeding strategy can be managed to limit opportunistic pathogens and improve health in totoaba culture.

II.6. References

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Capítulo IV

Conclusiones generales

La temperatura influyó de manera significativa en la tasa de crecimiento larvario, particularmente del notocordio, observándose una mayor incidencia de lordosis a temperaturas bajas. Estas alteraciones en la tasa de crecimiento pueden afectar la cinética de las larvas durante el proceso de alimentación, lo que incrementa el gasto energético asociado al desplazamiento y captura de alimento. En contraste, el cultivo a una temperatura óptima favorece un crecimiento alométrico negativo entre la talla y el peso, que mejora la homeostasis energética y podría facilitar un desplazamiento más eficiente durante la alimentación.

Se observó que las temperaturas subóptimas (20 y 28°C) tuvieron un efecto negativo sobre la integridad estructural de las lamelas primarias y secundarias de las branquias. Estas alteraciones podrían comprometer la excreción de iones (Na^+, CL^-, K^+) y desechos metabólicos que pueden actuar como sustratos para la microbiota asociada (NH_3 y HCO_3^-) a nivel de las lamelas principales, así como la difusión de gases (O_2 y CO_2), principalmente en lamelas secundarias.

El mayor consumo de oxígeno a temperaturas fuera del rango Q_{10} óptimo, no se reflejó en un incremento de la talla. Por el contrario, estas condiciones culminaron en tallas significativamente menores al DPH 24, como ocurrió a 20ºC, donde el tamaño promedio de los juveniles fue significativamente menor que sus congéneres a 26ºC. Este patrón indica una menor eficiencia en el uso de reservas energéticas para el crecimiento y el desarrollo.

La reducción de reservas de glucógeno y lípidos en hígado podría comprometer la capacidad de las larvas durante la transición entre el metabolismo aeróbico y anaeróbico como mecanismo compensatorio en condiciones térmicas subóptimas. Dicha transición metabólica es indispensable para garantizar el aporte energético al músculo en desarrollo durante las fases de crecimiento, principalmente, durante el incremento exponencial de peso observado en el periodo de destete

El deterioro de la homeostasis energética generó un consumo crónico de las reservas energéticas por parte de la microbiota simbionte. En este contexto, la mayor abundancia relativa de bacterias anaerobias, como *Vibrio*, se asoció con un incremento en las rutas metabólicas relacionadas con el metabolismo de carbohidratos y lípidos, de acuerdo con las predicciones funcionales de la microbiota. En contraste, a temperaturas elevadas, el aumento en la abundancia relativa de bacterias aeróbicas estrictas como *Pseudomonas*, podría estar relacionado con un incremento en la producción de mucinas por parte del hospedero, como mecanismo de defensa frente al estrés térmico (Fig 1).

El alimento vivo promovió la dominancia de un número reducido de Unidades Taxonómicas Operativas (OTUs), principalmente del género *Vibrio* (60-68%). Por otro lado, el uso de alimento formulado favoreció una mayor homogeneidad en la abundancia de OTUs, con una comunidad microbiana más equilibarada, caracterizada por la presencia de *Alteromonas* (39.6%), *Thalassotales* (16%), *Vibrio* (9.9%) y *Pseudomonas* (9.1%).

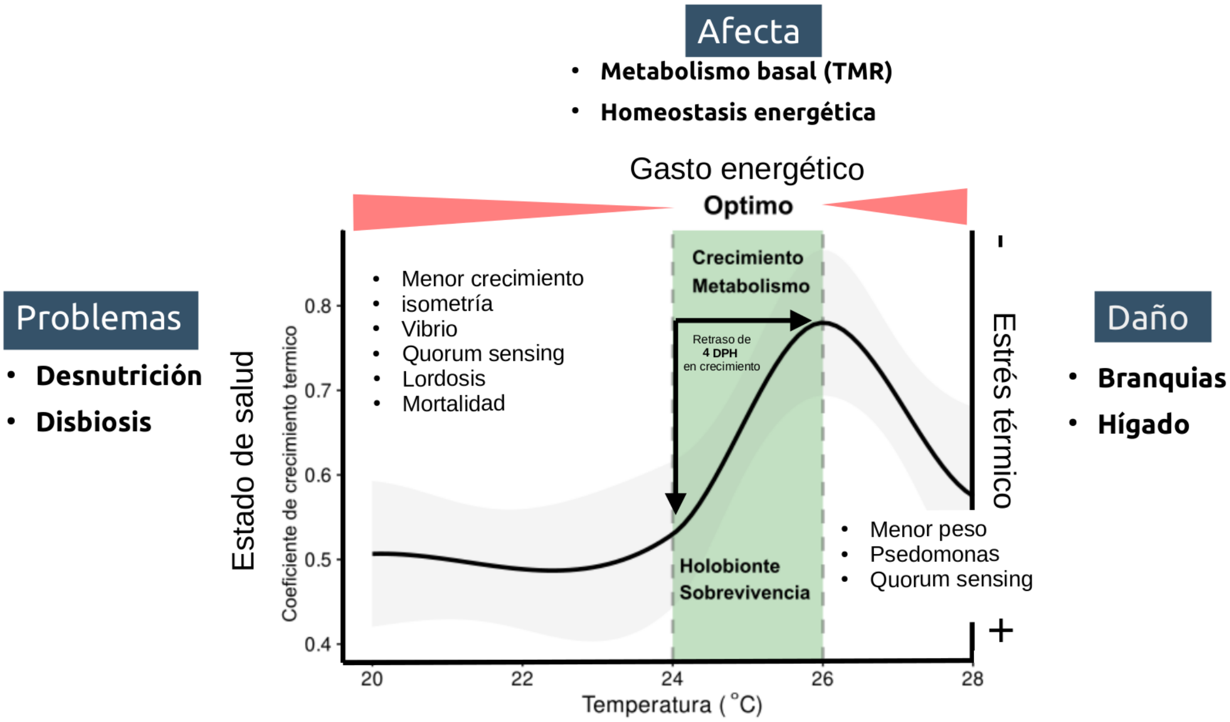

Fig 1. Coeficiente de crecimiento termico. Regresion Loess del TGC para 20, 24, 26 y 28ºC y variables involucradas con el gasto energetico de holobiontes.

La temperatura de 26°C generó los valores más altos de los índices de diversidad alfa, lo que sugiere mayor diversidad de OTUs y una mayor capacidad metabólica inferida de las comunidades microbianas asociadas al holobionte. En contraste, la temperatura de 28°C se caracterizó por un aumento en la riqueza de especies y una menor homogeneidad en la abundancia relativa de OTUs en comparación con las larvas expuestas a 26°C. No obstante, estos efectos se atenuaron cuando se aplicó un destete anticipado, lo cual se asoció lo cual se relaciona con una recuperación del equilibrio en la estructura de la comunidad microbiana.

Con base en los resultados obtenidos en las larvas analizadas en el presente estudio, se concluye que la temperatura afecta la tasa de crecimiento y desarrollo de holobiontes ectotérmicos. En este contexto, la temperatura y la microbiota simbionte desempeñan un papel crucial en el almacenamiento y la utilización de reservas energéticasa lo largo de la ontogenia del desarrollo larvario. Por lo que se recomienda priorizar el desarrollo y mantenimiento de la salud del holobionte en su conjunto, para disminuir los problemas asociados al cambio de dieta, particularmente, durante las etapas tempranas del desarrollo como la preflexión y la flexión, en las que se manifiestan con mayor intensidad los efectos negativos de las temperaturas subóptimas de cultivo.

Propuesta de investigación

A pesar de que este estudio establece los 26°C como la temperatura óptima para el desarrollo y crecimiento del holobionte Totoaba macdonaldi, persisten brechas de conocimiento críticas en torno a la relación entre el estrés oxidativo inducido por las microdietas y la salud hepática a largo plazo, así como la ausencia de estudios comparativos que contrasten la estructura de la microbiota entre organismos silvestres y cultivados. En consecuencia, se propone que las futuras investigaciones se centren en validar la eficacia de los protocolos de destete anticipado a 26°C como estrategia para minimizar el canibalismo y la mortalidad, además de evaluar parámetros metabólicos avanzados como la acción dinámica específica (SDA) y la tasa metabólica máxima (MMR) para cuantificar con precisión el presupuesto energético total durante la transición larva-juvenil. Asimismo, resulta imperativo investigar el papel funcional de géneros bacterianos específicos como Thalassotalea, Nautella y Ruegeria en la degradación de nutrientes y la supresión de patógenos oportunistas como Vibrio, integrando análisis de rentabilidad económica que optimicen las tasas de alimentación bajo regímenes térmicos controlados.

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