UNIVERSIDAD JUÁREZ AUTÓNOMA DE TABASCO
División Académica de Ciencias Biológicas

"CARACTERÍSTICAS FISIOLÓGICAS Y VENTANAS CRÍTICAS EN EL DESARROLLO DEL PEJELAGARTO *Atractosteus tropicus*"

TESIS

Para obtener el grado de Doctor en Ciencias en Ecología y Manejo de Sistemas Tropicales

Presenta:

M. en C. Gil Martínez Bautista

Director:

Dr. Carlos Alfonso Álvarez González

Co-director:

Dr. Warren William Burggren

Villahermosa, Tabasco, México Mayo de 2021

MAYO 12 DE 2021

C. GIL MARTÍNEZ BAUTISTA
PAS. DEL DOCTORADO EN CIENCIAS EN ECOLOGÍA Y MANEJO DE SISTEMAS TROPICALES
P R E S E N T E

En virtud de haber cumplido con lo establecido en los Arts. 80 al 85 del Cap. III del Reglamento de titulación de esta Universidad, tengo a bien comunicarle que se le autoriza la impresión de su Trabajo Recepcional, en la Modalidad de Tesis de Doctorado en Ciencias en Ecología y Manejo de Sistemas Tropicales titulado: **"CARACTERÍSTICAS FISIOLÓGICAS Y VENTANAS CRÍTICAS EN EL DESARROLLO DEL PEJELAGARTO *Atractosteus tropicus*"**, asesorado por el Dr. Carlos Alfonso Álvarez González y Dr. Warren William Burggren sobre el cual sustentará su Examen de Grado, cuyo jurado está integrado por el Dr. Emyr Saúl Peña Marín, Dr. Rafael Martínez García, Dr. Carlos Alfonso Álvarez González, Dr. Luis Daniel Jiménez Martínez, Dra. Susana Camarillo Coop, Dra. Susana del Carmen de la Rosa García y Dra. Carina Shianya Álvarez Villagómez.

Por lo cual puede proceder a concluir con los trámites finales para fijar la fecha de examen.

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
ESTUDIO EN LA DUDA. ACCIÓN EN LA FE

U.J.A.T.
DIVISIÓN ACADÉMICA
DE CIENCIAS BIOLÓGICAS
DIRECCIÓN

DR. ARTURO GARRIDO MORA
DIRECTOR

C.c.p.- Expediente del Alumno.
C.c.p.- Archivo

CARTA AUTORIZACIÓN

El que suscribe, autoriza por medio del presente escrito a la Universidad Juárez Autónoma de Tabasco para que utilice tanto física como digitalmente el Trabajo Recepcional en la modalidad de Tesis de doctorado denominado: **"CARACTERÍSTICAS FISIOLÓGICAS Y VENTANAS CRÍTICAS EN EL DESARROLLO DEL PEJELAGARTO *Atractosteus tropicus*"**, de la cual soy autor y titular de los Derechos de Autor.

La finalidad del uso por parte de la Universidad Juárez Autónoma de Tabasco el Trabajo Recepcional antes mencionada, será única y exclusivamente para difusión, educación y sin fines de lucro; autorización que se hace de manera enunciativa más no limitativa para subirla a la Red Abierta de Bibliotecas Digitales (RABID) y a cualquier otra red académica con las que la Universidad tenga relación institucional.

Por lo antes manifestado, libero a la Universidad Juárez Autónoma de Tabasco de cualquier reclamación legal que pudiera ejercer respecto al uso y manipulación de la tesis mencionada y para los fines estipulados en éste documento.

Se firma la presente autorización en la ciudad de Villahermosa, Tabasco a los 12 días del mes de mayo de dos mil veintiuno.

AUTORIZO

GIL MARTÍNEZ BAUTISTA

Índice

Resumen

Las alteraciones en las trayectorias de desarrollo de los peces ocurren como respuesta a cambios genéticos o del ambiente, especialmente durante periodos sensibles de desarrollo denominados 'ventanas críticas de desarrollo'. En esta investigación se utilizó a la ontogenia temprana del pejelagarto *Atractosteus tropicus* como modelo para estudiar la supervivencia, crecimiento y desarrollo de la especie para ampliar el panorama sobre la biología de los peces, especialmente de aquellos que respiran aire. Se analizaron características morfológicas (masa, longitud y factor de condición), fisiológicas (ritmo cardiaco, ventilación branquial y ventilación aérea) y moleculares (expresión de genes) en función de tres estresores como la temperatura, la disponibilidad de oxígeno y la salinidad. Así mismo, se analizó el tiempo de ocurrencia de eventos morfológicos (eclosión, alimentación exógena, absorción del vitelo, nado libre y cambio en la forma del hocico) y fisiológicos (latidos del corazón, movimientos operculares y respiración aérea) clave para el desarrollo de la especie, denominado 'heterokairy'. Los ensayos se realizaron durante tres periodos importantes en el desarrollo: 1) fertilización a eclosión, 2) 1 a 6 días post eclosión (dpe) y 3) 7 a 12 dpe. Los resultados de esta investigación demostraron que el pejelagarto es un pez modelo para estudios en etapas tempranas de desarrollo. Del mismo modo, los resultados sugieren ventanas críticas identificables para cada una de las variables, observando que en general los periodos de fertilización a eclosión y 1-6 dpe son los más sensibles desde perspectivas morfológica, fisiológica y molecular, mientras que el periodo que comprende los 7-12 dpe se mostró como el más resiliente. Este trabajo contribuye al conocimiento sobre la ecomorfofisiología y biología molecular de las etapas tempranas de los peces y las interacciones entre individuos × estresores × tiempo de exposición. Por otro lado, los resultados generados en la presente investigación sugieren parámetros importantes bajo los cuales puede favorecerse el larvicultivo de la especie y mejorar los sistemas de producción en el sureste del país, promoviendo una mayor supervivencia y crecimiento de los peces en sus primeras etapas de vida.

Introducción

La plasticidad en el desarrollo es la habilidad de los organismos en sus etapas tempranas de modificar su fenotipo en función de fatores intrínsecos (genéticos) o extrínsecos ambientales (Burggren, 2019; Burggren y Reyna, 2011; Hutchins, 2011). Las modificaciones en las trayectorias de desarrollo como resultado de la plasticidad fenotípica tienen la capacidad de alterar características en los adultos, las cuales pueden ser adaptativas o perjudiciales (Vehaskari *et al*., 2011; Morgan *et al*., 1998; Sallout y Walker, 2003). Algunas alteraciones en ciertas características de los organismos en desarrollo ocurren durante periodos de tiempo específico, las cuales se conocen como ventanas críticas. Las ventanas críticas se definen como periodos sensibles en el desarrollo donde el fenotipo puede alterarse por diferentes factores (Pinkerton y Joad, 2000; Hogan *et al*., 2008; Burggren y Reyna, 2011; Buggren y Mueller, 2015).

La mayoría de los estudios que han analizado ventanas críticas en el desarrollo se han centrado en el efecto de un solo factor (estresor) en diferentes tiempos de desarrollo (*e.g*,, Dzialowski *et al*., 2002; Chan y Burggren, 2005; Liu y Wong-Riley, 2010; Tate *et al*., 2015). Sin embargo, un diseño experimental para ventanas críticas que es 'multifactorial' (*e.g*., múltiples factores variando simultáneamente) ofrecen un panorama de circunstancias más realista que permite entender y visualizar la interacción del desarrollo, la dosis de los estresores y la respuesta en características fenotípicas de interés (Burggren y Mueller, 2015; Mueller *et al*., 2016).

Además de determinar el efecto de los estresores en las ventanas críticas de ciertas características, es importante analizar cómo esos factores pueden alterar las trayectorias de desarrollo de los organismos, especialmente en cuanto al inicio y tiempo de ocurrencia de eventos en el desarrollo (anatómicos, fisiológicos, etc,). Las modificaciones en estas características de los individuos se denominan 'heterokairy', que se define cómo '…la plasticidad en el tiempo de inicio de diferentes eventos en el desarrollo a nivel de organismos' (Spicer y Burggren, 2013). Del mismo modo, 'heterokairy' puede ser evidente en cambios que ocurren entre poblaciones de una misma especie.

Con base en lo anterior, las larvas de los Lepisosteidos presentan desarrollo embrionario y larval rápidos, lo cual los convierte en modelos ideales para el estudio de

etapas tempranas de desarollo (Burggren *et al*. 2016). El pejelagarto *Atractosteus tropicus* Gill 1863 es una de las siete especies existentes de los Lepisosteii y se distribuye desde el Sur de México hasta América Central (Barrientos-Villalobos y Espinoza de los Monteros, 2008; Bussing, 1998; Miller *et al*., 2005). Esta especie se encuentra en masas de agua con movimiento lento como ríos, lagos, lagunas y remansos. El pejelagarto puede sobrevivir en ambientes con baja saturación de oxígeno y moderada alta temperatura (Mora *et al*., 2015). Además de la función ecológica de la especie en su hábitat, *A. tropicus* representa importancia socioeconómica y cultural en Tabasco y áreas aledañas con costumbres de civilizaciones Olmeca y Maya. Por ejemplo, el pejelagarto es un alimento popular debido a su alta calidad nutrimental a bajo precio. Del mismo modo, se venden como mascotas, se realizan joyas con su piel y escamas y organismos completos son preservados como souvenirs. Además, el pejelagarto es una de las cinco fuentes pesqueras y de cultivo en México y es una especie importante en la industria de la pesca recreacional (Barrientos-Villalobos y Espinosa de los Monteros, 2008; Bussing, 1998; Miller *et al*., 2005).

Antecedentes

Supervivencia y crecimiento

La supervivencia de los organismos puede afectarse por numerosos factores y su efecto combinado. Por ejemplo, cambios en la salinidad y oxígeno disuelto están aunados a incremento en la temperatura del agua debido al cambio climático y actividades antropogénicas (Breigtburg *et al*., 2018; Díaz, 2001). Varios estudios han demostrado como la temperatura y el oxígeno disuelto disminuyen la supervivencia (especialmente durante la incubación) en Teleósteos y Holósteos (*e.g.*, Comabella *et al*., 2014; Del Río et al., 2019; Eme *et al*., 2015; Hassell *et al*., 2008; Hou *et al*., 2019; Levesque *et al*., 2019; Mueller *et al*., 2011a). En el caso de los Lepisosteidos, las larvas y jóvenes de *Lepisosteus oculatus* presentaron supervivencia disminuida al exponerse a altas concentraciones de oxígeno (hiperoxia), mientras que en bajas concentraciones (hipoxia) la supervivencia no presentó diferencias significativas con los peces mantenidos en normoxia (Rimoldi *et al*., 2016).

Con respecto a la salinidad, varias especies de peces primitivos pueden tolerar salinidades superiores o inferiores que la osmolalidad de su plasma. Sin embargo, existe muy poca información sobre la salinidad y las etapas tempranas de vida de los peces (Allen *et al*., 2011; Allen y Cech, 2007; Beamish, 1980; McDowall, 1988; Richards y Beamish, 1981; Schwarz y Allen, 2014). Un factor importante es que los peces recién eclosionados carecen de la capacidad de tolerar salinidades fácilmente toleradas por los adultos, ya que las branquias son el principal órgano para iono/osmorregulación en larvas de peces y de su madurez depende el éxito de este proceso y, por consecuencia, la supervivencia de los peces (Brauner y Rombough, 2012; Kupsco y Schlenk, 2016; Kwan *et al*., 2018; Melo *et al*., 2019; Tresguerres *et al*., 2020).

El crecimiento de los Leposisteii se caracteriza por un desarrollo embrionario rápido (~72 horas), un incremento de talla significativo antes de la metamorfosis. Por otro lado, después de la metamorfosis la tasa de crecimiento disminuye durante la transición en el tipo de alimentación. Este patrón es dependiente de la temperatura y se ha descrito para *Atractosteus tristoecus* (Comabella *et al*., 2014), *A. spatula* (Mendoza *et al*., 2002), *L. osseus* (Pearson *et al*., 1979) y *L. oculatus* (Simon y Tyberghein, 1991). Por otro lado,

algunos Teleósteos no muestran relación entre las temperaturas de incubación y la masa y longitud al momento de eclosionar (Jonassen *et al*., 1999; Keckeis *et al*., 2001; Mendiola *et al*., 2007; Person-Le Ruyet *et al*., 2006).

Del mismo modo, la disponibilidad de oxígeno tiene un efecto directo sobre el crecimiento de los peces en desarrollo. Una reducción en el crecimiento puede suceder debido a la respuesta compensatoria de los embriones para priorizar otras actividades en función de la disponibilidad de oxígeno (Rombough, 1988). Además, los peces menor crecimiento presentan menores posibilidades de supervivencia, especialmente debido a que presentan natación lenta, baja competitividad, pueden presentar deformidades y mayor riesgo de depredación (Del Río *et al*., 2019; Hassell *et al*., 2008; Mason, 1969; Pepin, 1991).

Además, la tasa de crecimiento de los peces puede incrementar en salinidades tolerables debido a la reducción en ellos costos de osmorregulación. Sin embargo, cuando los peces se exponen a concentraciones más altas, los costos de osmorregulación pueden interferir con el crecimiento (Boeuf y Payan, 2001). Por ejemplo, las larvas de *Cyprinus carpio* incrementan su masa corporal si se exponen a salinidades entre 5.0 y 20.0 (Malik *et al*., 2018), en contraste, su longitud no se modifica. Algunos estudios han mostrado que larvas de peces incubadas y criadas en mayores salinidades que en sus ambientes naturales presentan una reducción en su longitud total (Hernández-Rubio y Figueroa-Lucero, 2013; Vetemaa y Saat, 1996). Las variaciones en la masa corporal o longitud han sido atribuidas a diferencias en el contenido de agua de las especies (Holliday, 1969), los cuales dependen de la permeabilidad de las branquias y la superficie corporal al agua y sales en el ambiente (Eddy y Handy, 2012). Con relación a los Lepisosteiformes se conoce que existe una relación entre el crecimiento, la respiración aérea y la salinidad en peces jóvenes (Schwarz y Allen, 2014; Smatresk y Cameron, 1982), sin embargo, no se han descrito datos en embriones y larvas. Incluso cuando la temperatura se combina con la salinidad, el efecto de la temperatura sobre la absorción del vitelo y la utilización de la energía son mucho mayores que aquellos de la salinidad (Alderdice y Velsen, 1971; Collins y Nelson, 1993; Hart y Purser, 1995; May, 1974).

Desarrollo y heterokairy

Los Lepisosteidos y otras especies de peces con desarrollo indirecto deben atravesar una etapa de eleuteroembrión y pasar cierto tiempo adheridos al sustrato para madurar (Batty, 1984). El tiempo de ocurrencia de los diferentes eventos clave (morfológicos y fisiológicos) en el desarrollo de los peces (incluso después de la eclosión) puede verse afectado por la acción de diversos factores y tener consecuencias a largo plazo en los peces (Eddy y Handy, 2021; Eme *et al*., 2015). A medida que se desarrollan los peces, el presupuesto de energía proveído por el saco vitelino disminuye, el metabolismo incrementa y las larvas en desarrollo deben buscar presas para compensar sus demandas energéticas (Rønnestad *et al*., 2013). Por ejemplo, temperaturas mayores a las que se presentan de manera natural pueden acelerar su desarrollo, lo cual no en todos los casos es favorable, ya que un desarrollo acelerado requiere de un incremento en el gasto energético (Camacho *et al*., 2011; Dou *et al*., 2002, 2005). Por otro lado, se ha descrito que la variación en la disponibilidad de oxígeno y la salinidad pueden retrasar el desarrollo (Del Río *et al*., 2019; Massa *et al*., 1999; Polymeropoulos *et al*., 2017; Roussel *et al*., 2007) dando como resultado peces de menor tamaño y más susceptibles a las demandas del ambiente (Del Río *et al*., 2019; Hassell *et al*., 2008; Mason, 1969; Pepin, 1991). Estas alteraciones pueden limitar el desempeño de los peces y disminuir su supervivencia. Por ejemplo, un evento crucial en el desarrollo de los peces es el inicio de la natación (Voesenek *et al*., 2018) que puede verse afectado por la hipoxia y la salinidad, ya que ambas retrasan el desarrollo y disminuyen la actividad de los peces (Holliday, 1969). El desarrollo lento incluye una disminución en la tasa de absorción del vitelo (Del Río *et al*., 2019; Massa *et al*., 1999; Polymeropoulos *et al*., 2017; Roussel *et al*., 2007), la cual provee la energía necesaria para forrajear (Moteki *et al*., 2001; Williams *et al*., 2004) y para competir por recursos (Coughlin, 1991; Makrakis *et al*.,2005).

En el caso de los Lepisosteidos, las diferencias en el tiempo de desarrollo (especialmente el inicio temprano de la alimentación exógena) promueven el canibalismo una alta variación en la relación masa-longitud (Aguilera *et al*., 2002; Márquez-Couturier *et al*., 2015; Burggren *et al*., 2016). Además, las diferencias también pueden observarse en el tiempo de la apertura de la boca entre especies de este grupo. (Aguilera *et al*., 2002),

donde *A. spatula* exhibe crecimiento alométrico del hocico en proporción al tamaño de los adultos en tallas y edades menores que *A. tropicus*. Esta característica está relacionada con la rápida metamorfosis de *A. spatula* que mejora la oportunidad de cazar, capturar presas y la aparición temprana del canibalismo (Busch, 1996; Dabrowski and Bardega, 1984; Hecht y Pienaar, 1993).

Respuesta molecular y disponibilidad de oxígeno

Los animales acuáticos tienen acceso relativamente menor al oxígeno en su ambiente, comparado con animales terrestres (Eddy y Handy, 2012; Jonz *et al*., 2016). Debido a ello, los peces han desarrollado una serie de adaptaciones que les permite explotar diferentes hábitats y satisfacer sus demandas de oxígeno, tales como modificaciones en las branquias o la piel, y la habilidad de consumir oxígeno atmosférico (Damsgaard *et al*., 2020; Eddy y Handy, 2012; Garduño *et al*., 2020; Jonz *et al*., 2016). Sin embargo, existe muy poca información que relacione los procesos respiratorios de los peces con los mecanismos moleculares que los regulan. Actualmente existen más estudios que relacionan ambas características como respuesta a la hipoxia en peces con respiración acuática (*e.g*., Chen *et al*., 2012; Davies *et al*., 2011; Geng *et al*., 2014; Kodama *et al*., 2012; Rimoldi *et al*., 2012; Terova *et al*., 2008) que en peces con respiración aérea (Chi *et al*., 2013; Huang *et al*., 2015a, 2015b; Rimoldi *et al*. 2016).

El mantenimiento del equilibrio de oxígeno en organismos acuáticos es un proceso importante, cuya respuesta involucra factores de transcripción como las proteínas Hif (Chen, *et al*., 2020; Kaelin y Ratcliffe, 2008; Schofield and Ratcliffe, 2004; Whitehouse y Manzon, 2019). Las proteínas Hif (*hif-1α* y *hif-*2*α* por mencionar las más representativas) presentan una ruta conservada desde elasmobranchios hasta tetrápodos para la respuesta fisiológica a la disminución de oxígeno en el medio (Prabhakar y Semenza, 2015; Rytkonen *et al*., 2011; Semenza, 2010). Además, estas proteínas regulan la expresión de otros genes relacionados con procesos como la glucólisis, metabolismo del hierro, contracción muscular, así como el inicio de a respuesta a bajos niveles de oxígeno (Porporato *et al*., 2011; Semenza, 2000). En el caso de los Lepisosteidos, *L. oculatus* muestra un incremento en los niveles de expresión de proteínas Hif (*hif-2α*) como

respuesta a 71 días de exposición a bajas concentraciones de oxígeno (Rimoldi *et al*., 2016). Sin embargo, la expresión es variable entre las diferentes especies de peces que respiran aire (Huang *et al*., 2015a, 2015b).

La actividad transcripcional de *hif-1α* pueden regularse por el inhibidor del factor de inducción de hipoxia *fih-1* (Lando *et al*., 2002). Por ejemplo, la tilapia del Nilo (*Oreochromis niloticus*) y el bagre de canal (*Ichtalurus punctatus*) presentan un incremento en la expresión de *fih-1* después de someterse a estrés hipóxico (Feng *et al*., 2019; Geng *et al*., 2014; Li *et al*., 2017). Aunado a lo anterior, el estrés hipóxico puede promover un consumo branquial y transporte de oxígeno ineficiente debido a la acidosis en el plasma y la reducción de la afinidad de la hemoglobina al oxígeno (efecto Root). Las branquias son órganos multifuncionales responsables del intercambio de gases, el balance ácido-base, así como la iono- y osmorregulación (Evans *et al*., 2005; Laurent and Perry, 1991). Desde esta perspectiva, las proteínas Hif pueden participar en el transporte de iones a través del epitelio ya que la hipoxia altera la homeostasis y *hif-1α* está involucrado en la remodelación de las branquias bajo estrés hipóxico (Nilsson, 2007). Además, las proteínas Hif pueden regular la expresión de intercambiadores iónicos para mediar el flujo de Na^+ o K^+ a cambio de iones H^+ a través de las membranas (Brauner y Baker, 2009; Evans *et al*., 2005). Una de las mayores afectaciones ocurre cuando el intercambio de iones transepitelial disminuye durante eventos de acidosis, lo cual limita la capacidad de las branquias para regular el pH extracelular, lo cual puede disminuir el desempeño e incluso la supervivencia de los peces (Brauner y Baker, 2009; Shartau y Brauner, 2014).

De igual forma, las proteínas Hif puedne desencadenar la expresión de otros genes como respuesta a la variación en la disponibilidad de oxígeno, incluyendo aquellos relacionados con la eritropoyesis, mediada por la eritropoyetina. La eritropoyetina (*epo*) es una glucoproteína bien descrita para mamíferos (Haase 2010, 2013; Semenza 1999), en contraste, su función en los peces es poco conocida. Son pocos los estudios que describen el incremento en la expresión de *epo* en peces como respuesta a la hipoxia (Chou *et al*. 2004; Chu *et al*. 2007; Paffet-Lugassy *et al*. 2007; Pierron *et al*. 2007) y su función no solo se limita a la producción de glóbulos rojos, sino que participa en procesos como la neo-vascularización (Ribatti *et al*., 1999), la morfogénesis cardiaca (Wu *et al*., 1999), la

proliferación de mioblastos (Oglivie *et al*., 2010) y capacidades regenerativas (Buemi *et al*., 2009).

Respuesta fisiológica a cambios en la disponibilidad de oxígeno, la salinidad y la temperatura

La mayoría de los estudios realizados en peces con respiración aérea se han enfocado en etapas adultas (Little, 2009; Milsom, 2012; Perry *et al*., 2001; Randall *et al*., 1994; Randall *et al*., 1981; Randall e Ip, 2006; Shartau y Brauner, 2014). De acuerdo con Lefevré *et al*. (2014) existe una necesidad urgente de estudiar la fisiología (especialmente respiratoria) de los peces que respiran aire, en particular de aquellos empleados en proyectos de acuacultura. La mayoría de estos estudios se han enfocado en descripciones morfológicas y el valor nutrimental de las especies (Blank, 2009; Blank y Burggren, 2014; Burleson *et al*., 1998; Echelle y Rigs, 1972; González et al., 2010; Long y Ballard, 2001; Méndez-Sánchez y Burggren, 2014; Mendoza *et al*., 2007; Ramos *et al*., 2013), muy pocos en su bioquímica o su biología celular y molecular (Ahmad y Hasnain, *et al*., 2005; Arias-Rodríguez *et al*. 2009; Frías-Quintana *et al*., 2015; Frick *et al*., 2007; Guerrero-Zárate *et al*., 2014; Revol *et al*., 2005). En contraste, muy pocos estudios se han llevado a cabo en etapas tempranas, las cuales son las mas vulnerables en el desarrollo de los peces (Burggren and Bagatto, 2008; Johnson *et al*., 2010; Rudneva, 2014). Dentro de estas investigaciones destacan trabajos que describen las respuestas metabólicas en función del ambiente, incluyendo el efecto de la hipoxia, del desarrollo *per se*, y los costos de este proceso (Blank, 2009; Liem, 1981; Méndez-Sánchez, 2015; Méndez-Sánchez *et al*., 2014; Mueller *et al*., 2011a; Mueller *et al*., 2011b).

En el caso de los Lepisosteidos, particularmente jóvenes y adultos, existen diversos estudios que describen su morfología y fisiología con respecto a la toxicidad (Boudreux *et al*., 2007), tasa de ventilación (Burleson *et al*., 1998), respiración aérea (De Roth, 1973; McCormack, 1967; Rahn *et al*., 1971; Renfro, 1967; Saksena, 1967, Wiston, 1967), locomoción (Hill *et al*., 1973), estructura y función branquial (Landolt y Hill, 1975), función respiratoria de la vejiga gaseosa (Potter, 1927), crecimiento e inoregulación (Schwarz y Allen, 2014); respuestas a cambios en la temperatura e hipercapnia (Smatresk,

1982), respuestas quimio-receptivas (Smatresk *et al*., 1986; Smatresk y Cameron, 1982), morfología e inervación del corazón (Zaccone *et al*., 2011) y osmorregulación (Zawodny, 1975).

Con relación a la fisiología cardio respiratoria en el pejelagarto durante sus etapas tempranas de desarrollo destaca el trabajo de Burggren *et al*. (2016), donde se muestra que el ritmo cardiaco (f_H) y la ventilación branquial (f_G) incrementan rápidamente durante los primeros 5 días después de la eclosión; el f_H permanece constante hasta los 30 días, mientras que la f_G disminuye con la edad y la respiración aérea (f_{AB}) incrementa significativamente a los 15 días post eclosión. El f_H y la f_G incrementan después de actividad forzada mientras que se mantiene constante f_{AB}. Del mismo modo, el f_H y la f_G incrementan conforme incrementa la temperatura del ambiente en todas las edades menores a los 30 días posteclosión. Además, el f_H y la f_G se ven afectadas por la baja disponibilidad de oxígeno. Por un lado, los peces de menos de 5 días muestran un incremento de f_G al incrementar la hipoxia. Por otro lado, peces de15 y 30 días muestran una disminución de la f_G y un incremento de f_{AB} al disminuir el nivel de oxígeno. El f_H también incrementa al exponerse a los peces a estrés hipóxico.

Con relación a estudios sobre ventanas críticas en peces y su relación con su fisiología existen muy pocos estudios. Por ejemplo, Eme *et al*. (2015) evaluaron el f_H y el consumo de oxígeno (MO_2) en *Coregonus clupeaformis* durante tres periodos sensibles en la incubación y en función de cambios en la temperatura. La disminución de la temperatura incrementa el tiempo de cada ventana, mientras que temperaturas más altas lo reducen. Del mismo modo, los cambios en la temperatura de incubación modificaron el f_H y MO_2 se observaron incrementos y decrementos en esta característica. De esta manera, los autores sugieren que el desarrollo embrionario entre la fertilización y la organogénesis representa una ventana crítica de plasticidad fenotípica en embriones y peces recién eclosionados. Un ejemplo más sugiere que los cambios de temperatura en el desarrollo temprano de los peces pueden alterar el tiempo para la eclosión y la masa seca de los peces al eclosionar (Mueller *et al*., 2015). Así mismo, la tasa de conversión del vitelo disminuye y el costo de desarrollo incrementa al incrementar la temperatura. El periodo de organogénesis a los primeros movimientos de las aletas se ve afectado por la relación entre el desarrollo y la temperatura, lo cual sugiere que este intervalo de tiempo puede

representar una ventana crítica donde sucede el mayor impacto en los procesos energéticos.

Justificación

En peces, específicamente, es relativamente poco lo que se conoce acerca de su biología básica, respuestas fisiológicas y moleculares con relación a las ventanas críticas de desarrollo, a pesar de que este grupo ha sido ampliamente utilizado como modelos de investigación. Por otro lado, es poco el conocimiento que se tiene sobre heterokairy como una variación a nivel individual o poblacional y no entre especies (Spicer y Burggren, 2003; Spicer y Rundle, 2007; Spicer *et al*., 2001; Rundle y Spicer, 2016). La información sobre heterokairy y ventanas críticas en peces con respiración aérea -tanto Holosteii como Teleosteii- es limitada. La mayoría de los estudios en estos grupos se ha enfocado en la morfología y fisiología de peces adultos (*e.g*., Lefevre *et al*., 2016; Little, 1983; Milsom, 2012; Perry *et al*., 2001; Randall e Ip, 2006; Shartau y Brauner, 2014). Algunos estudios se han enfocado en embriones y larvas, que son las etapas más vulnerables y en donde las presiones selectivas actúan severamente (*e.g*., Johnson *et al*., 2010). Esos estudios han analizado la morfología y fisiología de embriones y larvas de Teleosteos (*e.g*., Blank y Burggren, 2014; Gonzáles *et al*., 2010; Joss, 2006; Méndez-Sánchez y Burggren, 2014; Mueller *et al*., 2011; Smith, 1985) y algunos Holósteos, (*e.g.*, Aguilera *et al*., 2002; Burggren *et al*., 2016; Comabella *et al*., 2013; Dean, 1895; Echelle y Rigs, 1972; Long y Ballard, 2001; Mendoza *et al*., 2007). Es por ello por lo que la presente investigación se enfoca en el estudio de las respuestas fisiológicas, morfológicas y moleculares en respuesta a la temperatura, disponibilidad de oxígeno y salinidad durante las ventanas críticas de desarrollo utilizando como modelo al pejelagarto *Atractosteus tropicus*. Los resultados de esta tesis contribuyen al conocimiento sobre la ecomorfofisiología y biología molecular de las etapas tempranas de los peces y las interacciones entre individuos × estresores × tiempo de exposición. Del mismo modo, los resultados sugieren parámetros importantes que pueden adoptarse para eficientizar el larvicultivo de la especie.

Objetivos

General

Determinar los efectos fisiológicos, morfológicos y moleculares promovidos por la exposición a alta temperatura, salinidad y diferente saturación de oxígeno durante los periodos sensibles en el desarrollo de *Atractosteus tropicus*.

Específicos

Evaluar la supervivencia y algunos parámetros de crecimiento (masa, longitud, factor de condición y tasa de crecimiento específico) en el desarrollo temprano del pejelagarto durante tres periodos clave en el desarrollo (fertilización a eclosión, 1 a 6 días post eclosión (dpe) y 7 a 12dpe) en función de los tres factores.

Analizar el ritmo cardiaco, la ventilación branquial y la ventilación aérea durante los tres periodos de desarrollo del pejelagarto en función de los tres factores.

Identificar las ventanas críticas para la supervivencia, crecimiento, características morfofisiológicas y moleculares en el desarrollo del pejelagarto en función de los tres factores

Evaluar la regulación de la expresión de genes implicados en la tolerancia a la hipoxia e hiperoxia en embriones y larvas de pejelagarto para cada periodo de desarrollo.

Determinar la variación en el tiempo de inicio del ritmo cardiaco, ventilación branquial, ventilación aérea, eclosión, alimentación exógena, absorción del vitelo, nado libre y cambio en la forma del hocico, como respuesta a la exposición a alta temperatura, salinidad y diferente saturación de oxígeno durante los periodos sensibles en el desarrollo del pejelagarto.

Referencias

Aguilera, C., Mendoza, R., Rodríguez, G., Márquez, G. 2002. Morphological description of alligator gar and tropical gar larvae, with an emphasis on growth indicators. Transactions of the American Fisheries Society, 131(5), 899-909.

Ahmad, R., Hasnain, A.U. 2005. Ontogenetic changes and developmental adjustments in lactate dehydrogenase isozymes of an obligate air-breathing fish Channa punctatus during deprivation of air access. Comparative Biochememistry and Physiology B Biochemistry and Molecular Biology, 140, 271–278.

Alderdice, D. F., Velsen, F. P. J. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). Journal of the Fisheries Board of Canada, 28(10), 1545-1562.

Allen, P. J., Cech, J. J. 2007. Age/size effects on juvenile green sturgeon, *Acipenser medirostris*, oxygen consumption, growth, and osmoregulation in saline environments. Environmental Biology of Fishes, 79(3-4), 211–229.

Allen, P. J., McEnroe, M., Forostyan, T., Cole, S., Nicholl, M. M., Hodge, B., & Cech, J. J. 2011. Ontogeny of salinity tolerance and evidence for seawater-entry preparation in juvenile green sturgeon, *Acipenser medirostris*. Journal of Comparative Physiology B, 181(8), 1045-1062.

Arias-Rodríguez, L., Páramo-Delgadillo, S., Contreras-Sánchez, W.M., Álvarez-González, C.A. 2009. Karyotype of the tropical gar *Atractosteus tropicus* Lepisosteiformes: Lepisosteidae) and chromosomal variation in their larval and adults. Revista de Biología Tropical, 5, 529–539.

Barrientos-Villalobos, J., Espinosa de los Monteros, A. 2008. Genetic variation and recent population history of the tropical gar *Atractosteus tropicus* Gill (Pisces: Lepisosteidae). Journal of Fish Biology, 73(8), 1919-1936.

Batty, R. S. 1984. Development of swimming movements and musculature of larval herring (*Clupea harengus*). Journal of Experimental Biology, 110(1), 217-229.

Beamish, F. W. H. 1980. Osmoregulation in juvenile and adult lampreys. Canadian Journal of Fisheries and Aquatic Sciences, 37(11), 1739-1750.

Blank, T., Burggren, W.W. 2014. Hypoxia-induced developmental plasticity of the gills and air-breathing organ of the air-breathing fish blue gourami (*Trichopodus trichopterus*). Journal of Fish Biolology, 84, 808–826.

Blank, T.M. 2009. Cardio-respiratory ontogeny and the transition to bimodal respiration in an air-breathing fish, the blue gourami (*Trichogaster trichopterus*): morphological and physiological development in normoxia and hypoxia. In: Biological Sciences. Denton, TX: University of North Texas.

Boeuf, G., Payan, P. 2001. How should salinity influence fish growth? Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 130(4), 411-423.

Boudreux, P.J., Ferrara, A.M., Fontenot, Q.C. 2007. Acute toxicity of ammonia to spotted gar, *Lepisosteus oculatus*, alligator gar, *Atractosteus spatula*, and paddlefish, *Polyodon spathula*. Journal of the World Aquaculture Society, 38, 322–325.

Brauner, C., Baker, D. 2009. Patterns of acid–base regulation during exposure to hypercarbia in fishes. In: Glass, M., Wood, S. (Eds). Cardio-respiratory control in vertebrates. Springer, Berlin, Heidelberg.

Brauner, C. J., Rombough, P. J. 2012. Ontogeny and paleophysiology of the gill: new insights from larval and air-breathing fish. Respiratory Physiology & Neurobiology, 184(3), 293-300.

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., et al. 2018. Declining oxygen in the global ocean and coastal waters. Science, 359(6371).

Buemi, M., Lacquaniti, A., Bolignano, D., Maricchiolo, G., Favaloro, A., Buemi, A., Grasso, G., Donato, V., Giorgianni, G., Genovese, L., Coppolino, G., Sfacteria, A. 2009. The erythropoietin and regenerative medicine: a lesson from fish. European Journal of Clinical Investigation, 39(11), 993-999.

Burggren, W.W. 2019. Phenotypic switching resulting from developmental plasticity: fixed or reversible? Frontiers in Physiology, 10, 1634.

Burggren, W.W., Bagatto, B. 2008. Cardiovascular anatomy and physiology. In Fish Larval Physiology; Finn, R.N., Kapoor, B.J., Eds.Science Publishers: Enfield, NH, USA, pp. 119–161.

Burggren, W.W., Bautista, G.M., Coop, S.C., Couturier, G.M., Delgadillo, S.P., García, R.M., González, C.A.A. 2016. Developmental cardiorespiratory physiology of the air-breathing tropical gar, *Atractosteus tropicus*. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 311(4), R689-R701.

Burggren, W.W., Elmonoufy, N.A. 2017. Critical developmental windows for morphology and hematology revealed by intermittent and continuous hypoxic incubation in embryos of quail (*Coturnix coturnix*). PloS One, 12(9), e0183649.

Burggren, W.W., Mueller, C.A. 2015. Developmental critical windows and sensitive periods as three-dimensional constructs in time and space. Physiological and Biochemical Zoology, 88(2), 91-102.

Burggren, W.W., Reyna, K.S. 2011. Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. Respiratory Physiology & Neurobiology, 178(1), 13-21.

Burleson, M., Shipman, B., Smatresk, N. 1998. Ventilation and acid-base recovery following exhausting activity in an air-breathing fish. Journal of Experimental Biology, 201, 1359–1368.

Busch, A. 1996. Transition from endogenous to exogenous nutrition: larval size parameters determining the start of external feeding and size of prey ingested by Ruegen spring herring *Clupea harengus*. Marine Ecology Progress Series, 130, 39-46.

Bussing, W.A. 1998. Freshwater fishes of Costa Rica (Vol. 46). Editorial Universidad de Costa Rica.

Camacho, S., Carmona, R., Llorente, J. I., Sanz, A., García-Gallego, M., Domezain, A., et al. 2011. Stomach development in the sturgeon *Acipenser naccarii*: histoenzymatic and ultrastructural analysis. Journal of Applied Ichthyology, 27(2), 693-700.

Chan, T., Burggren, W.W. 2005. Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (*Gallus gallus*). Respiratory Physiology & Neurobiology, 145(2-3), 251-263.

Chen, J., Guan, L., Zou, M., He, S., Li, D., Chi, W. 2020. Specific cyprinid HIF isoforms contribute to cellular mitochondrial regulation. Scientific Report, 10(1),17246.

Chen, N., Chen, L.P., Zhang, J., Chen, C., Wei, X.L., Gul, Y., Wang, W.M., Wang, H.L. 2012. Molecular characterization and expression analysis of three hypoxia-inducible factor alpha subunits, HIF-1α/2α/3α of the hypoxia-sensitive freshwater species, Chinese sucker. Gene 498, 81–90.

Chi, W., Gan, X.N., Xiao, W.H., Wang, W., He, S.P. 2013. Different evolutionary patterns of hypoxia-inducible factor α (HIF- α) isoforms in the basal branches of Actinopterygii and Sarcopterygii. FEBS Open Bio. 3, 479–483.

Chou, C.F., Tohari, S., Brenner, S., Venkatesh, B. 2004 Erythropoietin gene from a teleost fish, *Fugu rubripes*. Blood, 104, 1498–1503.

Chu, C.Y., Cheng, C.H., Chen, G.D., Chen, Y.C., Hung, C.C., Huang, K.Y., Huang, C.J. 2007. The zebrafish erythropoietin: functional identification and biochemical characterization. FEBS Letters, 581, 4265–4271

Collins, L. A., Nelson, S. G. 1993. Effects of temperature on oxygen consumption, growth, and development of embryos and yolk-sac larvae of *Siganus randalli* (Pisces: Siganidae). Marine Biology, 117(2), 195-204.

Comabella, Y., Azanza, J., Hurtado, A., Canabal, J., García-Galano, T. 2013. Allometric growth in cuban gar (*Atractosteus tristoechus*) larvae. Universidad & Ciencia, 29(3): 2227-2690.

Comabella, Y., Hurtado, A., Canabal, J., García-Galano, T. 2014. Effect of temperature on hatching and growth of Cuban gar (*Atractosteus tristoechus*) larvae. Ecosistemas y Recursos Agropecuarios, 1(1), 19-32.

Coughlin, D. J. 1991. Ontogeny of feeding behavior of first-feeding Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences, 48(10), 1896-1904.

Dabrowski, K., Bardega, R. 1984. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. Aquaculture, 40(1), 41-46.

Damsgaard C., Baliga, V.B., Bates, E., Burggren, W., McKenzie, D.J., Taylor, E., Wright, P.A. 2020. Evolutionary and cardio-respiratory physiology of air-breathing and amphibious fishes. Acta Physiologica, 228(3),e13406.

Davies, R., Moyes, C.D., Wang, Y.S. 2011. Intra- and inter-specific variation in metabolic gene expression in relationship to environmental hypoxia. Comparative Biochemistry and Physiology A 159, 25–31.

De Roth, G. 1973. Effects of temperature and light on aerial breathing behavior of the spotted gar, *Lepisosteus oculatus*. Ohio Journal of Science, 73, 35–41.

Dean, B. 1895. The early development of garpike and sturgeon. Ginn

Del Río, A. M., Davis, B. E., Fangue, N. A., Todgham, A. E. 2019. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. Conservation Physiology, 7(1), coy078.

Diaz, R. J. 2001. Overview of hypoxia around the world. Journal of Environmental Quality, 30(2), 275-281.

Dou, S. Z., Masuda, R., Tanaka, M., Tsukamoto, K. 2005. Effects of temperature and delayed initial feeding on the survival and growth of Japanese flounder larvae. Journal of Fish Biology, 66(2), 362-377.

Dou, S., Masuda, R., Tanaka, M., Tsukamoto, K. 2002. Feeding resumption, morphological changes and mortality during starvation in Japanese flounder larvae. Journal of Fish Biology, 60(6), 1363-1380.

Dzialowski, E.M., von Plettenberg, D., Elmonoufy, N.A., Burggren, W.W. 2002. Chronic hypoxia alters the physiological and morphological trajectories of developing chicken embryos. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 131(4), 713-724.

Echelle, A.A., Riggs, C.D. 1972. Aspects of the early life history of gars (*Lepisosteus*) in Lake Texoma. Transactions of the American Fisheries Society, 101, 106–112.

Eddy, F. B., Handy, R. D. 2012. Ecological and environmental physiology of fishes. OUP Oxford.

Eme, J., Mueller, C.A., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y. 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings. Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 179,71-80.

Evans, D.H., Piermarini, P.M., Choe, K.P. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste? Physiological Reviews, 8, 97-177.

Feng, X., Yu, X., Pang, M., Tong, J. 2019. Molecular characterization and expression regulation of the factor-inhibiting HIF-1 (FIH-1) gene under hypoxic stress in bighead carp (*Aristichthys nobilis*). Fish Physiology Biochemistry, 45(2), 657-665.

Frías-Quintana, C.A., Márquez-Couturier, G., Alvarez-González, C.A., Tovar-Ramírez, D., Nolasco-Soria, H., Galaviz-Espinosa, M.A., Martínez-García, R., Camarillo-Coop, S., Martínez-Yañez, R., Gisbert, E. 2015. Development of digestive tract and

enzyme activities during the early ontogeny of the tropical gar *Atractosteus tropicus*. Fish Physiology and Biochemistry, 41, 1075–1091.

Frick, N.T., Bystriansky, J.S., Ballantyne, J.S. 2007. The metabolic organization of a primitive air-breathing fish, the Florida gar (*Lepisosteus platyrhincus*). Journal of Experimental Zoololy, Ecology and Genetic Physiology, 307, 7–17.

Garduño-Paz, M.V., Méndez Sánchez, J.F., Burggren, W., García Martínez, J.L.A. 2020. Metabolic rate and hypoxia tolerance in *Girardinichthys multiradiatus* (Pisces: Goodeidae), an endemic fish at high altitude in tropical Mexico. Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 239,110576.

Geng, X., Feng, J., Liu, S., Wang, Y., Arias, C., Liu, Z. 2014. Transcriptional regulation of hypoxia inducible factors alpha (HIF-α) and their inhibiting factor (FIH-1) of channel catfish (*Ictalurus punctatus*) under hypoxia. Comparative Biochemistry and Physiology. B 169, 38–50.

González, C.A., Cruz, J., Alfaro, R.M. 2015. Physiological response of alligator gar juveniles (*Atractosteus spatula*) exposed to sub-lethal doses of pollutants. Fish Physiology and Biochemistry, 83, 322–332.

Gonzalez, R.J., Brauner, C.J., Wang, Y.X., Richards, J.G., Patrick, M.L., Xi, W., Matey, V., Val, A.L. 2010. Impact of ontogenetic changes in branchial morphology on gill function in *Arapaima gigas*. Physiological and Biochemical Zoology, 83, 322–332.

Guerrero-Zárate, R., Alvarez-González, C.A., Olvera-Novoa, M.A., Perales-Garciá, N., Frías-Quintana, C.A., Martínez-García, R., Contreras-Sánchez, W.M. 2014. Partial characterization of digestive proteases in tropical gar *Atractosteus tropicus* juveniles. Fish Physiology and Biochemistry, 40, 1021–1029.

Hart, P. R., Purser, G. J. 1995. Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Günther, 1862). Aquaculture, 136(3-4), 221-230.

Haase, V.H. 2010. Hypoxic regulation of erythropoiesis and iron metabolism. American Journal of Physiology Renal Physiology, 299, F1-F13.

Haase, V.H. 2013. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Reviews, 27, 41-53.

Hassell, K. L., Coutin, P. C., Nugegoda, D. 2008. Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). Marine Pollution Bulletin, 57(6-12), 302-306.

Hecht, T., Pienaar, A. G. 1993. A review of cannibalism and its implications in fish larviculture. Journal of the World Aquaculture Society, 24(2), 246-261.

Hernández-Rubio, M. C., Lucero, G. F. 2013. Effects of temperature and salinity during the embryonic period of *Chirostoma humboldtianum* and *Chirostoma riojai* (Atherinopsidae) until hatching. Hidrobiológica, 23(3), 365-373.

Hill, L.G., Schnell, G.D., Echelle, A.A. 1973. Effect of dissolved oxygen concentration on locomotory reactions of the spotted gar, *Lepisosteus oculatus* (Pisces: Lepisosteidae). Copeia, 1, 119–124.

Hogan, N.S., Duarte, P., Wade, M.G., Lean, D.R., Trudeau, V.L. 2008. Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): identifying critically vulnerable periods of development. General and Comparative Endocrinology, 156(3), 515-523.

Holliday, F. G. T. 1969. The effect of salinity on the eggs and larvae of teleost. Fish Physiology, 1: 293-309.

Hou, Z. S., Wen, H. S., Li, J. F., He, F., Li, Y., Qi, X. 2020. Environmental hypoxia causes growth retardation, osteoclast differentiation and calcium dyshomeostasis in juvenile rainbow trout (*Oncorhynchus mykiss*). Science of The Total Environment, 705, 135272.

Huang, C.Y., Lin, H.C., Lin, C.H. 2015a. Effects of hypoxia on ionic regulation, glycogen utilization and antioxidative ability in the gills and liver of the aquatic air-breathing fish *Trichogaster microlepis*. Comparative Biochemistry and Physiology A, 179, 25–34.

Huang, C.Y., Lin, H.H., Lin, C.H., Lin, H.C. 2015b. The absence of ionregulatory suppression in the gills of the aquatic air-breathing fish *Trichogaster lalius* during oxygen stress. Comparative Biochemistry and Physiology A, 179, 7–16.

Hutchings, J.A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. Heredity, 106(3), 421-437.

Johnson, D.W., Christie, M.R., Moye, J. 2010. Quantifying evolutionary potential of marine fish larvae: heritability, selection, and evolutionary constraints. Evolution, 64, 2614–2628.

Jonassen, T. M., Imsland, A. K., Stefansson, S. O. 1999. The interaction of temperature and fish size on growth of juvenile halibut. Journal of Fish Biology, 54(3), 556-572.

Jonz MG, Buck LT, Perry SF, Schwerte T, Zaccone G. 2016. Sensing and surviving hypoxia in vertebrates. Annals of the New York Academy of Sciences. 1365(1),43-58.

Joss, J. M. 2006. Lungfish evolution and development. General and Comparative Endocrinology, 148(3), 285-289.

Kaelin, W.G., Ratcliffe, P.J. 2008. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Molecular Cell, 30, 393–402.

Keckeis, H., Kamler, E., Bauer-Nemeschkal, E., Schneeweiss, K. 2001. Survival, development and food energy partitioning of nase larvae and early juveniles at different temperatures. Journal of Fish Biology, 59(1), 45-61.

Kodama, K., Rahman, M.S., Horiguchi, T., Thomas, P. 2012. Upregulation of hypoxia-inducible factor (HIF)-1a and HIF-2a mRNA levels in dragonet *Callionymus valenciennei* exposed to environmental hypoxia in Tokyo Bay. Marine Pollution Bulletin, 64, 1339–1347.

Kupsco, A., Schlenk, D. 2016. Stage susceptibility of Japanese medaka (*Oryzias latipes*) to selenomethionine and hypersaline developmental toxicity. Environmental Toxicology and Chemistry, 35(5), 1247-1256.

Kwan, G. T., Wexler, J. B., Wegner, N. C., Tresguerres, M. 2019. Ontogenetic changes in cutaneous and branchial ionocytes and morphology in yellowfin tuna (*Thunnus albacares*) larvae. Journal of Comparative Physiology B, 189(1), 81-95.

Lando, D., Peet, D.J., Gorman, J.J., Whelan, D.A., Whitelaw, M.L., Bruick, R.K. 2002. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes & Development, 16: 1466-1471.

Laurent, P., Perry, S.F. 1991. Environmental effects on fish gill morphology. Physiolical Zoology, 64, 4-25.

Lefevre, S., Bayley, M., McKenzie, D. J. 2016. Measuring oxygen uptake in fishes with bimodal respiration. Journal of Fish Biology, 88(1), 206-231.

Levesque, K. D., Wright, P. A., Bernier, N. J. 2019. Cross talk without cross tolerance: effect of rearing temperature on the hypoxia response of embryonic zebrafish. Physiological and Biochemical Zoology, 92(4), 349-364.

Li, H.L., Gu, X.H., Li, B.J., Chen, X., Lin, H.R., Xia, J.H. 2017. Characterization and functional analysis of hypoxia-inducible factor HIF1α and its inhibitor HIF1αn in tilapia. PloS one, 12(3), e0173478.

Little, C. 1983. The colonisation of land: origins and adaptations of terrestrial animals. Cambridge University Press.

Landolt, J.C., Hilll, L.G. 1975. Observations of the gross structure and dimensions of the gills of three species of gars (Lepisosteidae). Copeia, 3, 47–75.

Liem, K.F. 1981. Larvae of air-breathing fishes as countercurrent flow devices in hypoxic environments. Science, 211, 1177–1189.

Little, C.H. 2009. The Colonisation of Land: Origins and Adaptations of Terrestrial Animals. Cambridge, UK: Cambridge University Press.

Liu, Q., Wong-Riley, M.T. 2010. Postnatal changes in the expressions of serotonin 1A, 1B, and 2A receptors in ten brain stem nuclei of the rat: implication for a sensitive period. Neuroscience, 165(1), 61-78.

Long, W.L., Ballard, W.W. 2001. Normal embryonic stages of the longnose gar, *Lepisosteus osseus*. BMC Developmental Biology, 1, 6.

Makrakis, M. C., Nakatani, K., Bialetzki, A., Sanches, P. V., Baumgartner, G., Gomes, L. C. 2005. Ontogenetic shifts in digestive tract morphology and diet of fish larvae of the Itaipu Reservoir, Brazil. Environmental Biology of Fishes, 72(1), 99-107.

Malik, A. B. D. U. L., Abbas, G. H. U. L. A. M., Jabbar, A. B. D. U. L., Sajjad Shah, S., Ali Muhammad, A. 2018. Effect of different salinity level on spawning, fertilization, hatching and survival of common carp, *Cyprinus carpio* (Linnaeus, 1758) in semi-artificial environment. Iranian Journal of Fisheries Science, 17(4), 790-804.

Mason, J. C. 1969. Hypoxial stress prior to emergence and competition among coho salmon fry. Journal of the Fisheries Board of Canada, 26(1), 63-91.

Márquez-Couturier, G., Vázquez-Navarrete, C. J., Contreras-Sánchez, W. M., Álvarez-González, C. A. 2015. Acuicultura tropical sustentable: Una estrategia para la producción y conservación del pejelagarto (*Atractosteus tropicus*) en Tabasco, México. Colección José Narciso Rovirosa (2ª Ed. Vol. 3). México: Universidad Juárez Autónoma de Tabasco.

Massa, F., Delorme, C., Baglinière, J. L., Prunet, P., Grimaldi, C. 1999. Expositions d'oeufs de truite commune (*Salmo trutta*) à des hypoxies temporaires ou continue: effets sur la branchie, la résorption de la vésicule vitelline et les caractéristiques morphométriques des alevins. Bulletin Français de la Pêche et de la Pisciculture, 355: 421-440.

May, R. C. 1974. Effects of temperature and salinity on yolk utilization in *Bairdiella icistia* (Jordan & Gilbert) (Pisces: Sciaenidae). Journal of Experimental Marine Biology and Ecology, 16(3), 213-225.

McCormack, B.T. 1967. Aerial respiration in the Florida spotted gar. Quartertly Journal of Florida Academy of Sciciences, 30, 68–72.

McDowall, R. M. 1988. Diadromy in fishes: migrations between freshwater and marine environments. Croom Helm.

Melo, L. H., Martins, Y. S., Melo, R. M. C., Prado, P. S., Luz, R. K., Bazzoli, N., Rizzo, E. 2019. Low salinity negatively affects early larval development of Nile tilapia, *Oreochromis niloticus*: insights from skeletal muscle and molecular biomarkers. Zygote, 27(6), 375-381.

Mendez-Sanchez, J.F. 2015. Environmental modulation of the onset of air-breathing of the Siamese fighting fish and the blue gourami (Tesis Doctoral). Denton, TX: University of North Texas.

Mendez-Sanchez, J.F., Burggren, W.W. 2014. Environmental modulation of the onset of air breathing and survival of the Siamese fighting fish *Betta splendens* and the three spot gourami *Trichopodus trichopterus*. Journal of Fish Biology, 84, 794–807.

Mendiola, D., Alvarez, P., Cotano, U., Martínez de Murguía, A. 2007. Early development and growth of the laboratory reared north-east Atlantic mackerel *Scomber scombrus* L. Journal of Fish Biology, 70(3), 911-933.

Mendoza, R., Aguilera, C., Rodríguez, G., González, M., Castro, R. 2002. Morphophysiological studies on alligator gar (*Atractosteus spatula*) larval development as a basis for their culture and repopulation of their natural habitats. Reviews in Fish Biology and Fisheries, 12(2-3), 133-142.

Mendoza, R., Aguilera, C., Carreon, L., Montemayor, J., Gonzalez, M. 2007. Weaning of alligator gar (*Atractosteus spatula*) larvae to artificial diets. Aquaculture Nutrition, 14, 223–231.

Miller, R.R., Minckley, W.L., Norris, S.M. 2005. Freshwater fishes of Mexico (No. QL 629. M54 2005).

Milsom, W.K. 2012. New insights into gill chemoreception: receptor distribution and roles in water and air breathing fish. Respiration Physiology and Neurobiology, 184, 326–339.

Mora, M., Cabrera-Peña, J., Galeano, G. 1997. Reproducción y alimentación del gaspar *Atractosteus tropicus* (Pisces: Lepisosteidae) en el refugio nacional de vida Silvestre Caño Negro, Costa Rica. Revista de Biología Tropical, 45(2), 861-866.

Morgan, S.M., Huryn, V.M.B., Downes, S.R., Mercer, A.R. 1998. The effects of queenlessness on the maturation of the honey bee olfactory system. Behavioural Brain Research, 91(1-2), 115-126.

Moteki, M., Yoseda, K., Sahin, T., Üstündağ, C., Kohno, H. 2001. Transition from endogenous to exogenous nutritional sources in larval Black Sea turbot *Psetta maxima*. Fisheries Science, 67(4), 571-578.

Mueller, C.A., Joss, J.M., Seymour, R.S. 2011a. Effects of environmental oxygen on development and respiration of Australian lungfish (*Neoceratodus forsteri*) embryos. Journal of Comparative Physiology B Biochemistry, Systems and Environmental Physiology, 181, 941–952.

Mueller, C.A., Joss, J.M., Seymour, R.S. 2011b. The energy cost of embryonic development in fishes and amphibians, with emphasis on new data from the Australian lungfish, *Neoceratodus forsteri*. Journal of Comparative Physiology B Biochemistry, Systems and Environmental Physiology, 181, 43–52.

Mueller, C.A., Eme, J., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y. 2015. Embryonic critical windows: changes in incubation temperature alter survival,

hatchling phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). Joural of Comparative Physiology B, 185(3),315-31.

Mueller, C.A., Willis, E., Burggren, W.W. 2016. Salt sensitivity of the morphometry of *Artemia franciscana* during development: a demonstration of 3D critical windows. Journal of Experimental Biology, 219(4), 571-581.

Nilsson, G.E. 2007. Gill remodeling in fish-a new fashion or an ancient secret? Journal of Experimental Biology, 210, 2403-2409.

Ogilvie, M., Yu, X., Nicolas-Metral, V., Pulido, S.M., Liu, C., Ruegg, U.T., Noguchi, C.T. 2000. Erythropoietin stimulates proliferation and interferes with differentiation of myoblasts. Journal of Biological Chemistry, 275, 39754–61

Paffett-Lugassy, N., Hsia, N., Fraenkel, P.G., Paw, B., Leshinsky, I., Barut, B., Bahary, N., Caro, J., Handin, R., Zon, L.I. 2007. Functional conservation of erythropoietin signaling in zebrafish. Blood, 110, 2718–2726.

Pearson, W. D., Thomas, G. A., Clark, A. L. 1979. Early piscivory and timing of the critical period in postlarval longnose gar at mile 571 of the Ohio River. Transactions of the Kentucky Academy of Science, 40(3-4), 122-128.

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Canadian Journal of Fisheries and Aquatic Sciences, 48(3), 503-518.

Person-Le Ruyet, J., Buchet, V., Vincent, B., Le Delliou, H., Quemener, L. 2006. Effects of temperature on the growth of pollack (*Pollachius pollachius*) juveniles. Aquaculture, 251(2-4), 340-345.

Perry, S.F., Wilson, R.J., Straus, C., Harris, M.B., Remmers, J.E. 2001. Which came first, the lung or the breath? Comparative Biochememistry and Physiology Molecular Integrative Physiology 129, 37–47.

Pinkerton, K.E., Joad, J.P. 2000. The mammalian respiratory system and critical windows of exposure for children's health. Environmental Health Perspectives, 108(suppl 3), 457-462.

Pierron, F., Baudrimont, M., Gonzalez, P., Bourdineaud, J.P., Elie, P., Massabuau, J.C 2007. Common pattern of gene expression in response to hypoxia or cadmium in the

gills of the European glass eel (*Anguilla anguilla*). Environmental Science and Technology, 41, 3005–3011.

Polymeropoulos, E. T., Elliott, N. G., Frappell, P. B. 2017. Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 213, 28-35.

Porporato, P. E., Dhup, S., Dadhich, R. K., Copetti, T. and Sonveaux, P. 2011. Anticancer targets in the glycolytic metabolism of tumors: a comprehensive review. Frontiers in Pharmacoogy,. 2, 49.

Potter, G.E. 1927. Respiratory function of the swim bladder in *Lepisosteus*. Journa of Experimental Zoology, 49, 45–65.

Prabhakar, N.R., Semenza, G.L. 2015. Oxygen Sensing and Homeostasis. Physiology (Bethesda). 30(5), 340-348.

Rahn, H., Rahn, K.B., Howell, B.J., Gans, C., Tenney, S.M. 1971. Air breathing of the garfish (*Lepisosteus osseus*). Respiration Physiology, 11, 285–307.

Ramos, C.A., Fernandes, M.N., da Costa, O.T., Duncan, W.P. 2013. Implications for osmorespiratory compromise by anatomical remodeling in the gills of *Arapaima gigas*. The Anatomical Record, 296, 1664–1675.

Randall, D.J. 1994. Cardio-respiratory modeling in fishes and the consequences of the evolution of airbreathing. Cardioscience, 5, 167–171.

Randall, D.J., Burggren, W.W., Farrell, A.P., Haswell, M.S. 1981. The Evolution of Air Breathing in Vertebrates. Cambridge, UK: Cambridge University Press.

Randall, D.J., Ip, Y.K. 2006. Ammonia as a respiratory gas in water and airbreathing fishes. Respiratory Physiology and Neurobiology, 154, 216–225.

Rudneva, I. 2014. Biomarkers for Stress in Fish Embryos and Larvae; CRS Press: Boca Raton, FL, USA, pp. 206.

Renfro, J.L. 1967. Factors influencing aerial breathing and metabolism in gars (Tesis Doctoral). Norman, Oklahoma, USA: University of Oklahoma.

Revol, A., Garza-Rodríguez, M. de L., Hernandez-Montenegro, V. Aguilera, C., Barrera-Saldana, H., Mendoza, R. 205. Cloning of the growth hormone cDNA of alligator gar *Atractosteus spatula* and its expression through larval development.

Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 140, 423–429.

Ribatti, D., Presta, M., Vacca, A., Ria, R., Giuliani, R., Dell'Era, P., Nico, B., Roncali, L., Dammacco, F. 1999. Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood.;93, 2627–2636.

Richards, J. E., Beamish, F. W. H. 1981. Initiation of feeding and salinity tolerance in the Pacific lamprey *Lampetra tridentata*. Marine Biology, 63(1), 73-77.

Rimoldi, S., Terova, G., Ceccuzzi, P., Marelli, S., Antonini, M., Saroglia, M. 2012. HIF-1a mRNA levels in Eurasian perch (*Perca fluviatilis*) exposed to acute and chronic hypoxia. Molecular Biology Reports 39, 4009–4015.

Rimoldi, S., Terova, G., Zaccone, G., Parker, T., Kuciel, M., Dabrowski, K. 2016. The effect of hypoxia and hyperoxia on growth and expression of hypoxia-related genes and proteins in spotted gar *Lepisosteus oculatus* larvae and juveniles. Journal of Experimental Zoology B Molecular and Developmetal Evolution, 326(4),250-67.

Rombough, P. J. 1988. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. Canadian Journal of Zoology, 66(3), 651-660.

Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., Boglione, C. 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Reviews in Aquaculture, 5: S59-S98.

Roussel, J. M. 2007. Carry-over effects in brown trout (*Salmo trutta*): hypoxia on embryos impairs predator avoidance by alevins in experimental channels. Canadian Journal of Fisheries and Aquatic Sciences, 64(5), 786-792.

Rundle, S. D., Spicer, J. I. 2016. Heterokairy: a significant form of developmental plasticity?. Biology Letters, 12(9), 20160509.

Rytkönen, K.T., Akbarzadeh, A., Miandare, H.K., Kamei, H., Duan, C., Leder, E.H., Williams, T.A., Nikinmaa, M. 2013. Subfunctionalization of cyprinid hypoxia-inducible factors for roles in development and oxygen sensing. Evolution 67, 873-882.

Saksena, V.P. 1963. Effects of temperature, light, feeding, and activity on the rate of aerial breathing of gar (*Lepisosteus*). Norman, OK: University of Oklahoma.

Sallout, B., & Walker, M. 2003. The fetal origin of adult diseases. Journal of Obstetrics and Gynaecology, 23(5), 555-560.

Schofield, C.J., Ratcliffe, P.J. 2004. Oxygen sensing by HIF hydroxylases. Nature Reviews Molecular Cell Biology 5(5), 343-354.

Schwarz, D.E., Allen, P.J. 2014. Effects of salinity on growth and ion regulation of juvenile alligator gar *Atractosteus spatula*. Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 169, 44–50.

Semenza, G.L. 1999. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annual Reviews of Cell and Developmental Biology, 15, 551–578.

Semenza, G.L. 2000. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. Journal of Applied Physiology, 88(4), 1474-80.

Semenza, G.L. 2010. Oxygen homeostasis. WIREs Mechanisms of Disiese 2(3), 336-361.

Simon, T.P., Tyberghein, E.J. 1991. Contributions to the early life history of the spotted gar, *Lepisosteus oculatus* Winchell, from Hatchet Creek, Alabama. Journal of the Kentucky Academy of Science, 52(3 & 4):124-131.

Shartau, R.B., Brauner, C.J. 2014. Acid-base and ion balance in fishes with bimodal respiration. Journal of Fish Biology 84, 682–704.

Smatresk, N.J. 1982. Respiration and acid-base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. Journal of Experimental Biology, 96, 263–280.

Smatresk, N.J., Burleson, M.L., Azizi, S.Q. 1986. Chemoreflexive responses to hypoxia and NaCN in longnose gar: evidence for two chemoreceptor loci. American Journal of Physiology Regululatory Integrative and Comparative Physiology, 251, R116–R125.

Smith, M. M. 1985. The pattern of histogenesis and growth of tooth plates in larval stages of extant lungfish. Journal of Anatomy, 140(Pt 4), 627.

Spicer, J.I., Burggren, W.W. 2003. Development of physiological regulatory systems: altering the timing of crucial events. Zoology, 106(2), 91-99.

Spicer, J. I., Rundle, S. D., Tills, O. 2011. Studying the altered timing of physiological events during development: It's about time… or is it? Respiratory Physiology & Neurobiology, 178(1), 3-12.

Spicer, J. I., Rundle, S. D. 2007. Plasticity in the timing of physiological development: physiological heterokairy—what is it, how frequent is it, and does it matter? Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 148(4), 712-719.

Tate, K.B., Kohl, Z.F., Eme, J., Rhen, T., Crossley, D.A. 2015. Critical windows of cardiovascular susceptibility to developmental hypoxia in common snapping turtle (*Chelydra serpentina*) embryos. Physiological and Biochemical Zoology, 88(2), 103-115.

Terova, G., Rimoldi, S., Corà, S., Bernardini, G., Gornati, R., Saroglia, M. 2008. Acute and chronic hypoxia affects HIF-1a levels in sea bass (*Dicentrarchus labrax*). Aquaculture 279, 150–159.

Tresguerres, M., Clifford, A. M., Harter, T. S., Roa, J. N., Thies, A. B., Yee, D. P., Brauner, C. J. 2020. Evolutionary links between intra-and extracellular acid–base regulation in fish and other aquatic animals. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 333:449–465.

Vetemaa, M., Saat, T. 1996. Effects of salinity on the development of fresh-water and brackish-water ruffe *Gymnocephalus cernuus* (L.) embryos. In Annales Zoologici Fennici (pp. 687-691). Finnish Zoological and Botanical Publishing Board.

Voesenek, C. J., Muijres, F. T., Van Leeuwen, J. L. 2018. Biomechanics of swimming in developing larval fish. Journal of Experimental Biology, 221(1), jeb149583.

Whitehouse, L.M., Manzon, R.G. 2019. Hypoxia alters the expression of hif-1a mRNA and downstream HIF-1 response genes in embryonic and larval lake whitefish (*Coregonus clupeaformis*). Comparative Biochemistry and Physiology A Molecular Integrative Physiology, 230,81-90.

Williams, K., Papanikos, N., Phelps, R. P., Shardo, J. D. 2004. Development, growth, and yolk utilization of hatchery-reared red snapper *Lutjanus campechanus* larvae. Marine Ecology Progress Series, 275, 231-239.

Vehaskari, V.M., Aviles, D.H., Manning, J. 2001. Prenatal programming of adult hypertension in the rat. Kidney International, 59(1), 238-245.

Winston, W.D. 1967. Effects of temperature and light on the rate of aerial breathing of the alligator gar, *Lepiososteus spatula* (Tesis Doctoral). Norman, OK: University of Oklahoma.

Wu, H., Lee, S.H., Gao, J., Liu, X., Iruela-Arispe, M.L. 1999. Inactivation of erythropoietin leads to defects in cardiac morphogenesis. Development 126, 3597–605.

Zaccone, D., Grimes, A.C., Farrell, A.P., Dabrowski, K., Marino, F.M. 2011. Morphology, innervation and its phylogenetic step in the heart of the longnose gar *Lepisosteus osseus*. Acta Zoologica (Stockholm) 93, 1–9.

Zawodny, J.R. 1975. Osmoregulation in the Florida spotted gar, *Lepisosteus oculatus*. Miami, FL: University of Miami.

Capítulo 1

fishes

MDPI

Article

Survival, Growth, and Development in the Early Stages of the Tropical Gar *Atractosteus tropicus*: Developmental Critical Windows and the Influence of Temperature, Salinity, and Oxygen Availability

Gil Martínez [1,*], Emyr Peña [1], Rafael Martínez [1], Susana Camarillo [1], Warren Burggren [2] and Alfonso Álvarez [1,*]

[1] Laboratorio de Acuicultura Tropical, División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco (UJAT), Villahermosa, TAB 86089, Mexico; ocemyr@yahoo.com.mx (E.P.); biologomartinez@hotmail.com (R.M.); susana.camarillo.coop@gmail.com (S.C.)

[2] Developmental Physiology Laboratory, Developmental Integrative Biology Research Group, Department of Biological Sciences, University of North Texas, Denton, TX 76203-5017, USA; warren.burggren@unt.edu

* Correspondence: gil.martinez.bautista@live.com.mx (G.M.); alvarez_alfonso@hotmail.com (A.A.); UJAT Office Tel.: +52-993-358-15-00 (ext. 6480)

Citation: Martínez, G.; Peña, E.; Martínez, R.; Camarillo, S.; Burggren, W.; Álvarez, A. Survival, Growth, and Development in the Early Stages of the Tropical Gar *Atractosteus tropicus*: Developmental Critical Windows and the Influence of Temperature, Salinity and Oxygen Availability. *Fishes* **2021**, *6*, x. https://doi.org/10.3390/xxxxx

Received: 16 November 2020
Accepted: 26 January 2021
Published: date

Abstract: Alterations in fish developmental trajectories occur in response to genetic and environmental changes, especially during sensitive periods of development (critical windows). Embryos and larvae of *Atractosteus tropicus* were used as a model to study fish survival, growth, and development as a function of temperature (28 °C control, 33 °C, and 36 °C), salinity (0.0 ppt control, 4.0 ppt, and 6.0 ppt), and air saturation (control ~95% air saturation, hypoxia ~30% air saturation, and hyperoxia ~117% air saturation) during three developmental periods: (1) fertilization to hatch, (2) day 1 to day 6 post hatch (dph), and (3) 7 to 12 dph. Elevated temperature, hypoxia, and hyperoxia decreased survival during incubation, and salinity at 2 and 3 dph. Growth increased in embryos incubated at elevated temperature, at higher salinity, and in hyperoxia but decreased in hypoxia. Changes in development occurred as alterations in the timing of hatching, yolk depletion, acceptance of exogenous feeding, free swimming, and snout shape change, especially at high temperature and hypoxia. Our results suggest identifiable critical windows of development in the early ontogeny of *A. tropicus* and contribute to the knowledge of fish larval ecology and the interactions of individuals x stressors x time of exposure.

Keywords: ancestral fish; developmental phenotypic plasticity; heterokairy; sensitive periods; critical windows

1. Introduction

Developmental phenotypic plasticity is the ability of an organism in its early stages to modify its phenotype as a function of intrinsic (genetic) or extrinsic (environmental) factors [1–3]. Modifications in the developmental trajectory as a result of phenotypic plasticity have the capacity to alter traits in adulthood, being either adaptative or detrimental [4–6]. Alterations in certain characteristics of developing organisms occur during specific periods of time known as critical windows, which are sensitive periods during development when phenotype is responsive to intrinsic or extrinsic factors [1,7–9].

Most studies analyzing critical windows have focused on the effect of one factor (stressor) at different times in development [10–13]. However, an experimental design for critical windows that is multifactorial (e.g., multiple factors varying simultaneously) offers a more realistic set of circumstances that affords the opportunity to understand and visualize the interaction of development, stressor dose, and the response on phenotypic characteristics of interest [9,14]. Most studies investigating critical windows have targeted morphological and physiological characteristics, maturation, metabolism, etc. across the different animal taxa [12,13,15–17].

In fishes, specifically, relatively little is known about survival and growth during critical windows, even though fish species have long been used as animal models. Some examples of studies analyzing critical windows, survival, and growth described how survival and some growth parameters are affected by shifting incubation temperature in the lake whitefish *Coregonus clupeaformis* [16] or decreased survival and hatch in the Japanese medaka (*Ozyrias latipes*) exposed to selenomethionine and hypersaline environments. These experiments identified the early neurulation as the most susceptible stage for lethality and morphological alterations [18].

In addition to determining the effect of stressors on the critical windows of a given trait, it is important to analyze how these factors can alter the developmental trajectories of organisms, especially regarding the onset and timing of developmental events. Modifications in the onset and timing of the different physiological processes of individuals have been defined as "heterokairy", "…the plasticity in the timing of the onset of developmental events at the level of an individual during its development" [19]. However, heterokairy can also be evident in changes occurring between populations of a given species. Our understanding of heterokairy as variation at the individual or populational level and not between species remains meager [19–22]. Information on heterokairy and critical windows in air-breathing fishes—either Holosteans or Teleosteans—is especially incomplete. Most studies of these groups have focused on the morphology and physiology of the adult forms of the species [23–28]. Some studies have focused on embryonic and larval air-breathing fishes, which are the most vulnerable and where selective pressures act heavily [29]. Those studies have focused on larval and embryonic morphology and physiology of Teleosteans [30–35] and some Holosteans as Lepisosteids [36–42].

Lepisosteid larvae exhibit rapid embryonic and larval development, making them tractable models for studying early stages of fishes [37]. The tropical gar *Atractosteus tropicus* Gill 1863 is one of the seven extant Lepidosteid species and is distributed from Southern Mexico to Central America [43–45]. This species occurs in slow moving waters such as rivers, lakes, lagoons, and backwaters. The tropical gar can survive to low oxygen levels and moderately high temperatures [46]. In addition to the ecological role of the species in its habitat, *A. tropicus* represents socioeconomic and cultural significance in Tabasco (Southern Mexico) and surrounding areas

with Olmec and Mayan cultures. For example, *A. tropicus* is a popular food item, especially because of its high nutritional quality at a low price. This species is also sold as pets, jewelry is made with their skin and scales, and whole fish are preserved as souvenirs. Moreover, *A. tropicus* is one of the five main fishery resources and cultured species in Mexico and it is an important species in the recreational fishing industry [43–45].

Our study has focused on the effect of gradients of temperature, air saturation, and salinity on survival, growth, and timing to key developmental events in embryos and larvae of the tropical gar. We employed a critical windows design comprising three early developmental periods, which we hypothesize could be developmental phases when characteristics of embryos and larvae change as a function of the dose of the stressor and the time of exposure. Furthermore, we also hypothesize that the stressors will have their greatest effects during egg incubation, which can lead to long-term affectations in the fish since embryos and early larvae are the most vulnerable stages in fish development [16,47,48]. We identified the major critical windows for survival and growth as a function of temperature, oxygen saturation, and salinity. We additionally assessed how the onset of hatching, yolk depletion, acceptance of exogenous feeding, free swimming, and snout shape change is altered by the different stressors applied during different developmental periods and treatments. The data generated by the current research contribute to the knowledge and understanding of the ecology and physiology of larval fishes and the interaction between stressors, time of exposure, and individual response.

2. Results

2.1. Survival as a Function of Stressor

2.1.1. Temperature

The highest survival rates at the end of the experimental period occurred in the control group (84%), followed by larvae from experimental groups P2-33 °C, P3-33 °C, P2-36 °C, and P3-36 °C (76–81%; $p < 0.001$). In contrast, fish from experimental groups P1-33 °C and P1-36 °C showed lower survival (59% and 52% of survival, respectively) and CE-36 °C the lowest (46%; $p < 0.001$; Figure 1a).

2.1.2. Air Saturation Level

Survival at the end of the hypoxia experiment ranged from 79% to 83% in the fish from the control and experimental groups P2-hypoxia, P3-hypoxia, P2-hyperoxia, and P3-hyperoxia. These values were significantly higher ($p < 0.001$) compared to larvae from experimental groups P1-hypoxia, P1-hyperoxia, CE-hypoxia, and CE-hyperoxia (55% to 59%; Figure 1b).

2.1.3. Salinity

Major differences in survival occurred ($p < 0.001$) due to the high mortality in experimental groups P2-4.0, P2-6.0, CE-4.0, and CE-6.0. At the end of the experiment, larvae from experimental group P1-6.0 exhibited the lowest survival (52%) and the rest of the experimental groups showed no significant differences ($p > 0.05$), with percentages between 68% and 74% (Figure 1c).

2.2. Survival as a Function of Development Stage

2.2.1. Period 1 (P1)—Fertilization to Hatch

- Temperature. Control larvae exhibited the highest survival (95%; $p <$ 0.001) followed by larvae from experimental groups P2-33 °C, P3-33

°C, P2-36 °C, and P3-36 °C (74% to 89%). Lower survival ($p < 0.001$) was registered for fish from experimental groups P1-33 °C and P1-36 °C at 68% and 63%, respectively. The lowest survival ($p < 0.001$) occurred in larvae from experimental group CE-36 °C (49%; Figure 1a).

- Air saturation. Control larvae and those from experimental groups P2-hypoxia, P3-hypoxia, P2-hypeoxia, and P3-hyperoxia exhibited survival of 85–88%, which significantly differed ($p < 0.001$) from experimental groups P1-hypoxia, P1-hyperoxia, CE-hypoxia, and CE-hyperoxia (61–64%; Figure 1b).
- Salinity. No significant differences occurred in survival ($p > 0.05$), which ranged from 75% to 85% in the different groups (Figure 1c).

2.2.2. Period 2 (P2)—From 1 to 6 dph

- Temperature and air saturation. No significant differences were found ($p > 0.05$). Survival was >90% in both experiments (Figure 1a,b).
- Salinity. The highest salinity-induced mortality events occurred in this intermediate period ($p < 0.001$). By 2 days post hatch (dph), all larvae from experimental groups CE-4.0 and CE-6.0 had died. By 3 dph, the fish from experimental groups P2-4.0 and P2-6.0 had also died. The rest of the experimental groups showed no significant differences ($p > 0.05$; survival 90% to 97%; Figure 1c).

2.2.3. Period 3 (P3)—From 7 to 12 dph

- Developmental period 3 was the most resilient to stressors. No significant differences were found between experimental groups in any experiment ($p > 0.05$): temperature 91–98% survival, hypoxia-hyperoxia 92–98%, and salinity 93–97% (Figure 1a–c).

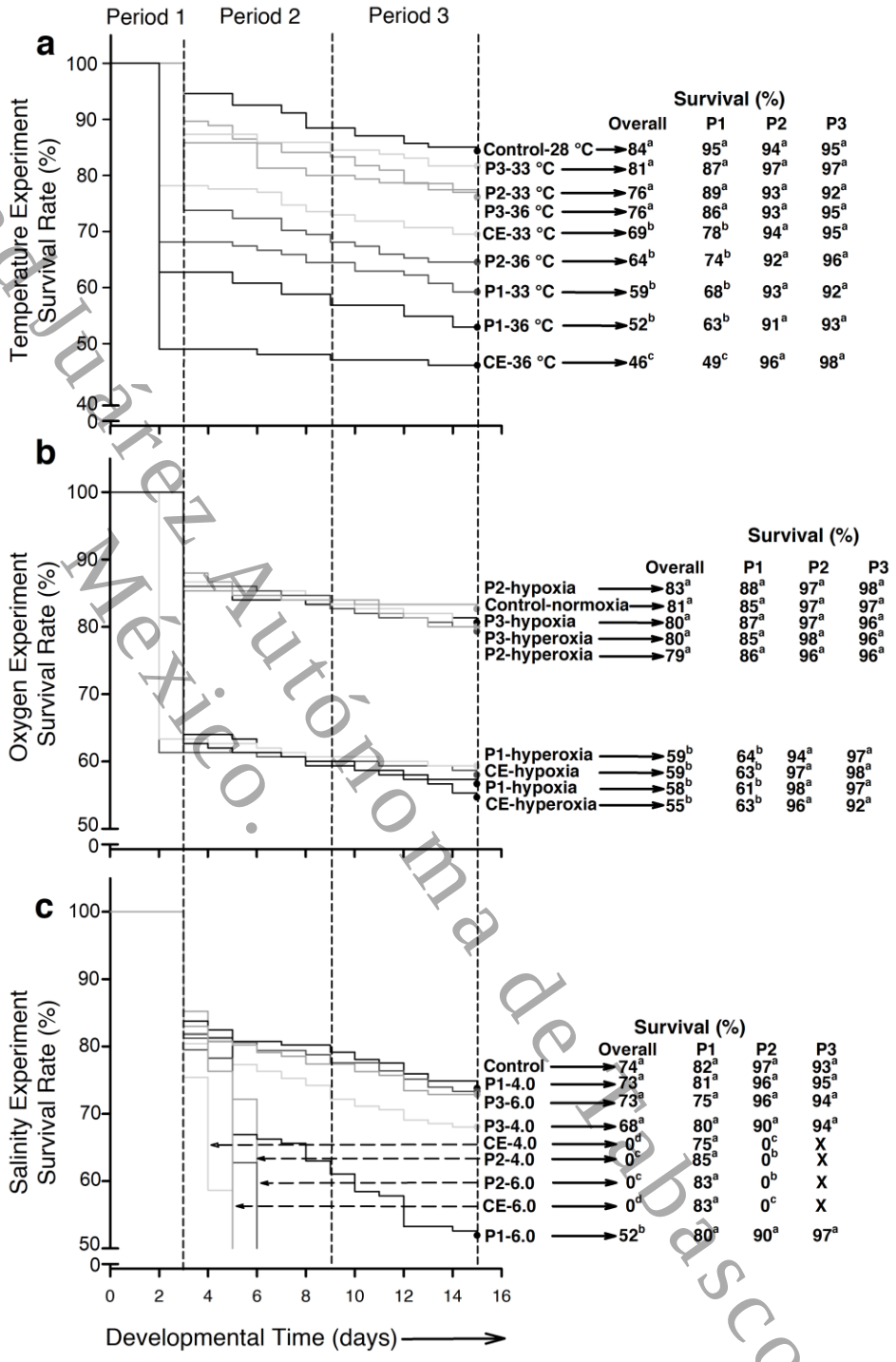


Figure 1. Survival of embryos and larvae of *Atractosteus tropicus* during each experimental condition. (a) Temperature, (b) air saturation, (c) salinity.

Differences in survival between groups for each experiment are represented by lowercase letters ($p < 0.001$). Black arrows indicate the corresponding survival percentages for each group. Dashed arrows in (**c**), indicate groups where all larvae died. $n = 1350$ fertilized eggs (150 per treatment) at the beginning of each experiment.

2.3. Body Morphology and Growth as a Function of Stressors and Stage of Exposure

2.3.1. Body Mass (BM)

- Temperature. At the end of developmental period 1, the control larvae displayed the lowest body mass (16.1 ± 0.6 mg), while larvae hatched in 33 °C and 36 °C exhibited the highest (BM ~19–23 mg; $p < 0.001$; Figure 2a). For developmental period 2, the highest BM was registered for larvae from experimental groups P1-33 °C (34.9 ± 3.9 mg) and CE-36 °C (39.2 ± 3,7 mg; $p < 0.001$; Figure 2a). By the end of developmental period 3, larvae from experimental groups P1-33 °C, P1-36 °C, CE-33 °C, and CE-33° showed higher BM (~38–43 mg) compared to the rest of the experimental groups (BM ~30 mg; $p < 0.001$; Figure 2a). Figure 2d illustrates how BM increased immediately after hatching in all the experimental groups, especially in CE-33 °C and CE-36 °C. At the beginning of developmental period 3, a slight increase in BM occurred in larvae from experimental groups P3-33 °C and P3-36 °C. However, after 2–3 days BM decreased to control values. The groups that exhibited the highest BM at the end of the experiment started increasing BM at ~9 dph.
- Air saturation. Larvae from experimental groups P1-hypoxia and CE-hypoxia displayed the lowest BM (~12 mg) at hatching ($p < 0.001$; Figure 2b). By developmental period 2, larvae from experimental groups P1-hyperoxia, P2-hyperoxia, and CE-hyperoxia exhibited the highest BM (~42 mg; $p < 0.001$; Figure 2b). By developmental period 3, fish from the control and P3-hyperoxia exhibited the lowest BM (~45 mg); conversely, individuals from experimental groups P1-hyperoxia and P2-hyperoxia showed the highest (BM ~64–73 mg; $p < 0.001$; Figure 2b). Figure 2e shows high variation in BM for all groups since the beginning of developmental period 2. Fish from experimental groups with the highest BM at the end of the experiment started showing differences at ~9 dph ($p < 0.001$). Body mass of larvae from experimental group P3-hyperoxia showed little variation through developmental period 3.
- Salinity. In developmental period 1, fish from experimental groups P1-6.0 and CE-6.0 showed the highest BM (18.12 ± 1.3 mg and 19.4 ± 1.6 mg respectively; $p < 0.001$; Figure 2c). At the end of developmental period 2, no significant differences in BM occurred between any of the five surviving groups (BM ~32–37 mg; $p > 0.05$). By developmental period 3, larvae from experimental groups P1-4.0 and P1-6.0 exhibited higher BM (48.2 ± 4.1 mg and 56.3 ± 6.1 mg, respectively) than the control and the rest of the groups (~37–40 mg; $p < 0.001$; Figure 2c). Figure 2f shows the BM to increase in P1-4.0 and P1-6.0 at ~7 dph and continued by the end of the experiment, while fish from the control and experimental groups P3-4.0 and P3-6.0 remained constant.

In summary, body mass was significantly increased by higher temperature during the three developmental periods, decreased in hypoxic groups during incubation and increased in hyperoxic experimental groups exposed during developmental periods 1 and 2, and increased at the end of the salinity experiment in fishes incubated at either 4.0 ppt and 6.0 ppt of salinity.

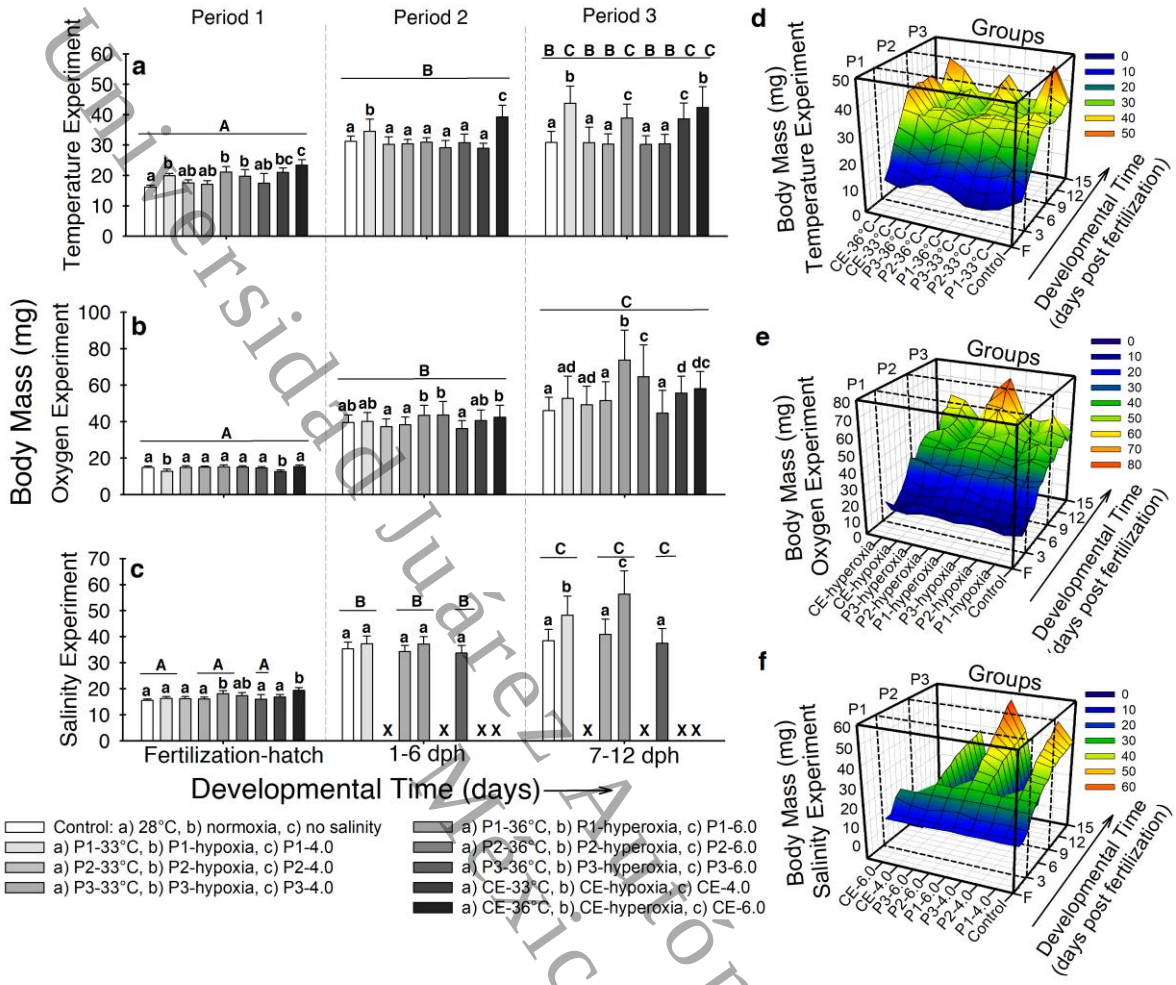


Figure 2. Body mass (BM) of *Atractosteus tropicus* at the end of each developmental period for (a) temperature, (b) air saturation, and (c) salinity experiments. Means ± SEM are presented. Lowercase letters represent differences between experimental groups from each developmental period ($p < 0.001$). Capital letters indicate differences within developmental time for each experimental group ($p < 0.001$). Panels (d-f) show the relation between development, treatments, and effect on the body mass of the fish in the whole experiments for temperature, air saturation, and salinity, respectively. Control conditions: 28 °C, 0.0 ppt of salinity, normoxia (95% air saturation); treatments 1: 33 °C, hypoxia (~30% air saturation), or 4.0 ppt of salinity respectively to each experiment; treatments 2 (independently for each experiment): 36 °C, hyperoxia (117% air saturation), or 6.0 ppt of salinity respectively to each experiment. Black crosses (X) in the salinity experiment represent groups where all fish died. $n = 20$ for each treatment.

2.3.2. Total Length (L_T)

- Temperature. Larvae exposed to either 33 °C or 36 °C during incubation exhibited higher total length (L_T) (~10–12 mm) compared to the controls (9.1 ± 0.36 mm; $p < 0.001$; Figure 3a). For developmental period 2, the highest L_T was observed in larvae from experimental groups CE-36 °C (22.9 ± 1.3 mm; $p < 0.001$; Figure 3a). By developmental period 3, larvae from experimental groups P1-33 °C, P1-36 °C, CE-33 °C, and CE-36 °C showed higher L_T (~20–22 mm) than the control larvae (19.1 ± 0.7 mm; $p < 0.001$; Figure 3a). The interaction of all variables during the complete experiment is presented in Figure 3d. Total length from fish from experimental groups P1-33 °C and CE-36 °C significantly increased from incubation through 12 dph, compared to the rest of the groups ($p <$

0.001). However, larvae from experimental group P1-36 °C showed increased L_T by hatching, but during developmental period 2, the values were closer to the control larvae (~19 mm).

- Air saturation. No significant differences were observed in fish L_T by developmental period 1 (L_T ~9 mm; $p > 0.05$; Figure 3b). In developmental period 2, larvae from experimental groups P1-hyperoxia and P2-hyperoxia showed higher L_T (19.6 ± 0.9 mm; 19.7 ± 1.2 mm, respectively) compared to the rest (L_T ~18.5 mm; $p < 0.001$; Figure 3b). For developmental period 3, L_T was higher in larvae from experimental groups P1-hyperoxia (24.8 ± 1.6 mm), P2-hyperoxia (23.6 ± 2.3 mm), CE-hyperoxia (23.8 ± 2.2 mm), and CE-hypoxia (23.3 ± 1.3 mm; $p < 0.001$) compared to the control (20 ± 1.8 mm; Figure 3b). Figure 3e shows the interaction of development, L_T, and time of exposure to hypoxia and hyperoxia throughout the experiment. All treatments showed a similar tendency in the L_T increase from incubation to ~9 dph. However, at this point, L_T of fishes from experimental group P1-hyperoxia, P2-hyperoxia, CE-hyperoxia, and CE-hypoxia showed major increase in L_T than the fish in the rest of the groups.
- Salinity. By the end of developmental period 1, larvae from experimental groups P1-4.0, CE-4.0, P1-6.0, and CE-6.0 showed higher L_T (~9.6-10 mm) compared to fish from the control and experimental groups P2-4.0, P3-4.0, P2-6.0, and P3-6.0 (LT ~9.2 mm; $p < 0.001$; Figure 3c). No significant differences in L_T were observed at developmental period 2 between experimental groups (L_T ~19.2 mm; $p > 0.05$; Figure 3c). At the end of the experiment, only larvae from experimental groups P1-4.0 and P1-6.0 showed significantly higher L_T values (~20.3–21-8 mm) compared to the control and larvae from experimental groups P3-4.0 and P3-6.0 (~19.7 mm; $p < 0.001$; Figure 3c). The interaction of L_T, development, and the groups during the whole experiment is presented in Figure 3f. From incubation to ~9 dph, all groups showed similar patterns of increase L_T. Right after this point, fish from experimental groups P1-4.0 and P1-6.0 showed higher values of L_T (~21 mm) than the control (19.2 ± 1.1 mm; $p < 0.001$), which continued through the experiment.

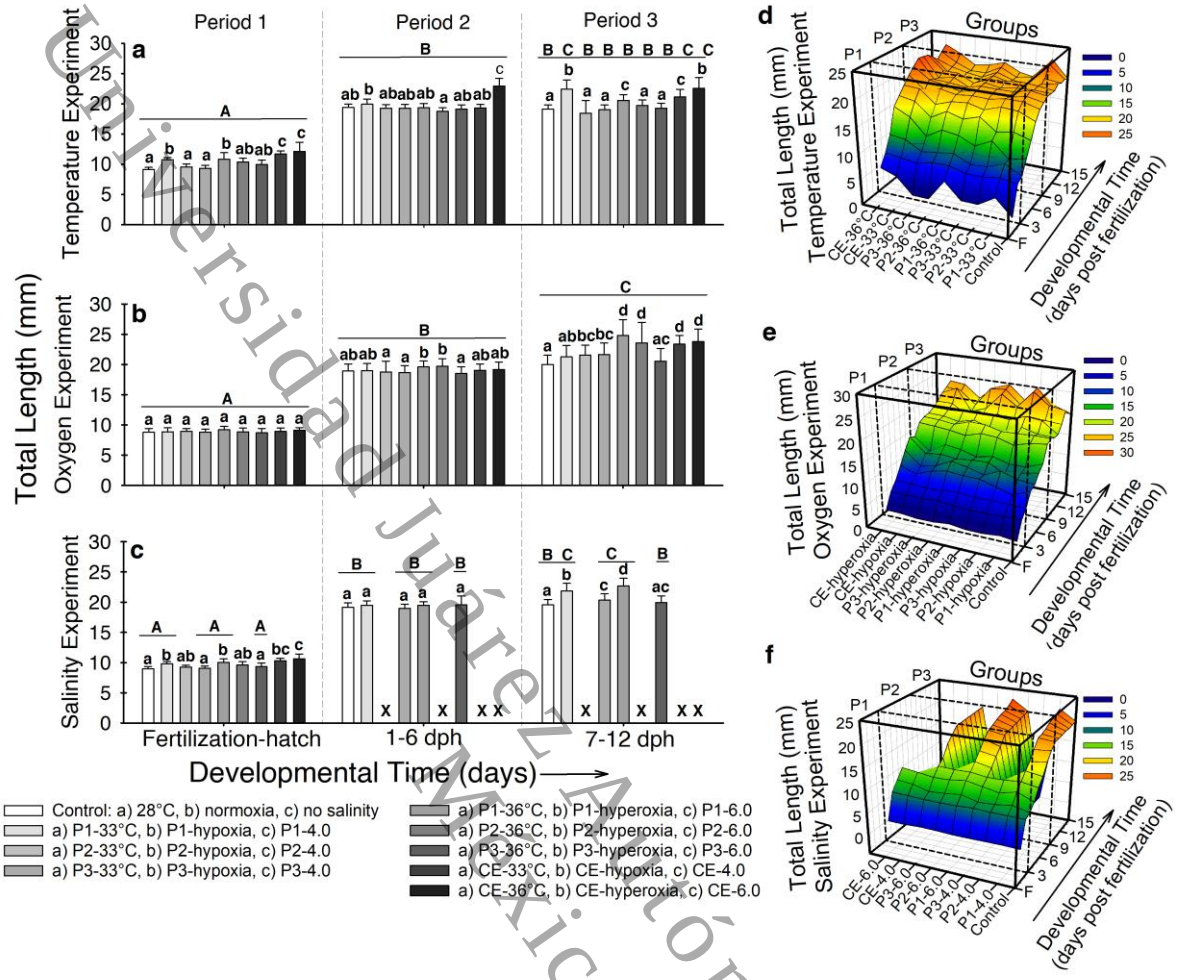


Figure 3. Total length (L_T) of *Atractosteus tropicus* at the end of each developmental period for the experiments with (a) temperature, (b) air saturation, and (c) salinity. Means ± SEM are presented. Lowercase letters represent differences between experimental groups from each developmental period ($p < 0.001$). Capital letters indicate differences within developmental time for each experimental group ($p < 0.001$). Panels (d-f) show the relation between development, treatments, and effect on the total length of the fish in the whole experiments for temperature, air saturation, and salinity, respectively. Control conditions: 28 °C, 0.0 ppt of salinity, normoxia (95% air saturation); treatments 1: 33 °C, hypoxia (~30% air saturation), or 4.0 ppt of salinity respectively to each experiment; treatments 2 (independently for each experiment): 36 °C, hyperoxia (117% air saturation), or 6.0 ppt of salinity respectively to each experiment. Black crosses (X) in the salinity experiment represent groups where all fish died. $n = 20$ for each treatment.

In summary, total length was increased by higher temperature, especially during continuous exposure. Hyperoxia during developmental periods 1 and 2, and continuous hypoxia and hyperoxia increased L_T increased L_T at the end of the experiment. Salinities of 4.0 ppt and 6.0 ppt during incubation increased L_T at the end of the experiment.

2.3.3. Specific Growth Rate (SGR)

- Temperature. During developmental period 1, fish from the control and experimental group P2-33 °C showed the lowest specific growth rate (SGR) (~0.7% d-1), while the highest SGR was observed in larvae from experimental group CE-36 °C (1.3 ± 0.2% d-1; $p < 0.001$; Figure 4a).

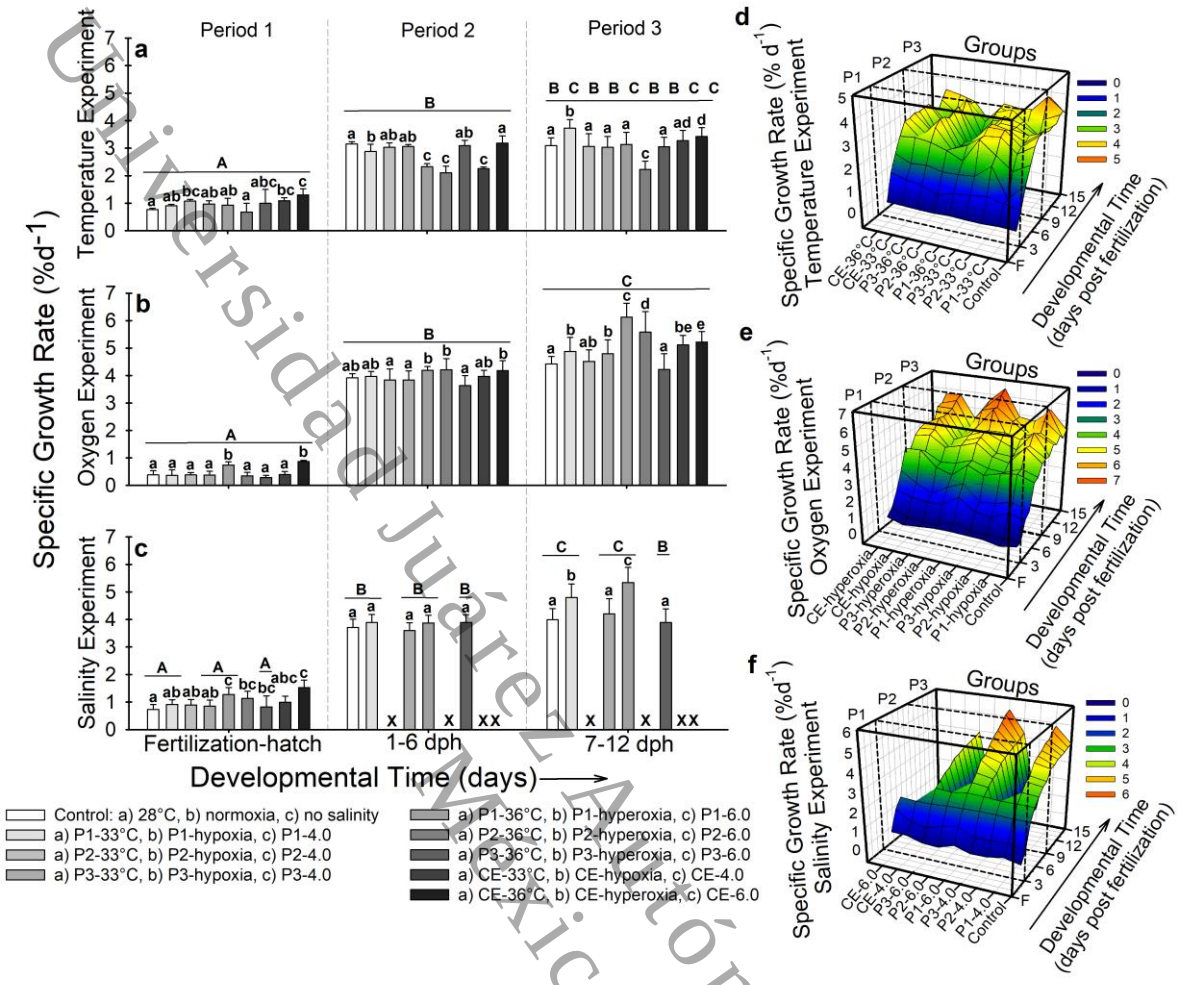


Figure 4. Specific growth rate (SGR) of *Atractosteus tropicus*. (a) Temperature, (b) air saturation, and (c) salinity experiments at the end of each developmental period. Means ± SEM are presented. Lowercase letters represent differences between experimental groups from each developmental period ($p < 0.001$). Capital letters indicate differences within developmental time for each experimental group ($p < 0.001$). Panels (d-f) show the relation between development, treatments, and effect on the specific growth rate of the fish in the whole experiments for temperature, air saturation, and salinity, respectively. Control conditions: 28 °C, 0.0 ppt of salinity, normoxia (95% air saturation); treatments 1: 33 °C, hypoxia (~30% air saturation), or 4.0 ppt of salinity respectively to each experiment; treatments 2 (independently for each experiment): 36 °C, hyperoxia (117% air saturation), or 6.0 ppt of salinity respectively to each experiment. Black crosses (X) in the salinity experiment represent groups where all fish died. $n = 20$ for each treatment.

In developmental period 2, the highest SGR was observed in the control (3.15 ± 0.1% d^{-1}) and experimental group CE-36 °C (3.17 ± 0.3% d^{-1}), and the lowest by experimental groups P1-36 °C, P2-36 °C, and CE-33 °C (~2.1–2.3% d^{-1}; $p < 0.001$; Figure 4a). At the end of developmental period 3, the highest SGR was registered in larvae from experimental groups P1-33 °C (3.7 ± 0.3% d^{-1}) and the lowest in larvae from experimental group P2-36 °C (SGR = 2.2 ± 0.2% d^{-1}; $p < 0.001$; Figure 4a). Figure 4d shows the interaction of temperature, time of exposure, and SGR. High variation can be observed in most groups compared to the control. Larvae from P2-36 °C (lowest SGR) showed low increases in SGR since the beginning of developmental period 2 ($p < 0.001$). Larvae from the group with highest SGR (P1-33 °C) at the end of the experiment started increasing its values at ~10 dph.

- Air saturation. At the end of developmental period 1, fish from experimental groups P1-hyperoxia and CE-hyperoxia showed the highest SGR (0.74–0.86% d-1; $p < 0.001$; Figure 4b). By developmental period 2, fish from experimental groups P1-hyperoxia, P2-hyperoxia, and CE-hyperoxia exhibited the highest SGR (~4.5% d-1) and the lowest was registered in larvae from P3-hyperoxia (3.5 ± 0.4% d-1), which did not present significant differences compared to the control (3.9 ± 0.2% d-1; $p > 0.05$; Figure 4b). At the end of the experiment, fishes from experimental groups P1-hyperoxia and P2-hyperoxia showed the highest SGR values (6.1 ± 0.4% d-1 and 5.4 ± 0.8% d-1 respectively; Figure 4b). Figure 4e shows the overall interactions in this experiment. From ~6 dph, larvae from experimental group P3-hyperoxia showed no increase in SGR throughout 12 dph. Fish from groups with the highest SGR (P1-hyperoxia and P2-hyperoxia) increased their SGR at ~9 dph. For the rest of the groups, a continuous but lower increase than P1-hyperoxia and P2-hyperoxia occurred ($p < 0.001$).
- Salinity. By developmental period 1, SGR in experimental groups P1-6.0 (1.28 ± 0.1% d-1) and CE-6.0 (1.53 ± 0.2% d-1) was significantly higher than larvae from the control (0.73 ± 0.1% d-1; $p < 0.001$; Figure 4c). During developmental period 2, no significant differences ($p > 0.05$) were observed (SGR ~3.4–3.9% d-1; Figure 4c). By developmental period 3, higher SGR was observed in experimental groups P1-4.0 (4.8 ± 0.6% d-1) and P1-6.0 (5.3 ± 0.8% d-1) compared to the rest of the fish (SGR ~3.9–4.2% d-1; $p < 0.001$; Figure 4c). Figure 4f shows similar values of SGR in all the groups by hatching and 1 dph for the seven surviving populations. The control group and the four surviving groups show a similar pattern in SGR along the experiment. However, by 8 dph, fish from P1-4.0 and P1-6.0 started to show higher SGR values ($p < 0.001$).

In summary, specific growth rate was increased by higher temperature during developmental periods 1 and 2 and slightly increased by the end of the experiment in continuous exposure groups and fish incubated at 33 °C. Hypoxia during developmental periods 1 and 3 slightly increased SGR at the end of the experiment, while hyperoxia increased SGR during developmental periods 1 and 2, which was constant at the end of the experiment. Salinities of 4.0 ppt and 6.0 ppt increased SGR at the end of the experiment in fish incubated under these conditions.

2.3.4. Fulton's Condition Factor (K)

- Temperature. In developmental period 1, larvae from the control group and experimental groups P2-33 °C and P3-33 °C showed the highest Fulton's condition factor (K) values (2.13 ± 0.3) and the larvae from experimental group CE-33 °C presented the lowest (1.32 ± 0.2; $p < 0.001$; Figure 5a). No significant differences were exhibited by developmental periods 2 and 3 ($p > 0.05$; Figure 5a). Figure 5d shows the interaction of K values, temperature, and time of exposure. Condition factor decreases in all the groups as development progresses. Peaks in experimental groups P3-33 °C, P3-36 °C, and CE-33 °C occurred at different times of development (7 dph, 9 dph, and 4 dph respectively). However, by the end of developmental periods 2 and 3, no difference was observed ($p > 0.05$).
- Air saturation. In developmental period 1, larvae from the control and experimental groups P2-hypoxia, P3-hypoxia, P2-hyperoxia, and P3-hyperoxia showed higher K values (K~2.1) than the larvae incubated in hypoxia and hyperoxia (K~1.8; $p < 0.001$; Figure 5b). No

significant differences in K occurred in developmental periods 2 and 3 and K values occurred as ~0.57 (p > 0.05; Figure 5b). Figure 5e shows how K values decreased in most of the groups from hatch to 5 dph. Two peaks were registered at 2 dph and 3 dph for experimental groups P1-hypoxia and P2-hypoxia, respectively. However, one day later K decreased to values close to the control group (K = 1.2 ± 0.1).

- Salinity. At the end of developmental period 1, the control and larvae from experimental groups P2-4.0, P3-4.0, P2-6.0, and P3-6.0 showed higher K values (~2.1) than larvae incubated in higher salinity (K ~1.7–1.9; p < 0.001; Figure 5c). No differences in K occurred by developmental periods 2 and 3 (K ~0.5; p > 0.05; Figure 5c). Figure 5f shows how K values decreased from hatching to 5 dph and remained constant for the rest of the experiment in the five surviving groups.

In summary, Fulton's condition factor was decreased by the three stressors during incubation. However, no differences occurred in developmental periods 2 and 3 for any experiment.

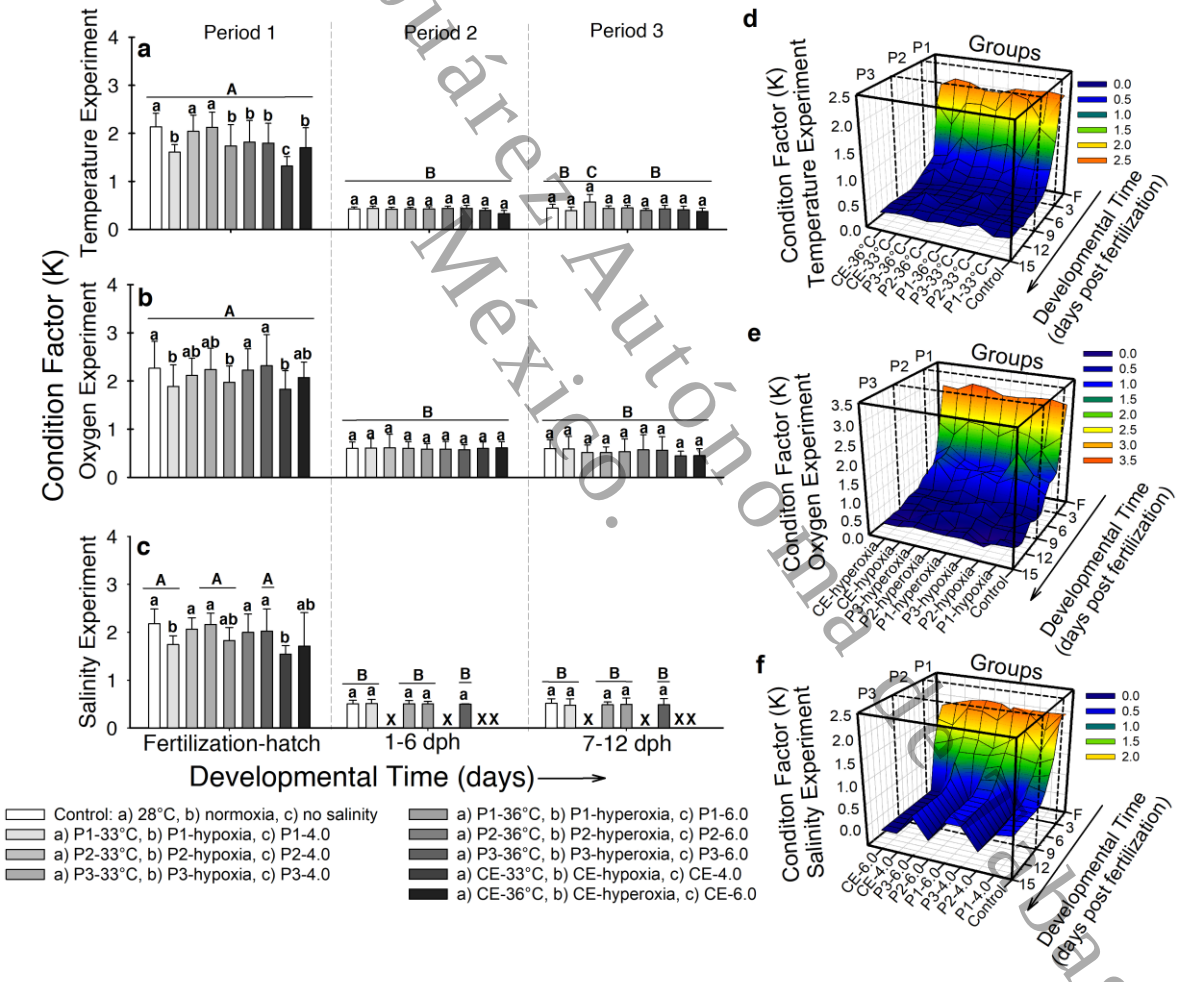


Figure 5. Fulton's condition factor (K) of *Atractosteus tropicus* at the end of each developmental period. (a) Temperature, (b) air saturation, and **(c)** salinity experiments. Means ± SEM are presented. Lowercase letters represent differences between experimental groups from each developmental period (p < 0.001). Capital letters indicate differences within developmental time for each experimental group (p < 0.001). Panels (d-f) show the relation between development, treatments, and effect on condition factor of the fish in the whole experiments for temperature, air saturation, and salinity, respectively. Control conditions: 28 °C, 0.0 ppt of

salinity, normoxia (95% air saturation); treatments 1: 33 °C, hypoxia (~30% air saturation), or 4.0 ppt of salinity respectively to each experiment; treatments 2 (independently for each experiment): 36 °C, hyperoxia (117% air saturation), or 6.0 ppt of salinity respectively to each experiment. Black crosses (X) in the salinity experiment represent groups where all fish died. $n = 20$ for each treatment.

2.4. Timing of Developmental Events as a Function of Stressors and Exposure Stage

The experimental treatments resulted in significant changes in the timing of developmental events (Table 1).

Table 1. Time of occurrence (hours post fertilization) of key developmental events in *Atractosteus tropicus*. Mean ± SD are presented. $n = 30$ fish per experiment.

Event	Experiment	Control	Treatment 1 (33 °C, Hypoxia, or Salinity = 4 ppt)				Treatment 2 (36 °C, Hyperoxia, or Salinity = 6 ppt)			
			P1-Treatment 1	P2-Treatment 1	P3-Treatmemt 1	CE-Treatment 1	P1-Treatment 2	P2-Treatment 2	P3-Treatment 2	CE-Treatment 2
Hatching	Temperature	63 ± 4 [a]	48 ± 2 [b]	63 ± 3 [a]	62 ± 3 [a]	48 ± 1 [b]	36 ± 1 [c]	62 ± 2 [a]	61 ± 2 [a]	36 ± 1 [c]
	Oxygen	60 ± 1 [a]	50 ± 1 [b]	61 ± 1 [a]	61 ± 1 [a]	49 ± 1 [b]	67 ± 2 [c]	60 ± 1 [a]	61 ± 1 [a]	67 ± 2 [c]
	Salinity	64 ± 1 [a]	72 ± 3 [b]	63 ± 1 [a]	62 ± 1 [a]	72 ± 3 [b]	78 ± 3 [b]	64 ± 1 [a]	63 ± 1 [a]	78 ± 3 [b]
Exogenous Feeding	Temperature	116 ± 5 [a]	98 ± 3 [b]	111 ± 6 [a]	109 ± 5 [a]	88 ± 2 [b]	92 ± 2 [b]	94 ± 4 [b]	112 ± 5 [a]	82 ± 1 [c]
	Oxygen	122 ± 3 [a]	122 ± 3 [a]	121 ± 3 [a]	121 ± 3 [a]	123 ± 2 [a]	123 ± 2 [a]	121 ± 3 [a]	122 ± 3 [a]	123 ± 3 [a]
	Salinity	122 ± 3 [a]	122 ± 1 [a]	-	121 ± 1 [a]	-	122 ± 2 [a]	-	123 ± 1 [a]	-
Yolk Depletion	Temperature	124 ± 1 [a]	118 ± 1 [b]	120 ± 2 [b]	125 ± 2 [a]	96 ± 1 [c]	96 ± 1 [c]	115 ± 2 [b]	124 ± 3 [a]	88 ± 1 [d]
	Oxygen	126 ± 2 [a]	132 ± 2 [b]	126 ± 3 [a]	126 ± 3 [a]	125 ± 2 [a]	125 ± 2 [a]	125 ± 3 [a]	127 ± 1 [a]	127 ± 1 [a]
	Salinity	125 ± 2 [a]	126 ± 3 [a]	-	127 ± 2 [a]	-	127 ± 3 [a]	-	126 ± 3 [a]	-
Free Swimming	Temperature	140 ± 2 [a]	125 ± 2 [b]	125 ± 1 [b]	138 ± 2 [a]	105 ± 1 [c]	102 ± 1 [c]	126 ± 1 [b]	140 ± 2 [a]	96 ± 1 [d]
	Oxygen	130 ± 3 [a]	136 ± 3 [a]	132 ± 2 [a]	132 ± 2 [a]	130 ± 3 [a]	136 ± 2 [b]	131 ± 3 [a]	132 ± 4 [a]	131 ± 2 [a]
	Salinity	142 ± 3 [a]	152 ± 3 [a]	-	143 ± 2 [a]	-	154 ± 3 [b]	-	145 ± 3 [a]	-
Snout Shape Change	Temperature	168 ± 3 [a]	156 ± 2 [b]	140 ± 2 [c]	168 ± 2 [a]	126 ± 1 [d]	155 ± 4 [b]	152 ± 2 [b]	168 ± 4 [a]	118 ± 1 [d]
	Oxygen	172 ± 3 [a]	183 ± 1 [b]	172 ± 3 [a]	171 ± 1 [a]	173 ± 3 [a]	171 ± 3 [a]	172 ± 2 [a]	171 ± 2 [a]	172 ± 3 [a]
	Salinity	174 ± 4 [a]	174 ± 3 [a]	-	174 ± 4 [a]	-	174 ± 4 [a]	-	173 ± 4 [a]	-

[a-d] Significant differences between groups from every experiment.

2.4.1. Time to Hatching

Time to hatching was significantly decreased by increasing temperature ($p < 0.001$). Embryos incubated at 28 °C hatched at ~63 h post fertilization (hpf), while embryos incubated in 33 °C and 36 °C hatched at ~48 hpf and ~36 hpf, respectively (Table 1). Temperature sensitivity for hatching showed higher Q_{10} values between 33 °C and 36 °C ($Q_{10} = 2.5$) while lower values were exhibited by intervals 28–33 °C ($Q_{10} = 1.5 \pm 0.24$) and 28–36 °C ($Q_{10} = 0.89 \pm 0.09$; $p < 0.001$; Table 2).

Hypoxia decreased the time to hatch compared to the control (50 hpf and 60 hpf respectively), while hyperoxia delayed it (~67 hpf; $p < 0.001$; Table 1). Salinities of 4.0 ppt and 6.0 ppt delayed the timing of hatching ($p < 0.001$) approximately 8–14 h compared to the control (~64 hpf; Table 1).

Table 2. Temperature sensitivity in key developmental events in the tropical gar in the temperature experiment. Mean ± SD are presented. n = 30 fish per group.

Event	Temperature Intervals		
	Q_{10} (28–33 °C)	Q_{10} (33–36 °C)	Q_{10} (28–36 °C)
Hatching	1.5 ± 0.2 [a]	3.1 ± 0.3 [b]	0. 9 ± 0.1 [c]
Exogenous Feeding	1.5 ± 0.3 [a]	3.1 ± 0.4 [b]	0.9 ± 0.1 [c]
Yolk Depletion	1.6 ± 0.2 [a]	3.1 ± 0.3 [b]	0.9 ± 0.1 [c]
Free Swimming	1.5 ± 0.4 [a]	3.1 ± 0.6 [b]	0.9 ± 0.1 [c]
Snout Shape Change	1.5 ± 0.3 [a]	3.1 ± 0.6 [b]	0.9 ± 0.1 [c]

[a-c] Significant differences between temperature intervals for each developmental event.

2.4.2. Time to Exogenous Feeding

In the temperature experiment, larvae from the control and experimental groups P2-33 °C, P3-33 °C, P2-36 °C, and P-36 °C began exogenous feeding at ~109–116 hpf. The larvae from experimental groups P1-33 °C, CE-33 °C, P1-36 °C, and CE-36 °C started feeding significantly earlier (~ 82–98 hpf; $p < 0.001$; Table 1). The largest temperature sensitivity occurred between 33 °C and 36 °C (Q_{10} = 3.1; $p < 0.001$; Table 2), with primarily temperature insensitivity at the highest temperature range. All larvae in the air saturation experiment showed no differences in the onset of exogenous feeding (~122 hpf; $p > 0.05$; Table 1). Fish from the control and the surviving larvae in the salinity experiment did not exhibit significant differences in the onset of feeding (~122 hpf; $p > 0.05$).

2.4.3. Time to Yolk Depletion

Larvae that hatched in 33 °C and 36 °C depleted the yolk sac in ~118 hpf and ~96 hpf, respectively, which was significantly sooner than the control (~ 124 hpf; $p < 0.001$; Table 1). Individuals from experimental groups P2-33 °C, P3-33 °C, P2-36 °C, and CE-36 °C depleted the yolk at ~122 hpf (Table 1). Temperature sensitivity was observed only between 33 °C and 36 °C (Q_{10} = 3.05; $p < 0.001$; Table 2). In the air saturation experiment, yolk sac depletion was significantly delayed in larva from experimental groups P1-hypoxia and CE-hypoxia (~132 hpf; $p < 0.001$; Table 1). However, fish from experimental group P2-hyperoxia exhibited the shortest depletion time (125 ± 2.5 hpf). No significant differences were observed in the salinity experiment ($p > 0.05$), with a time to yolk depletion of ~126 hpf (Table 1).

2.4.4. Time to Free Swimming

In the temperature experiment, time to free swimming in the control was ~140 hpf and differed from fish from experimental group CE-6.0 (~96 hpf; $p < 0.001$). Individuals from experimental groups P1-33 °C, P2-33 °C, P2-36 °C, and P3-36 °C started swimming significantly earlier than the control (~102 hpf to ~96 hpf; $p < 0.001$; Table 1). Large temperature sensitivity only occurred between 33 °C and 36 °C (Q_{10} = 3.05; $p > 0.001$; Table 2). In the air saturation experiment, larvae from the control started swimming at ~130 hpf and individuals from experimental groups P1-hypoxia and P1-hyperoxia started swimming ~6 h later ($p < 0.001$; Table 1). In the salinity experiment, larvae from the control and experimental groups P3-4.0 and P3-6.0 started swimming at ~144 hpf, while P1-4.0 and

P1-6.0 started later at ~152 hpf and ~154 hpf, respectively ($p < 0.001$; Table 1).

2.4.5. Time to Snout Shape Change

Change in the shape of the snout in larvae from the control occurred at ~168 hpf in the temperature experiment, at ~152 hpf in those from experimental groups P2-33 °C and P2-36 °C, and at ~120 hpf for the larvae in continuous exposure to 33 °C and 36 °C ($p < 0.001$; Table 1). Temperature sensitivity was exhibited primarily between 33 °C and 36 °C ($Q_{10} = 3.12$; $p < 0.001$; Table 2). In the air saturation experiment, snout shape change occurred at ~172 hpf in the control group and almost all the experimental groups (183 ± 1.3 in larvae from P1-hypoxia; $p < 0.001$; Table 1). No differences were observed for time to snout shape change in the salinity experiment (~174 hpf; $p > 0.05$; Table 1)

3. Discussion

3.1. Critical Windows for Survival

Organismal survival is affected by numerous factors and their combined effects. For example, changes in salinity and dissolved oxygen come together with water warming because of climate change and anthropogenic activities [49,50]. In the present study on *Atractosteus tropicus*, there were highly differential effects of environmental stressors. High temperature, hypoxia, and hyperoxia decreased survival during incubation; salinities of 4.0 ppt and 6.0 ppt after hatching killed all larvae (during developmental period 2). These data suggest that critical windows for survival vary with stressor type, as well as stressor dose. Moreover, the incubation period in early development of *A. tropicus* represents a critical window for temperature, hypoxia, and hyperoxia in contrast to a critical window of the first two days post hatch for salinity. Several studies have demonstrated how temperature and dissolved oxygen affect survival (especially during incubation) in Teleosteans and Holosteans [34,51–55]. When fish are incubated in high temperatures, survival can be affected because the yolk conversion efficiency can be reduced, and the cost of development increases with increasing temperature. Moreover, surviving individuals may show long-term alterations [16]. Furthermore, temperature acts as a lethal factor when the tolerance of individuals is exceeded [56]. Temperatures used in the current research did not reach the maximum temperature of 38 °C tolerated by *A. tropicus* [37]. However, a temperature of 36 °C may be lethal for embryos of the tropical gar in the current study since 50% of the individuals died before hatching, suggesting that 36 °C can represent the incipient lethal level for embryos of this species. Moreover, air saturation may act as a limiting factor, and can become lethal if severe enough [56]. Decreased survival in hypoxic treatments of the current study could have occurred since embryos' oxygen supply depends on oxygen diffusion pressure, and the rate of flow [56]. Moreover, decreased survival in hyperoxic treatments could be related to oxidant-related damage and/or minor gas bubble trauma [57,58].

Numerous primitive fishes can tolerate environmental salinities higher or lower than their plasma osmolality. However, there is little information regarding salinity and their early life stages and available information exists only for juveniles and adults [59–64]. Lack of maturity of their ionoregulatory and osmoregulatory organs likely explains why newly hatched larvae in the present study could not cope with environmental salinities that could be easily tolerated by adults, since gills are the principal organ for iono/osmoregulation in larval fishes [65–68]. *Atractosteus tropicus* has visible gills at hatch, but regular gill ventilation

did not start until ~5 dph [37]. Gill maturation was likely not complete at 5 dph, leading to the high mortality in the hatchlings.

3.2. Growth

Growth in the developing tropical gar was shaped by all three experimental stressors, but the growth responses differed with stressor dose and time of exposure. Generally, a major increase in BM, TL, and SGR tended to occur from fertilization to 6 dph. However, slow growth was observed during 7–12 dph, which corresponds to the period of the complete transition to exogenous feeding [51]. A similar growth pattern to *A. tropicus* occurred in *A. tristoechus* [51], *A. spatula* [42], *Lepisosteus osseus* [69], and *L. oculatus* [70], suggesting that this pattern is conserved among Lepisosteid species. These findings suggest that the period of transition from lecithotrophy to exotrophy could represent a critical window not just for growth but also for survival, especially when the young larvae must cope with the changing environment and the development of feeding strategies [71].

Temperatures close to the upper thermal limit (38 °C) [37] of *A. tropicus* during 1–6 dph drastically reduced SGR (Figure 4a), which suggests that this period may represent a critical window for growth at high temperatures. Despite the differences in BM, L_T, and SGR, Fulton's condition factor was constant from 1–12 dph for *A. tropicus* in the current study. Similar results were reported for *A. tristoechus* [51]. In contrast with our results, some Teleosteans show no significant relationship between incubation temperature and mass and length at hatch [72–75].

Growth rate of freshwater fishes can increase in tolerable salinities due to reduced osmoregulatory costs [76]. However, when exposed to higher concentrations, osmoregulatory costs can interfere with growth rate. This conjecture is consistent with the findings of the current study, where larvae incubated in low salinity showed higher SGR (~5 dph). There is a relationship between growth, air breathing, and salinity in juveniles of spotted gar and alligator gar [64,77], but no data are available for embryonic or larval gars. In the present study, BM, L_T, and SGR were larger in larvae incubated at salinity of 6.0. A similar result was observed in larvae of the common carp (*Cyprinus carpio*), where higher salinities (5.0 to 20.0) increased BM but not body length [78]. In contrast, some studies showed that larval fishes incubated and raised in higher salinity decreased body length, e.g., *Gymnocephalus cernuus* [79] and two endemic Mexican silversides, *Chirostoma humboldtianum* and *C. riojai* [80]. These findings of either increased or decreased mass or length have been attributed to differences in water content of the species [81], which depends on the permeability of gills and body surface to water and salts in the environment [82].

In the current study, several differences in growth rate occurred depending on the oxygen levels in the water and time of exposure. The incubation period in *A. tropicus* can represent a critical window for growth in fishes exposed to either hypoxia or hyperoxia. Our results suggest that (1) low oxygen during incubation causes a reduction in size of post hatched larvae, as described in other studies [83,84]; and (2) high oxygen during incubation and in pre-metamorphic larvae of the tropical gar favors growth. However, after metamorphosis high oxygen levels actually decreased growth. In the first case, a reduction in growth can occur due to a compensatory response of the embryos to prioritize other organismal activities as a function of oxygen availability [85]. Moreover, fishes with decreased growth have lower chances of survival, especially because of slower swimming, lower competitivity, the presence of deformities, and a major risk of predation [52,53,86,87].

3.3. Developmental Events

In the current study, differences in timing of occurrence of five developmental events occurred as a function of temperature, salinity, and air saturation level. However, no alterations of the sequence of these developmental events were observed, as described for mollusks [88].

3.3.1. Time to Hatching

We report that environmental factors altered time to hatch, either enhancing or delaying the event. This variation in time to hatch demonstrates that heterokairy is also dependent on stressor dose and type of stressor [9,14]. In our study, time to hatch was modified by higher temperature and is consistent with previous studies where individuals hatch between 36–72 hpf when incubated between 25 °C and 35 °C [89,90]. In other Lepisosteid species, variations in time to hatch occur in similar patterns to *A. tropicus*, suggesting that these responses to changes in temperature, air saturation and salinity are similar within Lepisosteids [38,41,42,51,91,92]. Moderate salinity promotes longer periods of incubation and can also promote a reduction in the developmental rate by redirecting the available energy to osmoregulation and/or as a response to the ionic/osmotic stress [93]. As in the current study, *Gymnocephalus cernua* show an increased incubation time and promoted morphological alterations, especially in body length [79]. In addition, some fish species incubated in different air saturations may hatch prematurely whereas hatching may be delayed in others [53]. Premature hatching in hypoxic conditions occurred in *A. tropicus*, as described in other fish species and in amphibians [34,53,94–96]. Moreover, decreased air saturation also produces a delay in developmental rate, resulting in larvae hatching at earlier stages of development with diminished body mass [34]. These data are consistent with the findings in the current study since larvae incubated as embryos in hypoxia showed decreased BM, which can be correlated with earlier hatching. Furthermore, these alterations are related to a respiratory response for coping with environmental hypoxia [34]. This is also relevant to the assumption that the oxygen levels shape the onset and maturation of gill ventilation and air-breathing in *A. tropicus*.

3.3.2. Time to Exogenous Feeding

The onset of exogenous feeding represents a crucial time point in the development of the fishes and is related to increased mortality [97]. As larvae develop, the energy budget provided by the yolk decreases, metabolism increases, and the developing larvae must look for prey to cope with the body's energy demands [98]. Exogenous feeding in *A. tropicus* occurs around 5 dph [36], which is when the control groups in the current study started consuming prey. However, higher temperature modified the onset of predation but, surprisingly, salinity and air saturation had no effect. Incubation period could represent a critical window for the onset of feeding at higher temperatures, since treatments P1-33 °C and P1-36 °C showed decreased time to predation compared to the control group and without considering the continuous exposure treatments (Table 1). Temperature accelerates fish development, which increases the energy demand of the organism for survival, growth, and maturation. Moreover, prey capture and consumption in the current study occurred when yolk sac was not completely absorbed, which prepares the larvae for the exotropic life and assists digestive tract maturation [99,100]. Lecitoexotrophy has also been described for *A. tristoechus* [101]. These yolk reserves provide the necessary energy of the young fishes for foraging [102,103] and competition for resources [104,105]. In natural populations of *A. tropicus*, an earlier onset of exogenous feeding promotes cannibalism and this

characteristic is the main reason for the high variation in mass-size relationship as reported in other studies [36,37,89].

3.3.3. Time to Yolk Depletion

In the present study, variations in the timing to this key developmental landmark occurred as a function of temperature and air saturation. Hypoxia and higher temperature during incubation, and higher temperature during the first days after hatching, could represent critical windows for yolk depletion, but further research is needed. Temperature is an environmental factor that promotes faster yolk absorption. However, there is a trade-off between rapid development and the efficient utilization of the energy provided by the yolk sac. Most of the species reared at higher temperature than their optimal face this issue. Even when temperature is combined with salinity, the effects on yolk absorption and utilization by temperature are much greater than those caused by salinity [106–108]. In terms of energy utilization and yolk depletion, indirect observation on the larvae incubated at 33 °C showed an increase in BM, L_T, and SGR, suggesting that energy was efficiently allocated in these larvae. However, weight and/or volume of the yolk sac was not measured and this increase in BM could be related to an advanced developmental stage rather than the control group.

Studies on salinity concentrations in freshwater fishes and yolk absorption are scarce. However, some freshwater fishes (e.g., *Salvelinus fontinalis*) are reared in low salinity where freshwater is not readily available, increasing yolk absorption efficiency and growth consequently, but significantly decreasing survival [109].

Hypoxia promotes a delayed development that can be observed through the delayed yolk absorption in several fish species [52,110–112]. In the present study, this association was partially true because larvae incubated under hypoxia and transferred to normoxia showed delayed yolk absorption. However, larvae continuously exposed to hypoxia showed values similar to the controls. These results suggest that it is a greater challenge for the larvae to cope with the increasing air saturation when incubated in hypoxia than when acclimating to longer hypoxic events as older individuals. This assertion is based on the ability of continuously exposed individuals to maintain similar timing for yolk depletion and growth parameters to the control group.

3.3.4. Time to Free Swimming

A crucial developmental event in fish is the onset of swimming [113]. Free swimming occurs at 5–6 dph in *A. tropicus* [36] and in a period of 4–10 days for *A. tristoechus* [101]. Lepisosteids and other species with indirect development must go through an eleuteroembryo stage and spend time attached to the substrate for the maturation of swimming structures [114]. Nonetheless, the onset of free swimming can be affected by several factors. In the present study, the first and second developmental periods could represent a critical window for the onset of swimming in the current study when embryos and larvae are exposed to high temperature, salinity, hypoxia, and hyperoxia. Acceleration in the onset of free swimming occurred due to increased temperature during incubation and the second period of exposure (Table 1). This is explained by the effect of increased temperature on muscle development, fin differentiation, and the maturation of other structures that facilitates fish kinematics for swimming [114,115].

Salinities of 4.0 ppt and 6.0 ppt delayed the timing of free swimming in larvae incubated under these conditions and then transferred to fresh water. Salinity reduces the activity of larval fish in response to ionic and osmotic disruption [81]. In *A. tropicus*, even though activity was not quantified, larvae that died in the second period appeared to show less

activity than the rest of the treatments, as well as characteristics such as bradycardia and ischemic stress.

Hypoxia diminishes activity in larvae and delays the onset of swimming, while hyperoxia presents little to no effect on swimming onset [116,117]. In the current experiments in *A. tropicus*, (1) larvae exposed to hypoxia during the second period did not show differences in timing to free swimming in comparison to the control group or the hyperoxic treatments; and (2) larvae that were incubated in hypoxia exhibited a delay in the onset of free swimming. The first case can be related to the onset of air-breathing (which occurred earlier than the control group) that helped the larvae to offset the aquatic hypoxia demands, which can occur as early as 2.5 dph [37]. In the second case, future studies are needed to assess the possible critical windows during incubation that promote these affectations in the organisms after hatching.

3.3.5. Time to Snout Shape Change

The timing in development of the pronounced snout of *A. tropicus* in this study corresponded to the previous reports of this event at around 7 dph [36,118]. Exposure to higher temperature during incubation and from 1–6 dph, as well as hypoxia during incubation, could represent critical windows for the onset of the change in the snout of *A. tropicus*.

Larvae exposed to higher temperature during 1–6 dph displayed the most rapid snout change. In contrast, larvae incubated in hypoxia and transferred to normoxia experienced a delay in this characteristic. This finding supports the need to study more specialized critical windows during incubation in *A. tropicus* to understand alterations induced by environment. Moreover, there is scant information on this topic, especially for Lepisosteids. Differences can occur in the timing of some characteristics, for example, the timing on mouth opening between species of gars [36]. The alligator gar (*A. spatula*) exhibited the allometric growth of the snout towards the proportion of the size of the adults earlier in size and age than *A. tropicus*. This characteristic was related to the faster metamorphosis of *A. spatula* that enhances the opportunity to hunt and catch prey and the earlier appearance of cannibalism [36,119–121]. However, *A. tropicus* shows high rates of cannibalism [37,89] but larvae in the current study did not show this behavior due to the feeding protocol, which can also favor the larviculture of this species.

4. Materials and Methods

4.1. Ethical Statement

Animals were handled in compliance with the Norma Oficial Mexicana NOM-062-ZOO-1999 from Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, the Mexican standards for good welfare practices of laboratory animals.

4.2. Fish Acquisition and Maintenance

Eggs and larvae of the tropical gar were obtained from three artificially induced spawnings of broodstock in May 2017, May 2018, and May 2019 carried out in the Laboratorio de Acuicultura Tropical of the Universidad Juárez Autónoma de Tabasco, Mexico. Each female was anesthetized with 200 mg L^{-1} of tricaine methanesulfonate, MS-222® (Agent Chemical Laboratories) and then injected with 35 µg kg^{-1} of luteinizing hormone-releasing analog (LNRHa) to induce egg laying. Each female and five males were placed for spawning into 2000 L tanks with artificial substrate for egg adhesion.

Three major experiments—one with each spawning and employing different experimental conditions (described below)—were performed to determine critical windows for survival, growth, and development. Sex cannot be determined in the embryos or larvae of *A. tropicus*, so data are reported assuming random mixing of sexes. Immediately after fertilization, fertilized eggs (3.08 ± 1.2 mm each) were transferred to 27 white 80 L holding tanks for incubation and development. For each experiment, one control and eight experimental groups were created (described in the next section). Each experiment was performed in triplicate (50 larvae per triplicate, 150 per group) with a total of 1350 fertilized eggs at the beginning of each experiment.

All tanks housing larvae contained non-chlorinated normoxic water (~95% air saturation; except in one experiment as described below), pH of 8.0, at ambient temperature (27–28 °C; except when indicated in one experiment). Photoperiod was 12 h light–12 h darkness and salinity of 0.0 ppt (with the exception of one experiment). After yolk absorption, larvae were fed ad libitum with nauplii of *Artemia* sp. every four hours from 8:00 a.m. to 8:00 p.m. Fifty percent of the water in the tanks was replaced every two days and feces and dead artemia were cleaned by siphoning one hour after every meal.

4.3. Developmental Stages and Experimental Design

Three developmental periods of the tropical gar were examined, based on a previously described scheme [36]: (1) the time from fertilization to hatching (60–72 h from fertilization); (2) yolk depletion stage (~from 1 day post hatch (dph) to ~6 days post hatch); and (3) pre-juvenile stage (~7–12 days post hatch). These three periods correspond to incubation, metamorphosis of the eleuteroembryo, and pre-juvenile stage, respectively. These stages are important to investigate the effect of environmental factors on the biology of developing fishes because these early stages in fishes are often the most vulnerable [47,48]. Embryos and larvae were exposed to control conditions (28 °C, normoxia, and salinity of 0.0 ppt for the three controls), to two treatments of a given stressor described below during each developmental period (six in total and returned to control conditions after exposure), and a group continuously exposed to each treatment (two per experiment). This yielded a total of nine groups for each experiment. Details of the protocol are shown in Figure 6.

The first experiment consisted of a temperature challenge to the embryos and larvae with temperatures of 33 °C and 36 °C. These temperatures are near the upper thermal limits for larvae of this species [37] and above its optimal incubation temperature (28–30 °C) [86]. In the second experiment, fish were exposed to hypoxia (~30% air saturation) and hyperoxia (117% air saturation; Figure 6), since Lepisosteids can tolerate environments with variable dissolved oxygen levels [77,122,123]. The third experiment consisted of exposure to salinities of 4.0 ppt and 6.0 ppt, because Lepisosteids can tolerate euryhaline environments and little information is available on early life stages under these conditions, especially regarding developmental critical windows. Temperature, air saturation, and salinity switches between developmental periods (as required for each experiment) occurred gradually within a three-hour period (e.g., from 28 °C to 33 °C).

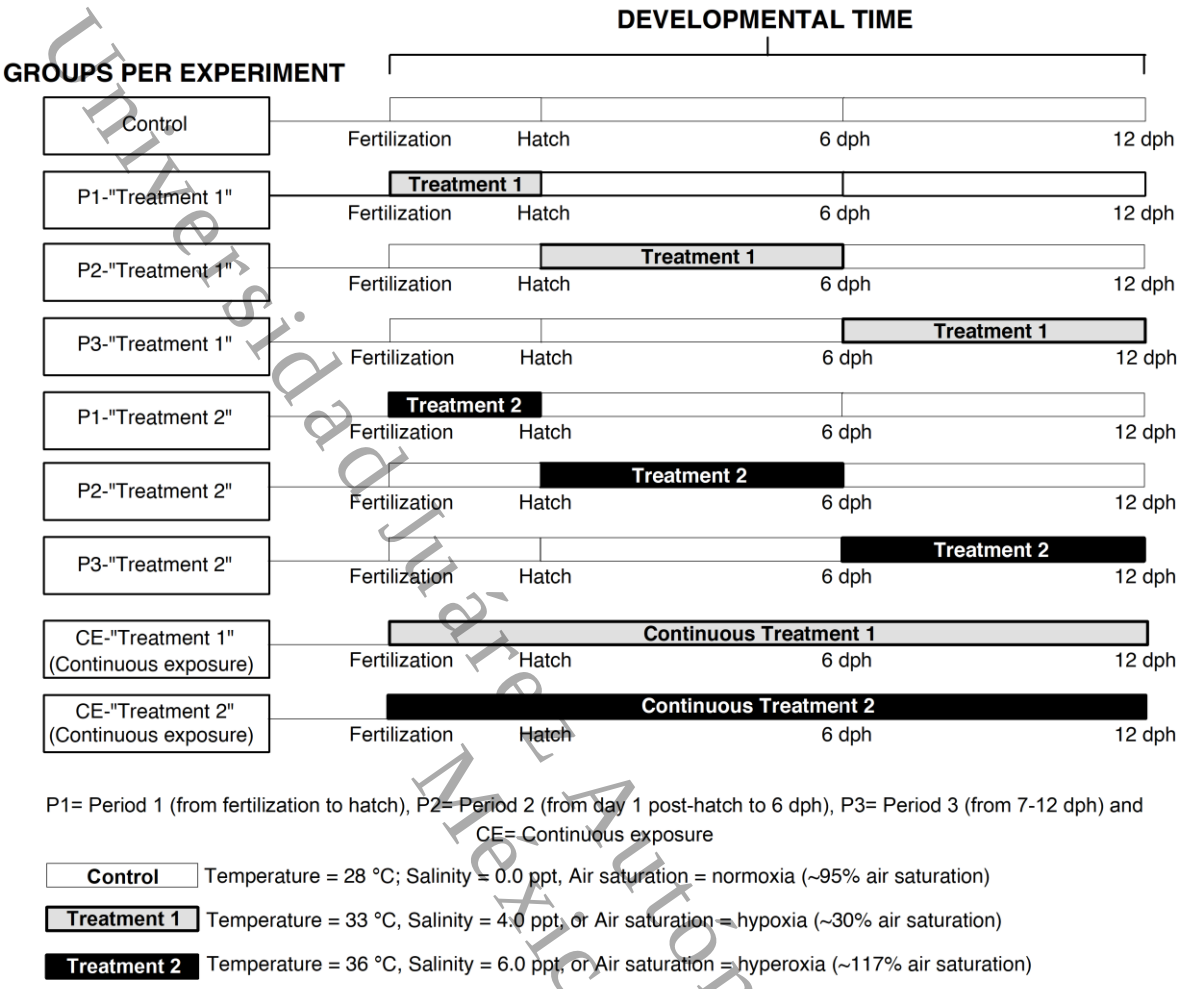


Figure 6. Experimental design for determining specific environmental effects on critical windows for development in the early ontogeny of *Atractosteus tropicus*. The first developmental period is from fertilization to hatching (~72 h), the second from 1 day post hatch (dph) to 6 dph, and the third from 7 to 12 dph. White boxes indicate control conditions during the experiments; light gray boxes represent exposure to treatments 1 (33 °C, hypoxia (~30% air saturation) or salinity of 4.0 ppt); darker boxes show exposure to treatments 2 (36 °C, hyperoxia (117% air saturation) or salinity of 6.0 ppt). Boxes at the bottom explain the conditions for control populations and treatments 1/treatments 2 during each independent experiment. See text for details.

4.4. Treatment Protocols

4.4.1. Temperature

Water temperature was regulated by a 12 Johnson Controls Thermostat attached to 12 immersion heaters (Volteck 46307 CAGU-5). Temperature in the experimental tanks was maintained at a constant temperature of either 33 ± 0.5 °C or 36 ± 0.5 °C. To enhance heat dispersal, the immersion heaters were placed immediately above the aeration stones.

Embryos and larvae were exposed to control conditions (control group), to two temperature treatments (33 °C and 36 °C) during developmental period 1 (experimental groups P1-33 °C and P1-36 °C), developmental period 2 (experimental groups P2-33 °C and P2-36 °C), developmental period 3 (experimental groups P3-33 °C and P3-36 °C), and to continuous exposure to both treatments (experimental groups CE-33 °C and CE-36 °C) as shown in Figure 6.

4.4.2. Air Saturation

Two treatments with different water air saturations were employed for this experiment, hypoxia (~30.2% air saturation) and hyperoxia (~117% air saturation). Hypoxia was generated by bubbling nitrogen gas directly into each tank until air saturation decreased to 30.2% of full air saturation and remained constant for each exposure. Hyperoxia was set at 117% of full air saturation and was created using a counter current water tower with an aeration stone for O_2 gas introduced to at the bottom of the tower. Water was sent from the tower to the tanks through a water pump and returned to the tower with a vent tube in the center of the tanks which was high enough to maintain a constant water level at the same air saturation. The tower promoted a high concentration of oxygen in the water simulating the function of a U-tube aeration system without the off-gas recycling mechanism [124].

Both hypoxia and hyperoxia were constantly measured with two oxygen meters YSI Pro2030. A 50% water change was performed every two days, adding water that was pre-equilibrated to the required oxygen levels.

Embryos and larvae were exposed to control conditions (control group), to two air saturation treatments (hypoxia and hyperoxia) during developmental period 1 (experimental groups P1-hypoxia and P1-hyperoxia), developmental period 2 (experimental groups P2-hypoxia and P2-hyperoxia), developmental period 3 (experimental groups P3-hypoxia and P3-hyperoxia), and to continuous exposure to both treatments (experimental groups CE-hypoxia and CE-hyperoxia) as shown in Figure 6.

4.4.3. Salinity

Water for the control population has a salinity of 0.0 ppt. Experimental salinity levels were set at 4.0 ppt for treatment 1 and 6.0 ppt for treatment 2, using industrial sea salt crystals (Grupo Industrial Roche) that were mechanically dissolved in the water of the tanks. Salinity was validated with a YSI Pro2030 multiparametric instrument. Water changes were made every 48 h at the proper validated salinity.

Embryos and larvae were exposed to control conditions (control group), to two salinity treatments (4.0 ppt and 6.0 ppt) during developmental period 1 (experimental groups P1-4.0 and P1-6.0), developmental period 2 (experimental groups P2-4.0 and P2-6.0), developmental period 3 (experimental groups P3-4.0 and P3-6.0), and to continuous exposure to both treatments (experimental groups CE-4.0 and CE-6.0) as shown in Figure 6.

4.5. Survival

Each experiment was carried out with 1350 fertilized eggs (150 fish per group, 50 fish per replicate). The unhatched embryos were counted and the number of dead larvae was recorded daily to calculate survival data. Sampled embryos for at 1 dph (described below) were not considered in this analysis.

4.6. Morphological Variables

Data from morphological variables (described below) were measured daily. However, data for treatments comparison (bars plots) are presented from measurements at the end of each developmental period (hatch, 6 dph, and 12 dph) using 20 individuals per treatment.

Body mass (BM) of the fish (n = 20 from each group) at the end of each developmental period was determined to the closest milligram with

an analytical balance (Denver Instruments). All individuals were taken from the tanks using an aquarium net and carefully deposited in plastic chambers (5 cm in diameter, 2 cm depth) before weighing. Then, fish were carefully taken from the chamber with a tip-truncated plastic bulb pipette and deposited on a tared plastic mesh to allow excess water drainage before weighing. Larvae were carefully deposited into a different chamber for taking high resolution photographs (14 megapixels, camera Sony Alpha 350) to measure total fish length (L_T) using Image J Software version 1.50 (NIH, Bethesda, Maryland). Millimeter graph paper underlying the larva was used to generate a scale on each image. Immediately after being photographed, individuals were carefully returned to holding tanks.

The specific growth rate (SGR) of the fish of each treatment was calculated as $SGR = ((Ln\, m_t - Ln\, m_i) \times t^{-1})) \times 100$ [125]; where m_t represents body mass at the end of each critical window, m_i the body mass of the embryos (removed from the chorion) 24 h after fertilization, t the duration of each experiment, and 100 a constant to obtain a percentage of growth. Fulton's condition factor was calculated for each organism as $K = 100*(mass/length^3)$ [126].

4.7. Developmental Events

The time of occurrence of five developmental events in the development of the tropical gar was recorded from the three experiments, for each control and each of the respective groups. The developmental events were hatching, exogenous feeding, yolk depletion, free swimming, and snout shape change. To measure the timing and onset of the five developmental events, 30 embryos per experiment were identified and separated from the rest using a plastic mesh. Data were collected when 100% of the fish had hatched, had accepted exogenous food, had completely absorbed the yolk sac, had started swimming, and when the shape of the snout rapidly changed from a typical larval piscine snout to that characteristic of gars. These events were previously described [36] and are important for understanding the effect of extrinsic factors in the development of *A. tropicus*.

Temperature sensitivity coefficient (Q_{10}) in the temperature experiment was calculated for all the developmental events with the data from the control group and the continuous exposure treatments as $Q_{10} = (R_2/R_1)^{(10/(T_2 - T_1))}$, where Q_{10} is the factor by which the time to a given developmental event increases with a rise in temperature; R_1 is the time to a given developmental event at temperature 1 (when $T_1 < T_2$); R_2 is the time to a given developmental event at temperature 2 (where $T_2 > T_1$); T_1 in the temperature at which R_1 is measured; and T_2 is the temperature at which R_2 was measured.

4.8. Statistical Analyses

Survival data were analyzed with the log-rank Kaplan–Meier method [127] for the overall experiments (individually) and within each developmental period per experiment. A Holm–Sidak multiple comparison test was used to assess differences between groups.

The effect of temperature, salinity, and oxygen availability on body mass, total length, SGR, and Fulton's condition factor was analyzed with a two-way ANOVA, followed by a Holm–Sidak multiple comparison test to assess differences between fish groups at the end of each developmental period and across development (n = 20 from every group, 180 fish in total for each experiment). Data on the timing of developmental events and Q_{10} were analyzed with a one-way ANOVA for each event between groups from a given experiment (10 fish per replicate, 30 fish from each group, per experiment). To determine differences between groups at a given

experiment, a multiple comparison Holm–Sidak test was carried out. All the analyses were made with the software SigmaPlot Version 11.0 with a significance level of 0.05.

5. Conclusions

Our results supported the hypothesis that environmental stressors early in ontogeny would affect key developmental processes. Developmental periods comprising from fertilization to hatch and 1 to 6 dph were identified as sensitive in the early ontogeny of *A. tropicus*. The greatest effects of high temperature, hypoxia, and hyperoxia occurred from fertilization to hatch, while for salinity occurred from 1 to 6 dph. In conclusion, developing fishes may exhibit different degrees of functional responses to stressor type, stressor dose, and time of stressor exposure. Moreover, the assumption is that if temperature increases in natural fish populations (either global warming or anthropogenic causes), then their susceptible stages of ontogeny can be affected. This increase in temperature can come with a decrease in the dissolved oxygen and a slight increase in the salinity of some brackish water environments that can drastically affect fishes, especially during their narrow critical windows for survival, development, and growth. In this regard, this study gives the first information integrating a 3D critical windows design, heterokairy, and functional developing fish responses to environmental factors. In addition, *A. tropicus* offers strong potential as biological model for studying fish early ontogeny. Nonetheless, identification during narrower developmental critical windows in fish early stages and the effect of intrinsic or extrinsic factors need further investigation to identify the possible morphological, physiological, and molecular responses of the fish.

Author Contributions: Conceptualization, G.M., W.B., and A.Á.; methodology, G.M.; validation, E.P., R.M., S.C., W.B., and A.Á.; formal analysis, G.M.; investigation, G.M. and E.P.; resources, A.Á.; data curation, G.M.; writing—original draft preparation, G.M.; writing—review and editing, G.M., W.B., and A.Á.; visualization, G.M.; supervision, A.Á. and W.B.; project administration, A.Á.; funding acquisition, A.Á. All authors have read and agreed to the published version of the manuscript.

Funding: National Council of Science and Technology of Mexico (CONACyT), Number CB-2016-01-282765.

Institutional Review Board Statement: Animals were handled in compliance with the Norma Oficial Mexicana NOM-062-ZOO-1999 from Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, the Mexican standards for good welfare practices of laboratory animals.

Acknowledgments: We appreciate the support of the National Council of Science and Technology in Mexico (CONACYT) for the scholarship for the doctoral studies to Martínez-Bautista G, and to the Laboratorio de Acuicultura Tropical from the Universidad Juárez Autónoma de Tabasco for providing the tropical gars for this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Burggren, W.W.; Reyna, K.S. Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. *Respir. Physiol. Neurobiol.* **2011**, 178, 13–21.
2. Hutchings, J.A. Old wine in new bottles: Reaction norms in salmonid fishes. *Heredity* **2011**, *106*, 421–437.
3. Burggren, W.W. Phenotypic switching resulting from developmental plasticity: Fixed or reversible? *Front. Physiol.* **2020**, *10*, 1634.

4. Vehaskari, V.M.; Aviles, D.H.; Manning, J. Prenatal programming of adult hypertension in the rat. *Kidney Int.* **2001**, *59*, 238–245.
5. Morgan, S.M.; Huryn, V.M.; Downes, S.R.; Mercer, A.R. The effects of queenlessness on the maturation of the honeybee olfactory system. *Behav. Brain Res.* **1998**, *91*, 115–126.
6. Sallout, B.; Walker, M. The fetal origin of adult diseases. *J. Obstet. Gynaecol.* **2003**, *23*, 555–560.
7. Pinkerton, K.E.; Joad, J.P. The mammalian respiratory system and critical windows of exposure for children's health. *Environ. Health Perspect.* **2000**, *108* (Suppl. 3), 457–462.
8. Hogan, N.S.; Duarte, P.; Wade, M.G.; Lean, D.R.; Trudeau, V.L. Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (*Rana pipiens*): Identifying critically vulnerable periods of development. *Gen. Comp. Endocrinol.* **2008**, *156*, 515–523.
9. Burggren, W.W.; Mueller, C.A. Developmental critical windows and sensitive periods as three-dimensional constructs in time and space. *Physiol. Biochem. Zool.* **2015**, *88*, 91–102.
10. Dzialowski, E.M.; von Plettenberg, D.; Elmonoufy, N.A.; Burggren, W.W. Chronic hypoxia alters the physiological and morphological trajectories of developing chicken embryos. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2002**, *131*, 713–724.
11. Chan, T.; Burggren, W.W. Hypoxic incubation creates differential morphological effects during specific developmental critical windows in the embryo of the chicken (*Gallus gallus*). *Respir. Physiol. Neurobiol.* **2005**, *145*, 251–263.
12. Liu, Q.; Wong-Riley, M.T. Postnatal changes in the expressions of serotonin 1A, 1B, and 2A receptors in ten brain stem nuclei of the rat: Implication for a sensitive period. *Neuroscience* **2010**, *165*, 61–78.
13. Tate, K.B.; Kohl, Z.F.; Eme, J.; Rhen, T.; Crossley, D.A. Critical windows of cardiovascular susceptibility to developmental hypoxia in common snapping turtle (*Chelydra serpentina*) embryos. *Physiol. Biochem. Zool.* **2015**, *88*, 103–115.
14. Mueller, C.A.; Willis, E.; Burggren, W.W. Salt sensitivity of the morphometry of *Artemia franciscana* during development: A demonstration of 3D critical windows. *J. Exp. Biol.* **2016**, *219 Pt 4*, 571–581.
15. Burggren, W.W.; Elmonoufy, N.A. Critical developmental windows for morphology and hematology revealed by intermittent and continuous hypoxic incubation in embryos of quail (*Coturnix coturnix*). *PLoS ONE* **2017**, *12*, e0183649.
16. Mueller, C.A.; Eme, J.; Manzon, R.G.; Somers, C.M.; Boreham, D.R.; Wilson, J.Y. Embryonic critical windows: Changes in incubation temperature alter survival, hatching phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). *J. Comp. Physiol. B* **2015**, *185*, 315–331.
17. Lee Pow, C.S.D.; Tilahun, K.; Creech, K.; Law, J.M.; Cope, W.G.; Kwak, T.J.; Kullman, S.W. Windows of susceptibility and consequences of early life exposures to 17β–estradiol on Medaka (*Oryzias latipes*) reproductive success. *Environ. Sci. Technol.* **2017**, *51*, 5296–5305.
18. Kupsco, A.; Schlenk, D. Stage susceptibility of Japanese medaka (*Oryzias latipes*) to selenomethionine and hypersaline developmental toxicity. *Environ. Toxicol. Chem.* **2016**, *35*, 1247–1256.
19. Spicer, J.I.; Burggren, W.W. Development of physiological regulatory systems: Altering the timing of crucial events. *Zoology* **2003**, *106*, 91–99.
20. Spicer, J.I.; Rundle, S.D. Plasticity in the timing of physiological development: Physiological heterokairy—what is it, how frequent is it, and does it matter? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2007**, *148*, 712–719.
21. Spicer, J.I.; Rundle, S.D.; Tills, O. Studying the altered timing of physiological events during development: It's about time… or is it? *Respir. Physiol. Neurobiol.* **2011**, *178*, 3–12.
22. Rundle, S.D.; Spicer, J.I. Heterokairy: A significant form of developmental plasticity? *Biol. Lett.* **2016**, *12*, 20160509.
23. Lefevre, S.; Bayley, M.; McKenzie, D.J. Measuring oxygen uptake in fishes with bimodal respiration. *J. Fish Biol.* **2016**, *88*, 206–231.
24. Little, C. *The Colonisation of Land: Origins and Adaptations of Terrestrial Animals*; Cambridge University Press: Cambridge, UK, 1983; p. 290.
25. Milsom, W.K. New insights into gill chemoreception: Receptor distribution and roles in water and air breathing fish. *Respir. Physiol. Neurobiol.* **2012**, *184*, 326–339.
26. Perry, S.F.; Wilson, R.J.; Straus, C.; Harris, M.B.; Remmers, J.E. Which came first, the lung or the breath? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2001**, *129*, 37–47.
27. Randall, D.J.; Ip, Y.K. Ammonia as a respiratory gas in water and air-breathing fishes. *Respir. Physiol. Neurobiol.* **2006**, *154*, 216–225.
28. Shartau, R.B.; Brauner, C.J. Acid–base and ion balance in fishes with bimodal respiration. *J. Fish Biol.* **2014**, *84*, 682–704.
29. Johnson, D.W.; Christie, M.R.; Moye, J. Quantifying evolutionary potential of marine fish larvae: Heritability, selection, and evolutionary constraints. *Evolution* **2010**, *64*, 2614–2628.
30. Blank, T.; Burggren, W.W. Hypoxia-induced developmental plasticity of the gills and air-breathing organ of *Trichopodus trichopterus*. *J. Fish Biol.* **2014**, *84*, 808–826.
31. Gonzalez, R.J.; Brauner, C.J.; Wang, Y.X.; Richards, J.G.; Patrick, M.L.; Xi, W.; Matey, V.; Val, A.L. Impact of ontogenetic changes in branchial morphology on gill function in *Arapaima gigas*. *Physiol. Biochem. Zool.* **2010**, *83*, 322–332.
32. Joss, J.M. Lungfish evolution and development. *Gen. Comp. Endocrinol.* **2006**, *148*, 285–289.

33. Méndez-Sanchez, J.F.; Burggren, W.W. Environmental modulation of the onset of air breathing and survival of *Betta splendens* and *Trichopodus trichopterus*. *J. Fish Biol.* **2014**, *84*, 794–807.
34. Mueller, C.A.; Joss, J.M.; Seymour, R.S. Effects of environmental oxygen on development and respiration of Australian lungfish (*Neoceratodus forsteri*) embryos. *J. Comp. Physiol. B* **2011**, *181*, 941–952.
35. Smith, M.M. The pattern of histogenesis and growth of tooth plates in larval stages of extant lungfish. *J. Anat.* **1985**, *140 Pt 4*, 627–643.
36. Aguilera, C.; Mendoza, R.; Rodríguez, G.; Márquez, G. Morphological description of alligator gar and tropical gar larvae, with an emphasis on growth indicators. *Trans. Am. Fish. Soc.* **2002**, *131*, 899–909.
37. Burggren, W.W.; Bautista, G.M.; Coop, S.C.; Couturier, G.M.; Delgadillo, S.P.; García, R.M.; González, C.A.A. Developmental cardiorespiratory physiology of the air-breathing tropical gar, *Atractosteus tropicus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2016**, *311*, R689–R701.
38. Comabella, Y.; Azanza, J.; Hurtado, A.; Canabal, J.; García-Galano, T. Allometric growth in Cuban gar (*Atractosteus tristoechus*) larvae. *Univ. Cienc.* **2013**, *29*: 2227–2690.
39. Dean, B. The early development of garpike and sturgeon. *J. Morphol.* **1895**, *11*, 1–62.
40. Echelle, A.A.; Riggs, C.D. Aspects of the early life history of gars (*Lepisosteus*) in Lake Texoma. *Trans. Am. Fish. Soc.* **1972**, *101*, 106–112.
41. Long, W.L.; Ballard, W.W. Normal embryonic stages of the longnose gar, *Lepisosteus osseus*. *BMC Dev. Biol.* **2001**, *1*, 6.
42. Mendoza, R.; Aguilera, C.; Rodríguez, G.; González, M.; Castro, R. Morphophysiological studies on alligator gar (*Atractosteus spatula*) larval development as a basis for their culture and repopulation of their natural habitats. *Rev. Fish Biol. Fish.* **2002**, *12*, 133–142.
43. Barrientos-Villalobos, J.; Espinosa de los Monteros, A. Genetic variation and recent population history of the tropical gar *Atractosteus tropicus* Gill (Pisces: Lepisosteidae). *J. Fish Biol.* **2008**, *73*, 1919–1936.
44. Bussing, W.A. *Freshwater Fishes of Costa Rica*; Editorial Universidad de Costa Rica: San José, Costa Rica, 1998; Volume 46, pp. 468.
45. Miller, R.R.; Minckley, W.L.; Norris, S.M. *Freshwater Fishes of Mexico*; University of Chicago Press: Chicago, IL, USA, 2005; p. 490.
46. Mora, M.; Cabrera-Peña, J.; Galeano, G. Reproducción y alimentación del gaspar *Atractosteus tropicus* (Pisces: Lepisosteidae) en el refugio nacional de vida silvestre Caño Negro, Costa Rica. *Rev. Biol. Trop.* **1997**, *45*, 861–866.
47. Burggren, W.W.; Bagatto, B. Cardiovascular anatomy and physiology. In *Fish Larval Physiology*; Finn, R.N., Kapoor, B.J., Eds.; Science Publishers: Enfield, NH, USA, 2008; pp. 119–161.
48. Rudneva, I. *Biomarkers for Stress in Fish Embryos and Larvae*; CRS Press: Boca Ratón, FL, USA, 2014; p. 206.
49. Breitburg, D.; Levin, L.A.; Oschlies, A.; Grégoire, M.; Chavez, F.P.; Conley, D.J.; Garçon, V.; Gilbert, D.; Gutiérrez, D.; Isensee, K.; et al. Declining oxygen in the global ocean and coastal waters. *Science* **2018**, *359*, eaam7240.
50. Diaz, R.J. Overview of hypoxia around the world. *J. Environ. Qual.* **2001**, *30*, 275–281.
51. Comabella, Y.; Hurtado, A.; Canabal, J.; García-Galano, T. Effect of temperature on hatching and growth of Cuban gar (*Atractosteus tristoechus*) larvae. *ERA* **2014**, *1*, 19–32.
52. Del Río, A.M.; Davis, B.E.; Fangue, N.A.; Todgham, A.E. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conserv. Physiol.* **2019**, *7*, coy078.
53. Hassell, K.L.; Coutin, P.C.; Nugegoda, D. Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). *Mar. Pollut. Bull.* **2008**, *57*, 302–306.
54. Hou, Z.S.; Wen, H.S.; Li, J.F.; He, F.; Li, Y.; Qi, X. Environmental hypoxia causes growth retardation, osteoclast differentiation and calcium dyshomeostasis in juvenile rainbow trout (*Oncorhynchus mykiss*). *Sci. Total Environ.* **2020**, *705*, 135272.
55. Levesque, K.D.; Wright, P.A.; Bernier, N.J. Cross talk without cross tolerance: Effect of rearing temperature on the hypoxia response of embryonic zebrafish. *Physiol. Biochem. Zool.* **2019**, *92*, 349–364.
56. Fry, J. The effect of environmental factors on the physiology of fish. In *Fish Physiology 5*; Hoar, W.S., Randall, D.J., Eds.; Academic Press: New York, NY, USA, 1974; pp. 1–98.
57. Matthews, M.D.; Prangnell, D.; Glenewinkel, H. Sensitivity of Guadalupe bass swim-up fry to hyperoxia. *N. Am. J. Aquac.* **2017**, *79*, 289–298.
58. Pleizier, N.; Nelson, C.; Cooke, S.; Brauner, C.J. Understanding gas bubble trauma in an era of hydropower expansion: How do fish compensate at depth? *Can. J. Fish. Aquat. Sci.* **2019**, *77*, 556–563.
59. Beamish, F.W.H. Osmoregulation in juvenile and adult lampreys. *Can. J. Fish. Aquat. Sci.* **1980**, *37*, 1739–1750.
60. Richards, J.E.; Beamish, F.W.H. Initiation of feeding and salinity tolerance in the pacific lamprey *Lampetra tridentata*. *Mar. Biol.* **1981**, *63*, 73–77.
61. McDowall, R.M. *Diadromy in Fishes: Migrations between Freshwater and Marine Environments*; Timber Press, Portland, OR, USA; 1988; p. 308.
62. Allen, P.J.; Cech, J.J. Age/size effects on juvenile green sturgeon, *Acipenser medirostris*, oxygen consumption, growth, and osmoregulation in saline environments. *Environ. Biol. Fishes* **2007**, *79*, 211–229.
63. Allen, P.J.; McEnroe, M.; Forostyan, T.; Cole, S.; Nicholl, M.M.; Hodge, B.; Cech, J.J. Ontogeny of salinity tolerance and evidence for seawater-entry preparation in juvenile green sturgeon, *Acipenser medirostris*. *J. Comp. Physiol. B* **2011**, *181*, 1045–1062.

64. Schwarz, D.E.; Allen, P.J. Effects of salinity on growth and ion regulation of juvenile alligator gar *Atractosteus spatula*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2014**, *169*, 44–50.
65. Brauner, C.J.; Rombough, P.J. Ontogeny and paleophysiology of the gill: New insights from larval and air-breathing fish. *Respir. Physiol. Neurobiol.* **2012**, *184*, 293–300.
66. Kwan, G.T.; Wexler, J.B.; Wegner, N.C.; Tresguerres, M. Ontogenetic changes in cutaneous and branchial ionocytes and morphology in yellowfin tuna (*Thunnus albacares*) larvae. *J. Comp. Physiol. B* **2019**, *189*, 81–95.
67. Melo, L.H.; Martins, Y.S.; Melo, R.M.C.; Prado, P.S.; Luz, R.K.; Bazzoli, N.; Rizzo, E. Low salinity negatively affects early larval development of Nile tilapia, *Oreochromis niloticus*: Insights from skeletal muscle and molecular biomarkers. *Zygote* **2019**, *27*, 375–381.
68. Tresguerres, M.; Clifford, A.M.; Harter, T.S.; Roa, J.N.; Thies, A.B.; Yee, D.P.; Brauner, C.J. Evolutionary links between intra-and extracellular acid–base regulation in fish and other aquatic animals. *J. Exp. Zool. A Ecol. Integr. Physiol.* **2020**, *333*, 449–465.
69. Pearson, W.D.; Thomas, G.A.; Clark, A.L. Early piscivory and timing of the critical period in postlarval longnose gar at mile 571 of the Ohio River. *Trans. Ky. Acad. Sci.* **1979**, *40*, 122–128.
70. Simon, T.P.; Tyberghein, E.J. Contributions to the early life history of the spotted gar, *Lepisosteus oculatus* Winchell, from Hatchet Creek, Alabama. *J. Ky. Acad. Sci.* **1991**, *52*, 124–131.
71. Hjort, J. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *Rapp. p.-v. Reun. Cons. Perm. Int. Explor. Mer*. **1914**, *20*, 1–228.
72. Jonassen, T.M.; Imsland, A.K.; Stefansson, S.O. The interaction of temperature and fish size on growth of juvenile halibut. *J. Fish Biol.* **1999**, *54*, 556–572.
73. Keckeis, H.; Kamler, E.; Bauer-Nemeschkal, E.; Schneeweiss, K. Survival, development and food energy partitioning of nase larvae and early juveniles at different temperatures. *J. Fish Biol.* **2001**, *59*, 45–61.
74. Mendiola, D.; Alvarez, P.; Cotano, U.; Martínez de Murguía, A. Early development and growth of the laboratory reared north-east Atlantic mackerel *Scomber scombrus* L. *J. Fish Biol.* **2007**, *70*, 911–933.
75. Person-Le Ruyet, J.; Buchet, V.; Vincent, B.; Le Delliou, H.; Quéméner, L. Effects of temperature on the growth of pollack (*Pollachius pollachius*) juveniles. *Aquaculture* **2006**, *251*, 340–345.
76. Boeuf, G.; Payan, P. How should salinity influence fish growth? *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2001**, *130*, 411–423.
77. Smatresk, N.J.; Cameron, J.N. Respiration and acid-base physiology of the spotted gar, a bimodal breather: III. Response to a transfer from fresh water to 50% sea water, and control of ventilation. *J. Exp. Biol.* **1982**, *96*, 295–306.
78. Malik, A.; Abbas, G.; Jabbar, A.; Sajjad Shah, S.; Ali Muhammad, A. Effect of different salinity level on spawning, fertilization, hatching and survival of common carp, *Cyprinus carpio* (Linnaeus, 1758) in semi-artificial environment. *Iran. J. Fish. Sci.* **2018**, *17*, 790–804.
79. Vetemaa, M.; Saat, T. Effects of salinity on the development of fresh-water and brackish-water ruffe *Gymnocephalus cernuus* (L.) embryos. *Ann. Zool. Fenn.* **1996**, *33*, 687–691.
80. Hernández-Rubio, M.C.; Figueroa-Lucero, G. Effects of temperature and salinity during the embryonic period of *Chirostoma humboldtianum* and *Chirostoma riojai* (Atherinopsidae) until hatching. *Hidrobiológica* **2013**, *23*, 365–373.
81. Holliday, F.G.T. The effect of salinity on the eggs and larvae of teleost. *Fish Physiol.* **1969**, *1*, 293–311.
82. Eddy, F.B.; Handy, R.D. *Ecological and Environmental Physiology of Fishes*; Oxford University Press: Oxford, UK, 2012; p. 252.
83. Shumway, D.L.; Warren, C.E.; Doudoroff, P. Influence of oxygen concentration and water movement on the growth of steelhead trout and coho salmon embryos. *Trans. Am. Fish. Soc.* **1964**, *93*, 342–356.
84. Marks, C.; Kaut, K.P.; Moore, F.B.; Bagatto, B. Ontogenetic oxygen changes alter zebra fish size, behavior, and blood glucose. *Physiol. Biochem. Zool.* **2012**, *85*, 635–644.
85. Rombough, P.J. Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, *Salmo gairdneri*. *Can. J. Zool.* **1988**, *66*, 651–660.
86. Mason, J.C. Hypoxial stress prior to emergence and competition among coho salmon fry. *J Fish. Res. Board Can.* **1969**, *26*, 63–91.
87. Pepin, P. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can. J. Fish. Aquat. Sci.* **1991**, *48*, 503–518.
88. Tills, O.; Spicer, J.I.; Rundle, S.D. Salinity-induced heterokairy in an upper-estuarine population of the snail *Radix balthica* (Mollusca: Pulmonata). *Aquat. Biol.* **2010**, *9*, 95–105.
89. Márquez-Couturier, G.; Vázquez-Navarrete, C.J.; Contreras-Sánchez, W.M.; Álvarez-González, C.A. *Acuicultura Tropical Sustentable: Una Estrategia para la Producción y Conservación del Pejelagarto (Atractosteus tropicus) en Tabasco, México*, 2nd ed.; Colección José Narciso Rovirosa. Universidad Juárez Autónoma de Tabasco: Tabasco, México, 2015; Volume 3, p. 223.
90. Frías-Quintana, C.A.; Álvarez-González, C.A.; Márquez-Couturier, G. Diseño de microdietas para el larvicultivo de pejelagarto *Atractosteus tropicus*, Gill 1863. *Univ. Cienc.* **2010**, *26*, 265–282.
91. Morales, G. *Reproducción y Desarrollo Embriológico del Catán* (*Lepisosteus spatula Lacepede): Primeros Resultados*; SEPES. Dirección General de Acuacultura: Ciudad de Mexico, Mexico, 1987; p. 70.
92. Morales, G. Especies nativas de México. *Acuavisión* **1999**, *12*, 7–9.

93. Rao, T.R. Influence of salinity on the eggs and larvae of the California killifish *Fundulus parvipinnis*. *Mar. Biol.* **1974**, *24*, 155–162.
94. Czerkies, P.; Brzuzan, P.; Kordalski, K.; Luczynski, M. Critical partial pressures of oxygen causing precocious hatching in *Coregonus lavaretus* and *C. albula* embryos. *Aquaculture* **2001**, *196*, 151–158.
95. Garside, E.T. Some effects of oxygen in relation to temperature on the development of lake trout embryos. *Can. J. Zool.* **1959**, *37*, 689–698.
96. Warkentin, K.M. Oxygen, gills, and embryo behavior: Mechanisms of adaptive plasticity in hatching. *Comp. Biochem. Physiol. A Mol. Integr. Physiol* **2007**, *148*, 720–731.
97. Yúfera, M.; Darias, M.J. The onset of exogenous feeding in marine fish larvae. *Aquaculture* **2007**, *268*, 53–63.
98. Rønnestad, I.; Yúfera, M.; Ueberschär, B.; Ribeiro, L.; Sæle, Ø.; Boglione, C. Feeding behaviour and digestive physiology in larval fish: Current knowledge, and gaps and bottlenecks in research. *Rev. Aquacult.* **2013**, *5*, S59–S98.
99. Camacho, S.; Carmona, R.; Llorente, J.I.; Sanz, A.; García-Gallego, M.; Domezain, A.; Dominguez, N.; Ostos-Garrido, M.V. Stomach development in the sturgeon *Acipenser naccarii*: Histoenzymatic and ultrastructural analysis. *J. Appl. Ichthyol.* **2011**, *27*, 693–700.
100. Dou, S.; Masuda, R.; Tanaka, M.; Tsukamoto, K. Feeding resumption, morphological changes and mortality during starvation in Japanese flounder larvae. *J. Fish Biol.* **2002**, *60*, 1363–1380.
101. Comabella Soto, Y. Desarrollo Larval del Manjuarí *Atractosteus tristoechus* (Bloch y Schneider, 1801) (Pisces: Lepisosteidae) en Condiciones de Cultivo: Aspectos Morfo-Fisiológicos. Ph.D. Thesis, Universidad de la Habana, La Habana, Cuba, 2011.
102. Moteki, M.; Yoseda, K.; Sahin, T.; Üstündag˘, C.; Kohno, H. Transition from endogenous to exogenous nutritional sources in larval black sea turbot *Psetta maxima*. *Fish. Sci.* **2001**, *67*, 571–578.
103. Williams, K.; Papanikos, N.; Phelps, R.P.; Shardo, J.D. Development, growth, and yolk utilization of hatchery-reared red snapper *Lutjanus campechanus* larvae. *Mar. Ecol. Prog. Ser.* **2004**, *275*, 231–239.
104. Coughlin, D.J. Ontogeny of feeding behavior of first-feeding Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **1991**, *48*, 1896–1904.
105. Makrakis, M.C.; Nakatani, K.; Bialetzki, A.; Sanches, P.V.; Baumgartner, G.; Gomes, L.C. Ontogenetic shifts in digestive tract morphology and diet of fish larvae of the Itaipu Reservoir, Brazil. *Environ. Biol. Fishes* **2005**, *72*, 99–107.
106. Collins, L.A.; Nelson, S.G. Effects of temperature on oxygen consumption, growth, and development of embryos and yolk-sac larvae of *Siganus randalli* (Pisces: Siganidae). *Mar. Biol.* **1993**, *117*, 195–204.
107. Hart, P.R.; Purser, G.J. Effects of salinity and temperature on eggs and yolk sac larvae of the greenback flounder (*Rhombosolea tapirina* Günther, 1862). *Aquaculture* **1995**, *136*, 221–230.
108. May, R.C. Effects of temperature and salinity on yolk utilization in *Bairdiella icistia* (Jordan & Gilbert) (Pisces: Sciaenidae). *J. Exp. Mar. Biol. Ecol.* **1974**, *16*, 213–225.
109. Khan, U. Effect of salinity on yolk utilization and growth of brook trout alevins (*Salvelinus fontinalis*). *J. Anatol. Environ. Animal Sci.* **2019**, *4*, 93–96.
110. Massa, F.; Delorme, C.; Baglinière, J.L.; Prunet, P.; Grimaldi, C. Expositions d'oeufs de truite commune (*Salmo trutta*) à des hypoxies temporaires ou continue: Effets sur la branchie, la résorption de la vésicule vitelline et les caractéristiques morphométriques des alevins. *Bull. Fr. Pech. Piscic.* **1999**, *355*, 421–440.
111. Polymeropoulos, E.T.; Elliott, N.G.; Frappell, P.B. Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2017**, *213*: 28–35.
112. Roussel, J.M. Carry-over effects in brown trout (*Salmo trutta*): Hypoxia on embryos impairs predator avoidance by alevins in experimental channels. *Can. J. Fish. Aquat. Sci.* **2007**, *64*, 786–792.
113. Voesenek, C.J.; Muijres, F.T.; van Leeuwen, J.L. Biomechanics of swimming in developing larval fish. *J. Exp. Biol.* **2018**, *221*, jeb149583.
114. Batty, R.S. Development of swimming movements and musculature of larval herring (*Clupea harengus*). *J. Exp. Biol.* **1984**, *110*, 217–229.
115. Vieira, V.I.A.; Johnston, I.A. Influence of temperature on muscle-fibre development in larvae of the herring *Clupea harengus*. *Mar. Biol.* **1992**, *112*, 333–341.
116. Domenici, P.; Herbert, N.A.; Lefrançois, C.; Steffensen, J.F.; McKenzie, D.J. The effect of hypoxia on fish swimming performance and behaviour. In *Swimming Physiology of Fish*; Springer: Berlin, Heidelberg, Germany, 2013; pp. 129–159.
117. Kaufmann, R.; Wieser, W. Influence of temperature and ambient oxygen on the swimming energetics of cyprinid larvae and juveniles. In *Environmental Biology of European Cyprinids*; Weiser, W., Scheimer, F., Goldschmidt, A., Kotrschal, K., Eds.; Springer Science & Business Media: Luxemburg, 1992; pp. 87–96.
118. Márquez-Couturier, G.; Álvarez-González, C.A.; Contreras-Sánchez, W.M.; Hernández-Vidal, U.; Hernández-Franyutti, A.A.; Mendoza-Alfaro, R.E.; Aguilera-González, C.; García-Galano, T.; Civera-Cerecedo, R.; Goytortua-Bores, E. Avances en la alimentación y nutrición del pejelagarto *Atractosteus tropicus*. In *Avances en Nutrición Acuícola VIII, Proceedings of the VIII Simposium Internacional de Nutrición Acuícola, Mazatlán, SI, México, 15–17 November 2006*; Cruz-Suárez, E., Ricque, D.M., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D.A., Puello-Cruz, A.C., García-Ortega, A., Eds.; Universidad Autónoma de Nuevo León: Monterrey, NL, Mexico, 2006; pp. 446–523.

119. Busch, A. Transition from endogenous to exogenous nutrition: Larval size parameters determining the start of external feeding and size of prey ingested by Ruegen spring herring *Clupea harengus*. *Mar. Ecol. Prog. Ser.* **1996**, *130*: 39–45.
120. Dabrowski, K.; Bardega, R. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. *Aquaculture* **1984**, *40*, 41–46.
121. Hecht, T.; Pienaar, A.G. A review of cannibalism and its implications in fish larviculture. *J. World Aquac. Soc.* **1993**, *24*, 246–261.
122. Hill, L.; Renfro, J.; Reynolds, R. Effects of dissolved oxygen tensions upon the rate of young spotted gar, *Lepisosteus oculatus* (Lepisosteidae). *Southwest. Nat.* **1972**, *17*, 273–278.
123. Rimoldi, S.; Terova, G.; Zaccone, G.; Parker, T.; Kuciel, M.; Dabrowski, K. The effect of hypoxia and hyperoxia on growth and expression of hypoxia-related genes and proteins in spotted gar *Lepisosteus oculatus* larvae and juveniles. *J. Exp. Zool. B Mol. Dev. Evol.* **2016**, *326*, 250–267.
124. Timmons, M.B.; Ebeling, J.M. *Recirculating Aquaculture*; Cayuga Aqua Ventures. Ithaca, NY, USA, **2010**; pp. 976.
125. Brett, J.R.; Groves, D.D. Physiological energetics. In *Volume VIII—Fish Physiology. Bioenergetics and Growth*; Hoar, W.S., Randall, D.J., Brett, J.R., Eds.; Academic Press: New York, NY, USA, **1979**; p. 344.
126. Ricker, W.E. Computation and interpretation of biological statistics of fish populations. *Bull. Fish. Res. Board Can.* **1975**, *191*, 1–382**.**
127. Kaplan, E.L.; Meier, P. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **1958**, *53*, 457–481.

Capítulo 2

Comparative Biochemistry and Physiology, Part A

Hypoxia- and Hyperoxia-Related Gene Expression Dynamics During Developmental Critical Windows of the Tropical Gar Atractosteus tropicus

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Article
Section/Category:	Development
Keywords:	development; critical windows; tropical gar; hypoxia; hyperoxia; gene expression; hif-1α
Corresponding Author:	https://www.facebook.com/gil.martinez.14 Martínez-Bautista Universidad Juarez Autonoma de Tabasco Villahermosa, Tabasco MEXICO
First Author:	Gil Martínez-Bautista
Order of Authors:	Gil Martínez-Bautista
	https://www.facebook.com/gil.martinez.14 Martínez-Burguete
	https://www.facebook.com/gil.martinez.14 Saul Peña-Marín
	https://www.facebook.com/gil.martinez.14 Daniel Jiménez Martínez
	https://www.facebook.com/gil.martinez.14 Camarillo-Coop
	Warren W. Burggren
	https://www.facebook.com/gil.martinez.14 Alfonso Álvarez-González
Abstract:	Aquatic hypoxia is both a naturally-occurring and anthropologically-generated event. Fish species have evolved different adaptations to cope with hypoxic environments, including gill modifications and air breathing. However, little is known about the molecular mechanisms involved in the respiration of embryonic and larval fishes during critical windows of development. We assessed expression of the genes hif-1α, fih-1, nhe1, epo, gr and il8 using the developing tropical gar as a piscine model during three key developmental periods (fertilization-hatch, 1-6 days post hatch (dph) and 7-12 dph) when exposed to normoxia (~95% air saturation), hypoxia (~30% air saturation) or hyperoxia (~117% air saturation). All genes showed higher expression when fish were exposed to either hypoxia or hyperoxia during the first two developmental periods. However, fish continuously exposed to hypoxia showed increased expression of the six genes by hatching and 6 dph, and by 12 dph only for hif-1α . The middle developmental period was the most hypoxia-sensitive, coinciding with several changes in physiology and morphology. The oldest larvae were the most resilient to gene expression change, with little variation in expression of the six genes compared. This is study is the first to relate the molecular response of an air-breathing fish to oxygen availability to developmental critical windows and contributes to our understanding of some molecular responses of developing fish to changes in oxygen availability.
Suggested Reviewers:	Simona Rimoldi Università degli Studi Di Cagliari: Universita degli Studi Di Cagliari simona.rimoldi@uninsubria.it Expert in gene expression related to hypoxia in fish
	Solomon David Nicholls State University solomon.david@nicholls.edu Expert in molecular biology and gene expression
	Francisco Moyano Universidad de Almería: Universidad de Almeria fjmoyano@ual.es Expert in physiology and biochemistry of aquatic organisms

	Maria Darias Institut de recherche pour le développement: Institut de recherche pour le developpement maria.darias@ird.fr Expert in physiology, nutrition and metabolism in tropical fish
	Ignacio Fernandez Instituto Agrario de Castilla y León nacfm@hotmail.com Expert in gene expression, physiology and bioinformatics in aquatic organisms
	Allyse Ferrara Nicholls State University allyse.ferrara@nicholls.edu Expert in molecular biology and bioinformatics
Opposed Reviewers:	

Hypoxia- and Hyperoxia-Related Gene Expression Dynamics During Developmental Critical Windows of the Tropical Gar *Atractosteus tropicus*

Gil Martínez-Bautista[1]*, Talhia Martínez-Burguete[1], Emyr Saul Peña-Marín[1], Luis Daniel Jiménez Martínez[2], Rafael Martínez-García[1], Susana Camarillo-Coop[1], Warren W. Burggren[3], Carlos Alfonso Álvarez-González[1]*

[1]Laboratorio de Acuacultura Tropical, División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México.

[2]División Académica Multidisciplinaria de Jalpa de Méndez, Universidad Juárez Autónoma de Tabasco, Jalpa de Méndez, Tabasco, México

[3]Developmental Physiology Laboratory, Developmental Integrative Biology Research Group, Department of Biological Sciences, University of North Texas, Denton, Texas, United States of America.

*Corresponding authors: gil.martinez.bautista@live.com.mx; alvarez_alfonso@hotmail.com.

ABSTRACT

Aquatic hypoxia is both a naturally-occurring and anthropologically-generated event. Fish species have evolved different adaptations to cope with hypoxic environments, including gill modifications and air breathing. However, little is known about the molecular mechanisms involved in the respiration of embryonic and larval fishes during critical windows of development. We assessed expression of the genes *hif-1α, fih-1, nhe1, epo, gr* and *il8* using the developing tropical gar as a piscine model during three key developmental periods (fertilization-hatch, 1-6 days post hatch (dph) and 7-12 dph) when exposed to normoxia (~95% air saturation), hypoxia (~30% air saturation) or hyperoxia (~117% air saturation). All genes showed higher expression when fish were exposed to either hypoxia or hyperoxia during the first two developmental periods. However, fish continuously exposed to hypoxia showed increased expression of the six genes by hatching and 6 dph, and by 12 dph only for *hif-1α*. The middle developmental period was the most hypoxia-sensitive, coinciding with several changes in physiology and morphology. The oldest larvae were the most resilient to gene expression change, with little variation in expression of the six genes compared. This is study is the first to relate the molecular response of an air-breathing fish to oxygen availability to developmental critical windows and contributes to our understanding of some molecular responses of developing fish to changes in oxygen availability.

Key words: development, critical windows, tropical gar, hypoxia, hyperoxia, gene expression, *hif-1α*

INTRODUCTION

Aquatic animals may experience lower absolute amounts of oxygen compared to terrestrial animals (Eddy and Handy, 2012; Jonz *et al.*, 2016). Limited oxygen availability can reduce fish activity and restrict the supply or removal of certain compounds in cellular metabolic cascades. Fishes have developed a series of hypoxia-related adaptations to maintain large scopes for activity and exploit different habitats, including modifications in the gills, skin and the ability to consume atmospheric oxygen (Damsgaard *et al.*, 2020; Eddy and Handy, 2012; Garduño *et al.*, 2020; Jonz *et al.*, 2016). Air-breathing is believed to arise as an adaptation to hypoxia and other extreme conditions in the environment (e.g., hypercapnia or variations in pH and presence of sulfides in the aquatic environment) (Brauner *et al.*, 1995; Graham 1997; Randall *et al.*, 1981; Val and Almeida-Val, 1995). Air-breathing has evolved ~70 times in bony fishes (Brauner and Rombough, 2012; Graham, 1997; Randall *et al.*, 1981). A variety of air-breathing organs have evolved for aerial respiration such as a modified swimming bladder, the gut (stomach and/or intestines), cephalic/opercular chambers and true lungs in lungfishes (e.g., Affonso and Rantin, 2005; Belaõ *et al.*, 2011; Burggren 1988; Burggren *et al.*, 1986; Graham, 1997; Johansen and Burggren, 1980; Johnson *et al.*, 2010; Lefevre *et al.*, 2011; Lefevre *et al.*, 2016; Little 2009; Lopes *et al.*, 2010; Milsom, 2012; Oliveira *et al.*, 2004; Perry *et al.*, 2001, Randall *et al.*, 1981; Shartau and Brauner, 2014). Yet, major gaps remain in our understanding of the molecular mechanisms related to respiratory processes and especially hypoxia tolerance in air-breathing species, with only a few such studies (Chi *et al.*, 2013; Huang *et al.*, 2015a,b; Rimoldi *et al.*, 2016). compared to those in strictly aquatic fishes

(e.g., Chen *et al.*, 2012; Davies *et al.*, 2011; Geng *et al.*, 2014; Kodama *et al.*, 2012; Rimoldi *et al.*, 2012; Terova *et al.*, 2008).

Compounding our limited knowledge of respiration in air-breathing fishes, we also know relatively little about early developmental stages compared to adults. Filling in this knowledge gap is not only important to understand the basic developmental biology of air breathing fish, but also because fish embryos and larvae are the most vulnerable developmental stages, and their biology can be altered by numerous environmental factors (Burggren and Bagatto, 2008; Johnson *et al.*, 2010; Martínez-Bautista *et al.*, 2021; Mueller *et al.*, 2015; Rudneva, 2014). Understanding the interplay of environment and development at the molecular/genetic level is especially important when considering critical windows of development - those periods during development when phenotype is particularly sensitive to changes of intrinsic or extrinsic factors (Burggren and Mueller, 2015).

The elucidation of tractable animal models has long been recognized as essential for research (Davidson *et al.*, 1987; Flores Santin and Burggren, 2021). The fishes known as gars, in the family Lepisosteidae, show low rates of speciation and phenotypic evolution (Rabowsky *et al.*, 2013) and have several additional characteristics that make them a useful animal model for developmental studies (Martínez-Bautista *et al.*, 2021). In the current study, the air-breathing tropical gar *Atractosteus tropicus* was used to study gene expression as a function of environmental oxygen availability during early development. This species inhabits slowly-moving waters from South Mexico to Costa Rica (Barrientos-Villalobos and Espinosa de los Monteros, 2008; Bussing, 1998; Miller *et al.*, 2005). The

tropical gar can survive to low oxygen levels and moderately high temperatures (Mora *et al.*, 1997). Moreover, it is characterized by extraordinarily rapid development (Burggren *et al.*, 2016; Martínez-Bautista *et al.* 2021).

Our objective in studying the tropical gar was to determine the expression of the genes hypoxia inducible factor 1-alpha (*hif.1α*), hypoxia inducible factor inhibitor (*fih-1*), erythropoietin (*epo*), sodium-hydrogen exchanger 1 (*nhe1*), glucocorticoid receptors (*gr*) and interleukin 8 (*il8*) as a function of hypoxia and hyperoxia during early development. These genes were chosen because they have important roles in the response to variations in oxygen availability, including normal development, regulation of ion exchange, stress and immune responses, and red blood cell production (Ayson et al, 1995; Brauner and Baker, 2009; Evans *et al.*, 2005; Haase, 2010, 2013; Pelster and Egg, 2018; Semenza, 1999; Tan *et al.*, 2017; Vizzini *et al.*, 2007). We employed a design to reveal developmental critical windows for hypoxia responses by investigating gene expression during three major developmental periods (fertilization to hatch, 1-6 days post hatch (dph) and 7-12 dph), as informed by studies on *A. tropicus* by Aguilera *et al.* (2002) and Martínez-Bautista *et al.* (2021). Our results provide the first insight into molecular mechanisms involved in respiration, stress, and immune responses in an air-breathing fish during its developmental critical periods.

MATERIAL AND METHODS

Ethical statement

Fish were handled in compliance with the Norma Oficial Mexicana NOM-062-ZOO-1999 from Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, the Mexican standards for appropriate welfare practices of laboratory animals.

Fish rearing and care

Embryos and larvae of *Atractosteus tropicus* were obtained from an artificially induced spawn (1 female and 5 males) in May 2019 from a broodstock held at the Tropical Aquaculture Laboratory, La Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco state, México. Immediately after fertilization, 1350 fertilized eggs were transferred from the breeding tank to experimental tanks in a mix of sexes (sex cannot be determined in embryos or larvae). One Control group and eight experimental groups were created as described in the experimental protocol below. Each group (including the Control) was carried out in triplicate (50 larvae per replicate, 150 per group).

Experimental tanks contained non-chlorinated water at 28°C. Embryos and larvae were kept in normoxia (~95% air saturation, except when indicated), and with a 12:12 h photoperiod. Larvae were fed to satiety with brine shrimp (*Artemia* sp.) nauplii every four hours from 8:00 h to 20:00 h. Dead brine-shrimp and feces were siphoned one hour after every meal. Fifty percent of the water in each tank was replaced every 48 h at the required oxygen level according to the experimental protocol (see below).

Experimental protocol

The developmental period from fertilization to 12 days post-hatch (dph) was divided into three divisions according to Aguilera *et al.* (2002) and Martínez-Bautista *et al.*, (2021) as follows: 1) from fertilization to hatch (~3 days), 2) from ~1-6 dph (yolk depletion), and 3) from ~7-12 dph (pre-juvenile stage). Embryos and larvae in each of these developmental periods were exposed to three major conditions: normoxia (~95% air saturation), hypoxia (~30% air saturation) and hyperoxia (~117% air saturation). The hypoxia and hyperoxia levels were chosen on the basis that Lepisosteids can tolerate variable oxygen availability in their environments (Hill, *et al.*, 1972; Rimoldi *et al.*, 2016; Smatresk and Cameron, 1982). Low oxygen was generated by bubbling nitrogen gas into the tanks until oxygen levels decreased to a constant ~30% of full air saturation. High oxygen was created using a counter current water tower with an aeration stone bubbling pure oxygen gas at the bottom which rose against a downward flow of water (e.g., Timmons and Ebeling, 2010). By adjusting water flow into the tower, this system produced a constant flow of water with an air saturation of ~117%. Oxygen levels were constantly validated with two oximeters (YSI Pro2030).

A control group was created for both embryos and larvae in normoxia. Of the eight experimental groups, six were exposed exclusively to hypoxia or hyperoxia during a specific developmental period (Figure 1). Two additional groups were continuously exposed to either hypoxia or hyperoxia. The experimental groups were designated as: normoxia (Control), period 1 hypoxia (P1-hypoxia), period 2 hypoxia (P2-hypoxia), period 3 hypoxia (P3-hypoxia), continuous hypoxia exposure (CE-hypoxia), period 1 hyperoxia (P1-hyperoxia), period 2 hyperoxia (P2-hyperoxia), period 3 hyperoxia (P3-hyperoxia), and continuous hyperoxia exposure (CE-hyperoxia). Naming conventions for

these periods of exposures are from Martinez-Bautista *et al*. (2021). Before and after the hypoxic or hyperoxic exposure (depending on the developmental period) embryos and larvae were maintained in normoxia as shown in Figure 1.

Sampling

Hatchlings and larvae of *A. tropicus* were sampled at the end of each developmental period (hatching, 6 dph and 12 dph). Nine hatchlings and six larvae from each experimental group were collected after 12 h of fasting, rinsed in distilled water, transferred to Eppendorf tubes with 1.5 mL of RNA Later (Life Technologies, Carlsbad, CA, USA) and stored at -80°C. A sub-sample of fish exposed to hypoxia or hyperoxia during 1-6 dph were not sampled at hatching, nor were those exposed during 7-12 dph sampled earlier, because we assumed that gene expression was the same as values of the control population *per se* – essentially, until their hypoxic/hyperoxic exposure, they were part of the control population.

Molecular procedures

RNA extractions were carried out by the TRIzol Reagent method (Invitrogen, Carlsbad, CA, USA) using pools of 6 hatchlings and 3 larvae per experimental group. RNA concentration and purity were calculated by 260/280 ratio in a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). Integrity was determined by electrophoresis on 1% agarose gel using 1 μL sample aliquot mixed with 1 μL of run

buffer for each sample at 100 volts for 30 minutes in an electrophoresis Mini SUb Cell GT chamber (Bio-Rad). Resulting bars were visualized in a Molecular Imagen Gel Doc XR + Imaging System (Bio-Rad). After RNA integrity validation, aliquots were sampled from the proper calculated concentration. In addition, 1 μg of RNA was used for reverse transcription with an iScript™ Select cDNA Synthesis Kit 170-8896 (Bio-Rad).

For gene expression analysis, nucleotide sequences for hypoxia-inducible factor 1 alpha (*hif-1α*), hypoxia-inducible factor 1 alpha inhibitor (*fih-1*), erythropoietin (*epo*), glucocorticoid receptor (*gr*), sodium/hydrogen exchanger 1 (*nhe1*) and interleukine 8 (*il8*) were obtained from the transcriptome of *Atractosteus tropicus* (Martínez-Burguete *et al*., 2021). Specific oligonucleotides for each gene (Table 1) were designed using Primer3web version 4.1.0 (Untergasser *et al*., 2012). Oligonucleotide specificity and potential secondary structures were validated using OligoAnalyzer version 3.1 (Integrated DNA Technologies). Expression analysis of the genes were performed in 96-well thermocycler (CFX96 Real-Time System Thermal Cycle; C1000, CA, USA). The reaction mixture contained 5 μL of Eva Green, 1 μL of cDNA, 3.2 μL of H_2O milli-Q and 0.40 μL of each oligonucleotide (total mixture = 10 μL).

Real-time PCRs were performed according to the following conditions: two minutes at 95°C, consequently in 36 cycles at 95°C for 10 seconds, 64°C for 30 seconds and extension at 60°C for five seconds. A negative control was included for each test, with the components of the reaction using distilled water instead of cDNA. Elongation factor 1 (*ef1*) was utilized as a housekeeping gene (Jiménez-Martínez *et al.*, 2019). The relative

expression of the genes was calculated by the delta-delta ct method (Livak and Schmittgen, 2001).

Statistical analysis

Gene expression data were tested for normality (Kolmogorov-Smirnov) and homoscedasticity (Levine). A two-way ANOVA on ranks was performed for the expression analysis of each gene as a function of development and the different hypoxia/hyperoxia exposure groups. A Tukey test for multiple comparison was performed to assess differences within treatments. All analyses were performed with a significance level of 0.05 in the software SigmaPlot 11.0.

RESULTS

Gene Expression and Oxygen Levels

The relationships between relative gene expression during normoxia, hypoxia, and hyperoxia as a function of development are indicated in Figure 2. Figure 3 presents a gene expression heat map. Specific gene expression patterns will now be described.

Hypoxia-inducible factor 1 alpha (*hif-1α*)

From fertilization to hatch, *hif-1α* expression was significantly ($P<0.001$) increased when compared to the Control in fish from experimental groups P1-hypoxia and CE-

hypoxia. Decreased expression was observed in fish from groups P1-hyperoxia and CE-hyperoxia (Figure 2a).

From 1-6 dph, larvae from groups P2-hypoxia and CE-hypoxia exhibited the highest expression, while higher expression than the Control occurred in larvae from groups P1-hyperoxia and P2-hyperoxia ($P<0.001$). Following the trend of *hif-1α* expression being related to ambient oxygen levels, fish from group CE-hyperoxia showed the lowest expression of *hif-1α* ($P<0.001$; Figure 2a).

For 7-12 dph, the highest expression of *hif-1α* occurred in larvae from group CE-hypoxia ($P<0.001$). Higher expression compared to Controls was observed in individuals from groups P1-hypoxia and CE-hyperoxia ($P<0.001$). Lower expression than the Control was shown by larvae from groups P3-hypoxia, P2-hyperoxia, and P3-hyperoxia, and the lowest was registered by those form P3-hyperoxia ($P<0.001$; Figure 2a). Fish from the experimental group CE-hypoxia exhibited a gradual increase in the expression of *hif-1α* through the experiment (Figure 2a).

Expression of hypoxia-inducible factor 1 alpha inhibitor (*fih-1*)

From fertilization to hatch, lower expression of *fih-1* compared to the control was observed in fish from groups P1-hypoxia and P1-hyperoxia ($P<0.001$; Figure 2b).

For 6-12 dph, a significant increase in *fih-1* (compared to the Control) was observed in fish from groups P1-hypoxia, CE-hyperoxia and P1-hyperoxia. Higher expression

compared to Controls, occurred in larvae from groups CE-hypoxia and P2-hyperoxia, while the highest was registered by fish from P2-hypoxia ($P<0.001$; Figure 2b).

By 7-12 dph, higher *fih-1* expression compared to Controls was exhibited by larvae from groups P1-hypoxia, CE-hypoxia and CE-hyperoxia (*P<0.001*). Lower expression occurred in individuals from groups P1-hyperoxia, P2-hyperoxia and P3-hyperoxia. However, the lowest expression was observed in fish from groups P2-hypoxia and P3-hypoxia ($P<0.001$; Figure 2b).

Expression of sodium/hydrogen exchanger 1 (*nhe1*)

Expression of *nhe1* generally followed the same patterns as *hif-1α* and *fih-1*. Thus, by hatching, increased expression of *nhe1* (compared to the Control) occurred in fish from groups P1-hypoxia and CE-hypoxia, while lower expression in individuals from P1-hyperoxia and CE-hyperoxia ($P<0.001$; Figure 2c).

For 1-6 dph, higher *nhe1* expression than Controls was observed in fish from P1-hypoxia, P2-hypoxia, and CE-hypoxia, while the highest was registered for individuals from P2-hyperoxia and the lowest for fish from CE-($P<0.001$; Figure 2c).

For 7-12 dph, fish from experimental groups P1-hypoxia and CE-hyperoxia showed higher expression of *nhe1* than Controls, while the rest of the groups exhibited lower expression ($P<0.001$; Figure 2c).

Expression of erythropoietin (*epo*)

From fertilization to hatch, *epo* expression increased in fish from groups P1-hypoxia and CE-hypoxia by hatching ($P<0.001$; Figure 2d).

For 1-6 dph, larvae from groups P2-hypoxia, CE-hypoxia and P1-hyperoxia showed higher *epo* expression than Controls and individuals from P1-hypoxia exhibited lower expression ($P<0.001$). The highest expression in this period was registered for larvae from group P2-hyperoxia and the lowest for fish from experimental group CE-hyperoxia ($P<0.001$; Figure 2d).

Lower expression than Controls was observed for all the experimental groups for 7-12 dph. However, the lowest expression occurred in fish from groups P1-hypoxia and P2-hypoxia ($P<0.001$; Figure 2d).

Expression of glucocorticoid receptor (*gr*)

From fertilization to hatch, *gr* expression increased in individuals from groups P1-hypoxia and CE-hypoxia compared to Controls ($P<0.001$; Figure 2e).

For 1-6 dph, higher *gr* expression than Controls occurred in larvae from groups P1-hypoxia, P2-hypoxia and P1-hyperoxia. Highest *gr* expression occurred in fish from groups CE-hypoxia and P2-hyperoxia, while larvae lower *gr* expression occurred in group CE-hyperoxia ($P<0.001$; Figure 2e).

For 7-12 dph, fish from group P3-hyperoxia showed higher *gr* expression than Controls, and the rest of the experimental groups exhibited lower *gr* expression

(P<0.001). The lowest *gr* expression from 7-12 dph was registered for larvae from group P2-hypoxia (Figure 2e).

Expression of interleukine 8 (*il8*)

By hatching, fish from experimental groups P1-hypoxia, CE-hypoxia, P1-hyperoxia and CE- hyperoxia showed higher expression of *il8* than the Control (P<0.001; Figure 2f).

For 1-6 dph, fish from most experimental groups (except from P3-hypoxia and P3-hyperoxia) exhibited increased expression of *il8* compared to the Control and the highest expression was observed in larvae from experimental group P2- hyperoxia (P<0.001; Figure 2f).

For 7-12 dph, all fish in all experimental groups showed lower expression of *il8* than the Control and larvae from experimental group P2-hypoxia exhibited the lowest (P<0.001; Figure 2f).

Gene Expression and Development Periods

Developmental period 1 (fertilization to hatch) resulted to be more sensitive for hypoxic than hyperoxic treatments compared to Controls. Genes *hif-1α, nhe1, epo, gr* and *il8* showed increased expression in hypoxic groups during this period, while hyperoxic groups showed increased expression of *il8*, decreased expression of *hif-1α, fih-1* and *nhe1*, and no differences in expression of *epo* and *gr* compared to Controls The developmental

period of 1-6 dph was characterized by the highest gene expression of all genes tested. This was especially the case in larvae exposed to both hypoxia and hyperoxia on developmental periods 1 and 2 (P1-hypoxia, P2-hypoxia, P1-hyperoxia and P2-hyperoxia), and larvae continuously exposed to hypoxia (CE-hypoxia) by 6 dph. In contrast, of all developmental periods examined, the oldest developmental period of 7-12 dph was the least changed by hypoxia or hypoxia, with all tested genes exhibited little variation compared to Controls.

DISCUSSION

Freshwater fish species inhabit aquatic environments where oxygen availability varies – sometimes stochastically and often greatly – across both time and space. Physiological hypoxia is a commonly occurring phenomenon for fishes due to water's low oxygen solubility, ineffective mixing in the water column and the respiratory processes of aquatic organisms, coupled with only intermittent plant photosynthetic activity that could alleviate aquatic hypoxia (Diaz and Breitburg, 2009; Jenny *et al.*, 2016; Val and Almeida-Val, 1995). Environmental oxygen variations can lead to decreased survival and growth. Survival and growth as a function of ambient oxygen levels have been previously presented for the tropical gar *A. tropicus* (Martínez-Bautista *et al.*, 2021). By hatching in *A. tropicus*, both hypoxia and hyperoxia decreased survival compared to control oxygen levels. However, hypoxic or hyperoxic exposure during 1-6 dph and 7-12 dph showed no differences compared to the Control population. Moreover, there were differences in body mass, total length, and specific growth rate as a function of hypoxia. In contrast to these

findings, juvenile *Lepisosteus oculatus* exposed to 71 days of hyperoxia exhibited decreased survival, but growth was unaffected by either hypoxia or hyperoxia (Rimoldi *et al.*, 2016). Importantly, these somewhat contrasting previously published results in air breathing gar species suggest that sensitivity to variations in oxygen level are dependent on developmental stage, which differed between these two studies.

The maintenance of oxygen equilibrium in vertebrates typically involves the transcriptional factor hypoxia inducible factor (*Hif*) (Chen, *et al.*, 2020; Kaelin and Ratcliffe, 2008; Schofield and Ratcliffe, 2004; Whitehouse and Manzon, *et al.*, 2019). *Hif* proteins represent an evolutionarily conserved pathway (from elasmobranchs to tetrapods) for the physiological response to hypoxia (Prabhakar and Semenza, 2015; Rytkonen *et al.*, 2011; Semenza, 2010). These proteins consist of two subunits (α and β), where α-units are hypoxia sensitive while β-subunits are insensitive (Uchida *et al.*, 2004). *Hif-α* proteins regulate the expression of other genes involved in several processes such as glycolysis, iron metabolisms and muscle contraction, as well as initiating the response to low oxygen levels (Porporato *et al.*, 2011; Semenza, 2000).

The function of *hif-1α* during development is not limited to the hypoxia-response because this transcriptional factor is also crucial for proper development and organogenesis (Pelster and Egg, 2018). For example, in yolk-free *Salmo salar*, reduction in *hif-1α* expression results in reduced vascular endothelial growth factor, implicated in vasculogenesis and angiogenesis (Vuori *et al.*, 2004). Knockdown *hif-1α* in the zebrafish produced morphological alterations (Tan *et al.*, 2017) and changes in the ventilatory response of larvae (Mandic *et al.* 2019). In the current study on the tropical gar, *hif-1α*

was upregulated in hypoxic hatchlings and downregulated in hyperoxic hatchlings, suggesting that increased *hif-1α* expression is related to ambient oxygen levels. Moreover, the hypoxic signaling pathway appears to be operational before the onset of cardiorespiratory activity and hemoglobin-dependent oxygen transport (Burggren *et al.*, 2016), as suggested by studies in other fish species (Pelster and Burggren, 1996; Rombough and Drader, 2009). *Hif-1α* expression in fish embryos varies between species and developmental stage, with increased expression observed as early as 12 hpf in the zebrafish (Chen *et al.*, 2012; Rytkönen *et al.*, 2013; Santhakumar *et al.*, 2012; Shen *et al.*, 2010). Collectively, these data suggest that the expression of *hif-1α* can vary between species according to the specific times of development and hypoxic response, where *Hif* protein levels change to maintain the early individual's basal physiological requirements (Pelster and Egg, 2018; Brusselmans *et al.*, 2001).

To better understand the role of *Hif* proteins in the developing tropical gar, we evaluated their expression across early developmental stages to differentiate/relate their importance in development during hypoxic and hyperoxic exposure. Higher expression levels of *hif-1α* were observed in hypoxia and hyperoxia in the 1-6 dph developmental period, similarly to those continuously exposed to hypoxia. For 7-12 dph larvae, only the continuous exposed group to hypoxia showed increased expression. These maintained high levels of *hif-1α* expression can result from prolonged hypoxic conditions, resulting in a slow-decreasing level of *hif-1α* as described for other fishes (Kopp *et al.*, 2011; Rahman and Thomas, 2007; Sollid *et al.*, 2006; Thomas and Rahman, 2009). The decreased *hif-1α* expression from fish with the highest expressions registered during 1-6 dph when measured at 12 dph (end of the experiment) are consistent with the assumption

that increased *hif-1α* levels return to their basal values as soon as 24 h of recovery in normoxia (Shen *et al.*, 2010; Terova *et al.*, 2008). These data taken in aggregate suggest that the period comprising the first 9 days post-fertilization (developmental periods 1 and 2) represents a key critical window for the molecular response to hypoxia and hyperoxia in *A. tropicus*. During this critical window, several key developmental events occur in the tropical gar, including gill maturation and the onset of gill ventilation, the onset of air breathing, the transition to lecitoexotrophy, yolk depletion, metamorphosis, free swimming, etc. (Aguilera *et al.*, 2002; Burggren et al, 2016; Martínez-Bautista et al, 2021; Martínez-Bautista *et al.*, *in preparation*). In contrast, the last developmental period tested (7-12 dph) was the most resilient, showing the least variation in gene expression of all tested genes and the 9 experimental groups. Moreover, these data show a similar pattern as described in Martínez-Bautista *et al.* (2021) for morphological characteristics of the tropical gar, with higher variation during developmental periods 1 and 2.

Despite *hif-1α* expression not being evaluated by Rimoldi *et al.*, (2016), their findings for Lepisosteids suggest that expression levels of *Hif* proteins may be similar to those we have measured in *A. tropicus*. Juvenile *Lepisosteus oculatus* showed increased expression of *hif-2α* after 71 days of exposure to hypoxia Rimoldi *et al.*, (2016), which is consistent with our data for the larval group continuously exposed to hypoxia. In contrast, however, the expression levels of *hif-1α* in juvenile *Trichopodus microlepis* and *Trichogaster lalius* did not vary during 72 h of hypoxic exposure (Huang *et al.*, 2015a,b). These findings suggest that different times of exposure during different periods of time can lead to different responses in *hif-1α* expression.

The transcriptional activity of *hif-1α* can be regulated by *fih-1*, the factor inhibiting *Hif* (Lando *et al.*, 2002). In our study high expression levels of *hif-1α* at hatching correlate with low expression levels of *fih-1* (Figure 2b). Curiously, high expression levels of *fih-1* were observed in fish exposed to either hypoxia or to hyperoxia during 1-6 dph and also in the continuously exposed group. The increased levels of *fih-1* could be being expressed to decrease the high levels of *hif-1α* in *A. tropicus*. Consistent with our findings for the tropical gar, some teleosts such as the Nile tilapia, the bighead carp, and the channel catfish show increased *fih-1* expression after hypoxic stress (Feng *et al.*, 2019; Geng *et al.*, 2014; Li *et al.*, 2017). However, *fih-1* expression levels varied between species and across developmental stages. This suggests that the activity of *fih-1* is tightly coupled with the activity of *hif-1α*, because both show similar expression tendencies under hypoxic conditions.

Hypoxic stress can lead to less efficient oxygen uptake at the gills due to blood acidosis and the reduction in haemoglobin oxygen-carrying capacity at low pH (Root effect). Fish gills are multifunctional organs responsible for gas exchange, acid-base balance and ion and osmoregulation (Laurent and Perry, 1991; Evans *et al.*, 2005). In fish gills, *Hif* proteins can account for the control of ion transport across the epithelium because hypoxia disrupts fish homeostasis and *hif-1α* is involved in gill remodeling under hypoxic stress (Nilsson, 2007). Uptake and plasma levels of ions such as Na^+ can decrease because of hypoxia (Iftikar *et al.*, 2010; Matey *et al.*, 2008; Wood *et al.*, 2007). In case of blood acidosis, fish improve oxygen uptake by regulating intracellular and extracellular erythrocyte pH through Na^+/H^+ exchangers such as *nhe*, which basically mediate the flux of Na^+ or K^+ ions in exchange for H^+ ions across the cell membrane (Evans *et al.*, 2005;

Brauner and Baker, 2009). *nhe1* expression levels in *A. tropicus* in the current study were upregulated in fish from hypoxic groups by the act of hatching, and the greatest expression levels occurred in fish exposed to either hypoxia or hyperoxia during 1-6 dph. These expression levels may be related to gill maturation, which occurs at ~5 dph in the tropical gar (Burggren *et al.*, 2016; Martínez-Bautista *et al.*, 2021). A reduced size in gill surface has been associated with the evolution of an accessory air-breathing organ. Moreover, reduced transepithelial ion exchange occurs due to the limiting gill capacity for extracellular pH regulation during acidosis (Brauner and Baker, 2009; Shartau and Brauner, 2014). In bimodal-breathing fish, the evolutionary transition to air breathing from strictly aquatic respiration creates a shift in acid-base balance with increased arterial PCO_2 and decreased extracellular pH, driven in part by the reduced gill surface area and associated reduction in CO_2 elimination (Randall *et al.*, 1981; Graham, 1997; Shartau and Brauner, 2014). This phenomenon occurs in the spotted gar (Smatresk and Cameron, 1982) as well as other facultative air-breathing fishes (Shartau and Brauner, 2014). However, in the current study, the *nhe1* expression levels in 7-12 dph were similar to those at hatching or 1-6 dph. Developmental period 2 (1-6 dph) could be considered a critical window for the activity of Na^+/H^+ exchangers because this is the period where the onset of air-breathing, gill ventilation and gill maturation occur (Burggren *et al.*, 2016; Martínez-Bautista *et al.*, 2021) and the role of the exchangers serves to cope with the low oxygen in the environment.

Hif-1 is important for triggering expression of different genes involved in post-hypoxic exposure processes such as iron homeostasis (Long *et al.* 2015; Shah and Xie 2014), haemoglobin formation and erythropoiesis. Erythropoiesis (production of new red

blood cells) is mediated by erythropoietin (*epo*). Erythropoietin is a well described glycoprotein hormone in mammals (Haase 2010, 2013; Semenza 1999) although its role in fish is poorly understood. In our study, higher expression of *epo* was registered for tropical gar exposed to hypoxia at hatching, 1-6 dph and in the continuously exposed larval population. The upregulation in *epo* can be related to higher production of red blood cells as a function of the low oxygen availability for the tropical gar, consistently with findings in studies on other teleosts (Chou *et al.* 2004; Chu *et al.* 2007; Paffet-Lugassy *et al.* 2007; Pierron *et al.* 2007). However, the greatest expression level of *epo* in the tropical gar occurred in fishes exposed to hyperoxia during 1-6 dph. Aquatic environments with supersaturation of atmospheric gases (such as in the hyperoxic treatments in the current research) can alter the physiology of fishes, including gas bubble trauma. Gas bubble trauma promotes the formation of bubbles in the cardiovascular system, super inflation of internal cavities (swimming bladder, intestines, and peritoneum), emphysema in muscles, internal organs and subdermal surfaces and extracorporeal bubble formation in the gills (Boyle 1970). These alterations cause blockage in blood flow and disruption of organ functions and neural activity (Stroud *et al.* 1975; Weitkamo and Katz 1980). Erythropoietin function is not limited to the erythroid linage and has been linked to neo-vascularization (Ribatti *et al.* 1999), cardiac morphogenesis (Wu *et al.*, 1999), mioblast proliferation (Ogilvie *et al.*, 2010) and regenerative capacities (Buemi et al 2009). For this reason, it is possible that the highest expression of *epo* in the tropical gar exposed to hyperoxia can be related to healing processes of possible damage caused by prolonged oxygen supersaturation.

Hif-1α is also involved in mineralocorticoid and glucocorticoid production (Tan *et al.*, 2017). Glucocorticoids are pivotal hormones in fish, regulating cell growth, bone density, metabolism (especially at first feeding in fish early stages), cardiovascular function, osmolarity, immune responses, and metamorphosis or direct development from larvae to juveniles. Glucocorticoids are also mediators of stress-associated responses and are tightly associated with their receptors (*gr*) (Charmandari *et al.*, 2005; Vizzini *et al.*, 2007). The greatest expression levels of *gr* in the developing tropical gar in this study occurred during 1-6 dph, which corresponds to the metamorphosis stage where important changes occur, including yolk depletion, change in the shape of the snout, lecitoexotrophy and free swimming (Aguilera *et al.*, 2002; Martínez-Bautista *et al.*, 2021). Consistent with our data, cortisol levels of the goldlined seabream (*Sparus sarba*) and chum salmon (*Oncorhynchus keta*) larvae increased between 1-7 dph and remain unchanged in the period 7-14 dph (de Jesús and Hirano, 1992; Deane and Woo, 2003). As *gr* is an important mediator of metamorphosis, the highest expression registered in tropical gar larvae continuously exposed to hypoxia, and to hyperoxia from 1-6 dph, suggests that the individuals exhibited some degree of physiological stress. Moreover, the continuously hypoxia exposed fish showed decreased heart rate, gill ventilation and increased air-breathing frequency at 6 dph, compared to Controls (Martínez-Bautista *et al.*, *in preparation*), which suggests that this group of individuals exhibited functional cardiorespiratory responses to cope the developmental demands and the stress response.

Immunity in fishes is mediated by several molecules including cytokines (Reyes-Cerpa *et al.*, 2012), and it is possible that their activation could occur via *Hif-1*, as in humans (Frede *et al.*, 2006; Zinkernagel *et al.*, 2007), since this molecule is highly

conserved from elasmobranchs to mammals (Prabhakar and Semenza, 2015; Rytkonen *et al.*, 2011; Semenza, 2010). Interleukin 8 (*il8*) is a cytokine associated with the inflammatory response, wound healing by increasing angiogenesis and the initiating the oxidative burst in neutrophiles (superoxide production) (Harun *et al.*, 2008; Mukaida *et al.*, 1988). In our study, larvae exposed to hypoxia and hyperoxia during fertilization to hatching exhibited increased expression of *il8* compared to Controls, and larvae exposed to both conditions during fertilization to hatching and 1-6 dph, as the continuous exposed group to hypoxia, showed increased overexpression of *il8* by 6 dph. *il8* overexpression is mainly attributed to bacterial infections (Chen *et al.*, 2005, Sun *et al.*, 2011), so our results suggests that for the tropical gar under hypoxia and hyperoxia, *il8* overexpression was related to the oxidative stress promoted by the lack or superabundance of oxygen in the environment, since these variations can induce the occurrence of reactive oxygen species (ROS) (Lushchak and Bagnyukova, 2006; Pelster *et al.*, 2018). Biomarkers of oxidative stress, such as the activity of antioxidant enzymes, have been documented in fishes (Rudneva, 2014; Rudneva *et al.*, 2010), including *Hoplerythrinus unitaeniatus* and *Hoplias malabaricus* (Pelster *et al.*, 2018), *Protopterus annectens* (Loong *et al.*, 2008) and *Carassius auratus* (Lushchak *et al.*, 2005). However, very little information exists on the role of hypoxia and hyperoxia and its effect on oxidative stress in fish development, because most studies have been focused on toxicants and pollutants (Chowdhury and Saikia 2020). Moreover, there is little information regarding the role of *il8* in the superoxide production under variable oxygen availability and the current study offers the first insight of the relative expression of *il8* in a developing fish.

CONCLUSION

The tropical gar (*Atractosteus tropicus*) is an excellent piscine model for studying early development of fishes because of its rapid development and metamorphosis which occurs at ~9 dpf. Our study suggests variable patterns of gene expression that are dependent not only on the oxygen availability, but also on developmental time and the time of exposure. Each developmental period exhibited particularities for gene expression:1) fertilization to hatch, the hypoxic groups showed increased expression of almost all genes (excluding *fih-1*); 2) 1-6 dph, the highest expression of all genes tested occur, principally in larvae exposed to both hypoxia and hyperoxia during fertilization to hatch, from 1-6 dph and continuous exposed to hypoxia; and 3) 7-12 dph, the lowest expression occurred for all tested genes compared to the Controls. Our study on the air-breathing tropical gar, with its rapid development, provides an entry into the molecular responses to variable oxygen availability using a series of basic genes for the respiratory, ionoregulatory, stress and immunity. Moreover, this manuscript provides guidelines for future research to increase our understanding of molecular pathways of air-breathing ancient fish responses to hypoxia and hyperoxia.

Acknowledgments: We appreciate the support of the National Council of Science and Technology in Mexico (CONACYT) for the scholarship for the doctoral studies to Martínez-Bautista G., and to the Laboratorio de Acuicultura Tropical from the Universidad Juárez Autónoma de Tabasco for providing the tropical gars for this research.

Declaration of competing interests: The authors declare no competing interests.

Funding: This research was supported by the Project Number CB-2016-01-282765 from the National Council of Science and Technology of Mexico (CONACyT).

REFERENCES

Affonso, E.G., Rantin, F.T. 2005. Respiratory responses of the airbreathing fish *Hoplosternum littorale* to hypoxia and hydrogen sulfide. Comp. Biochem. Physiol. 141C, 275–280.

Aguilera, C., Mendoza, R., Rodríguez, G., Márquez, G. 2002. Morphological description of alligator gar and tropical gar larvae, with an emphasis on growth indicators. Trans. Am. Fish. Soc. 131, 899–909.

Ayson, F.G., Kaneko, T., Hasegawa, S., Hirano, T. 1995. Cortisol stimulates the size and number of mitochondrion-rich cells in the yolksac membrane of embryos and larvae of tilapia (*Oreochromis mossambicus*) in vitro and in vivo. J Exp Zool 272, 419–425.

Barrientos-Villalobos, J., Espinosa de los Monteros, A. 2008. Genetic variation and recent population history of the tropical gar *Atractosteus tropicus* Gill (Pisces: Lepisosteidae). J. Fish Biol. 73, 1919–1936.

Belaõ, T.C., Leite, C.A.C., Florindo, L.H., Kalinin, A.L., Rantin, F.T. 2011. Cardiorespiratory responses to hypoxia in the African catfish, *Clarias gariepinus* (Burchell 1822), an air-breathing fish. J. Comp. Physiol. B 181, 905–916.

Boyle, R. 1670. New pneumatical observations concerning respiration. Physiological Transactions, 5, 2011-2031, 2035-2056.

Brauner, C., Baker, D. 2009. Patterns of acid–base regulation during exposure to hypercarbia in fishes. In: Glass, M., Wood, S. (Eds). Cardio-respiratory control in vertebrates. Springer, Berlin, Heidelberg.

Brauner, C.J., Ballantyne, C.L., Randall, D.J., Val, A.L. 1995. Air breathing in the armoured catfish (*Hoplosternum littorale*) as an adaptation to hypoxic, acidic, and hydrogen sulphide rich waters. Can. J. Zool. 73, 739–744.

Brauner, C.J., Rombough, P.J. 2012. Ontogeny and paleophysiology of the gill: new insights from larval and air-breathing fish. Respir. Physiol. Neurobiol. 184, 293–300.

Brusselmans, K., Bono, F., Maxwell, P., Dor, Y., Dewerchin, M., Collen, D., Herbert, J.M., Carmeliet, P. 2001. Hypoxia-inducible factor-2alpha (HIF-2α) is involved in the apoptotic response to hypoglycemia but not to hypoxia. J Biol Chem 276, 39192–39196.

Buemi, M., Lacquaniti, A., Bolignano, D., Maricchiolo, G., Favaloro, A., Buemi, A., Grasso, G., Donato, V., Giorgianni, G., Genovese, L., Coppolino, G., Sfacteria, A. 2009. The erythropoietin and regenerative medicine: a lesson from fish. Eur J Clin Invest. 39(11), 993-999.

Burggren, W.W. 1988 Cardiac design in lower vertebrates: what can phylogeny reveal about ontogeny? Experientia. 1;44(11-12), 919-930.

Burggren, W.W.; Bagatto, B. 2008. Cardiovascular anatomy and physiology. In: Finn, R.N., Kapoor, B.J., (Eds). Fish Larval Physiology. Science Publishers: Enfield, NH, USA, 2008; pp. 119–161.

Burggren, W.W., Johansen, K., McMahon, B.R. 1986. Respiration in primitive fishes. In: Foreman RE, Gorbman A, Dodd JM, and Olson R. (Eds.). The Biology of Primitive Fishes, New York: Plenum, pp. 217-236.

Burggren, W.W., Bautista, G.M., Coop, S.C., Couturier, G.M., Delgadillo, S.P., García, R.M., González, C.A.A. 2016 Developmental cardiorespiratory physiology of the air-breathing tropical gar, *Atractosteus tropicus*. Am J Physiol Regul Integr Comp Physiol. 311(4), R689-R701.

Burggren, W.W., Mueller, C.A. 2015. Developmental critical windows and sensitive periods as three-dimensional constructs in time and space. Physiol. Biochem. Zool. *88*, 91–102.

Bussing, W.A. 1998. Freshwater Fishes of Costa Rica; Editorial Universidad de Costa Rica: San José, Costa Rica, Volume 46, p. 468.

Charmandari, E., Tsigos, C., Chrousos, G. 2005. Endocrinilogy of the stress response. Ann. Rev. Physiol. 67(1), 259-284.

Chen, J., Guan, L., Zou, M., He, S., Li, D., Chi, W. 2020. Specific cyprinid HIF isoforms contribute to cellular mitochondrial regulation. Sci. Rep.10(1),17246.

Chen, L., He, C., Baoprasertkul, P., Xu, P., Li, P., Serapion, J., Waldbieser, G., Wolters, W., Liu, Z. 2005. Analysis of a catfish gene resembling interleukin-8: cDNA cloning, gene structure, and expression after infection with *Edwardsiella ictaluri*. Dev. Comp. Immunol. 29, 135e42.

Chen, N., Chen, L.P., Zhang, J., Chen, C., Wei, X.L., Gul, Y., Wang, W.M., Wang, H.L. 2012. Molecular characterization and expression analysis of three hypoxia-inducible factor alpha subunits, HIF-1α/2α/3α of the hypoxia-sensitive freshwater species, Chinese sucker. Gene 498, 81–90.

Chi, W., Gan, X.N., Xiao, W.H., Wang, W., He, S.P. 2013. Different evolutionary patterns of hypoxia-inducible factor α (HIF- α) isoforms in the basal branches of Actinopterygii and Sarcopterygii. FEBS Open Bio. 3, 479–483.

Chou, C.F., Tohari, S., Brenner, S., Venkatesh, B. 2004 Erythropoietin gene from a teleost fish, *Fugu rubripes*. Blood, 104, 1498–1503.

Chowdhury, S., Saikia, S.K. 2020. Oxidative stress in fish: a review. J. Sci. Res. 12 (1), 145-160.

Chu, C.Y., Cheng, C.H., Chen, G.D., Chen, Y.C., Hung, C.C., Huang, K.Y., Huang, C.J. 2007. The zebrafish erythropoietin: functional identification and biochemical characterization. FEBS Lett. 581, 4265–4271

Damsgaard C., Baliga, V.B., Bates, E., Burggren, W., McKenzie, D.J., Taylor, E., Wright, P.A. 2020. Evolutionary and cardio-respiratory physiology of air-breathing and amphibious fishes. Acta Physiol. 228(3),e13406.

Davies, R., Moyes, C.D., Wang, Y.S. 2011. Intra- and inter-specific variation in metabolic gene expression in relationship to environmental hypoxia. Comp. Biochem. Physiol. A 159, 25–31.

Davidson, M.K., Lindsey, J.R., Davis, J.K. 1987. Requirements and selection of an animal model. Isr J Med Sci. 23(6), 551-555.

de Jesus, E.G., Hirano, T. 1992. Changes in whole body concentration of cortisol, thyroid hormones, and sex steroid during early development of the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 85, 55–61.

Deane, E.E., Woo, N.Y.S. 2003 Ontogeny of thyroid hormones, cortisol, hsp70 and hsp 90 during silver sea bream larval development. Life Sci. 72, 805–818

Diaz, R.J., Breitburg, D.L. 2009. The Hypoxic Environment. In: Jeffrey G. Richards, Anthony P. Farrell, Colin J. Brauner (Eds.), Fish Physiology, Academic Press, Volume 27, pp. 1-23.

Eddy, F.B.; Handy, R.D. 2012. Ecological and Environmental Physiology of Fishes; Oxford University Press: Oxford, UK; p. 252.

Evans, D.H., Piermarini, P.M., Choe, K.P. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste? Physiol. Rev. 8, 97-177.

Feng, X., Yu, X., Pang, M., Tong, J. 2019. Molecular characterization and expression regulation of the factor-inhibiting HIF-1 (FIH-1) gene under hypoxic stress in bighead carp (*Aristichthys nobilis*). Fish Physiol Biochem. 45(2), 657-665.

Flores-Santin, J.; Burggren, W.W. 2021. Birds as models in biomedical research: Looking beyond the chicken. In Review with the Journal *Animals*.

Frede, S., Stockmann, C., Freitag, P., Fandrey, J. 2006. Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NK.kappaB. Biochem. J. 396, 517-527.

Garduño-Paz, M.V., Méndez Sánchez, J.F., Burggren, W., García Martínez, J.L.A. 2020. Metabolic rate and hypoxia tolerance in *Girardinichthys multiradiatus* (Pisces: Goodeidae), an endemic fish at high altitude in tropical Mexico. Comp Biochem Physiol A Mol Integr Physiol. 239,110576.

Geng, X., Feng, J., Liu, S., Wang, Y., Arias, C., Liu, Z. 2014. Transcriptional regulation of hypoxia inducible factors alpha (HIF-α) and their inhibiting factor (FIH-1) of

channel catfish (*Ictalurus punctatus*) under hypoxia. Comp. Biochem. Physiol. B 169, 38–50.

Graham JB, 1997. Air-Breathing Fishes: Evolution, Diversity and Adaptation. San Diego, CA: Academic Press.

Haase, V.H. 2010. Hypoxic regulation of erythropoiesis and iron metabolism. Am. J. Physiol. Renal. Physiol. 299, F1-F13.

Haase, V.H. 2013. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Rev. 27, 41-53.

Harun, N.O., Zou, J., Zhang, Y.A., Nie, P., Secombes, C.J. 2008. The biological effects of rainbow trout (*Oncorhynchus mykiss*) recombinant interleukin-8. Dev. Comp. Immunol. 32(6), 673e81.

Hill, L.; Renfro, J.; Reynolds, R. 1972. Effects of dissolved oxygen tensions upon the rate of young spotted gar, *Lepisosteus oculatus* (Lepisosteidae). Southwest. Nat. 17, 273–278

Huang, C.Y., Lin, H.C., Lin, C.H. 2015a. Effects of hypoxia on ionic regulation, glycogen utilization and antioxidative ability in the gills and liver of the aquatic air-breathing fish *Trichogaster microlepis*. Comp. Biochem. Physiol. A 179, 25–34.

Huang, C.Y., Lin, H.H., Lin, C.H., Lin, H.C. 2015b. The absence of ionregulatory suppression in the gills of the aquatic air-breathing fish *Trichogaster lalius* during oxygen stress. Comp. Biochem. Physiol. A 179, 7–16.

Iftikar, F.I., Matey, V., Wood, C.M. 2010. The ionoregulatory responses to hypoxia in the freshwater rainbow trout *Oncorhynchus mykiss*. Physiol. Biochem. Zool. 83, 343-355.

Jenny, J.P., Francus, P., Normandeau, A., Lapointe, F., Perga, M.E., Ojala, A:, Schimmelmann, A., Zolitschka, B. 2016. Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. Global Change Biol. 22, 1481-1489.

Jiménez-Martínez, L.D., Álvarez-González, C.A., De la Cruz-Hernández, E., Tovar-Ramírez, D., Galaviz, M.A., Camarillo-Coop, S., Martínez-García, R., Concha-Frías, B., Peña, E. 2019. Partial sequence characterization and ontogenetic expression of genes involved in lipid metabolism in the tropical gar (*Atractosteus tropicus*). Aquac Res 50(1),162–172.

Johansen, K., Burggren, W.W. 1980. Cardiovascular function in lower vertebrates. In: Bourne G (Ed). Hearts and Heart-Like Organs. New York: Academic Press.

Johnson, D.W., Christie, M.R., Moye, J. 2010. Quantifying evolutionary potential of marine fish larvae: heritability, selection, and evolutionary constraints. Evolution. 64(9), 2614-2628.

Jonz MG, Buck LT, Perry SF, Schwerte T, Zaccone G. 2016. Sensing and surviving hypoxia in vertebrates. Ann. N .Y. Acad. Sci. 1365(1):43-58.

Kaelin, W.G., Ratcliffe, P.J. 2008. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol. Cell. 30, 393–402.

Kodama, K., Rahman, M.S., Horiguchi, T., Thomas, P. 2012. Upregulation of hypoxia-inducible factor (HIF)-1a and HIF-2a mRNA levels in dragonet *Callionymus valenciennei* exposed to environmental hypoxia in Tokyo Bay. Mar. Poll. Bull. 64, 1339–1347.

Kopp, R., Köblitz, L., Egg, M., Pelster, B. 2011. Hif signaling and overall gene expression changes during hypoxia and prolonged exercise differ considerably. Physiol. Genomics 43, 506-516

Lando, D., Peet, D.J., Gorman, J.J., Whelan, D.A., Whitelaw, M.L., Bruick, R.K. 2002. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. 16: 1466-1471.

Laurent, P., Perry, S.F. 1991. Environmental effects on fish gill morphology. Physiol. Zool. 64, 4-25.

Lefevre, S., Bayley, M., McKenzie, D.J. 2016. Measuring oxygen uptake in fishes with bimodal respiration. J. Fish Biol. 88, 206–231.

Lefevre, S., Huong do, T.T., Wang, T., Phuong, N.T., Bayley, M. 2011. Hypoxia tolerance and partitioning of bimodal respiration in the striped catfish (*Pangasianodon hypophthalmus*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 158(2), 207–214.

Li, H.L., Gu, X.H., Li, B.J., Chen, X., Lin, H.R., Xia, J.H. 2017. Characterization and functional analysis of hypoxia-inducible factor HIF1α and its inhibitor HIF1αn in tilapia. PloS one, 12(3), e0173478.

Little, C. 2009. The Colonisation of Land: Origins and Adaptations of Terrestrial Animals. Cambridge University Press: Cambridge, UK, p. 290.

Livak, K. J., Schmittgen, T.D. 2001. Analysis of relative gene expression data using realtime quantitative PCR and the 2(−delta delta C(T)) method. Methods, 25, 402–408.

Long, Y., Yan, J., Song, G., Li, X., Li, X., Li, Q., Cui, Z. 2015. Transcriptional events co-regulated by hypoxia and cold stresses in Zebrafish larvae. BMC Genomics 16, 385.

Loong, A.M., Ang, S.F., Wong, W.P., Pönter, H.O., Bock, C., Wittig, R. Briges, C.R., Chew, S.F., Ip, Y.K. 2008. Effects of hypoxia on the energy status and nitrogen metabolism of African lungfish during aestivation in a mucus cocoon. J. Comp. Physiol. B 178, 853–865.

Lopes, J.M., Boijink, C.L., Florindo, L.H., Leite, C.A.C., Kalinin, A.L., Milsom, W.K., Rantin, F.T. 2010. Hypoxic cardiorespiratory reflexes in the facultative air-breathing

fish jeju (*Hoplerythrinus unitaeniatus*): role of branchial O2 chemoreceptors. J. Comp. Physiol. 180B, 797–811.

Lushchak, V.I., Bagnyukova, T.V. 2006. Effects of different environmental oxygen levels on free radical processes in fish. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 144, 283–289.

Lushchak, V.I., Bagnyukova, T.V., Husak, V.V., Luzhna, L.I., Lushchak, O.V., Storey, K.B. 2005. Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. Int. J. Biochem. Cel.l Biol. 37(8), 1670-1680.

Mandic, M., Tzaneva, V., Careau, V., Perry, S.F. 2019. *Hif-1α* paralogs play a role in the hypoxic ventilatory response of larval and adult zebrafish (*Danio rerio*). J. Exp. Biol. 222(Pt 2):jeb195198.

Matey, V., Richards, J.G., Wang, Y., Wood, C M., Rogers, J., Davies, R., Murray, B.W., Chen, X.Q., Du, J., Brauner, C.J. 2008. The effect of hypoxia on gill morphology and ionoregulatory status in the Lake Qinghai scaleless carp, *Gymnocypris przewalskii*. J. Exp. Biol. 211, 1063-1074.

Martínez-Bautista, G.; Peña, E.; Martínez, R.; Camarillo, S.; Burggren, W.; Álvarez, A. 2021. Survival, growth, and development in the early stages of the tropical gar *Atractosteus tropicus*: developmental critical windows and the influence of temperature, salinity, and oxygen availability. *Fishes 6*, 5.

Martínez-Bautista, G., Peña, E., Burggren, W.W., Álvarez, C.A. Physiological responses of the developing tropical gar to changes in the environment - Part 2: Oxygen Availability. *In preparation.*

Martinez-Burguete, T., Peña-Marín, S.E., Garcia-Gasca, A., Llera-Herrera, R., Alvarez-Gonzalez, C.A. 2021. Nutrigenomic marker discovery by de novo transcriptimic sequencing during early development of the tropical gar (*Atractosteus tropicus*). Aquaculture Research. *In Press.* Doi: 1'.1111/are.15228

Miller, R.R., Minckley, W.L., Norris, S.M. 2005. Freshwater Fishes of Mexico; University of Chicago Press: Chicago, IL, USA, p. 490.

Milsom, W.K. 2012. New insights into gill chemoreception: Receptor distribution and roles in water and air breathing fish. Respir. Physiol. Neurobiol. 184, 326–339.

Mora, M., Cabrera-Peña, J., Galeano, G. 1997. Reproducción y alimentación del gaspar *Atractosteus tropicus* (Pisces: Lepisosteidae) en el refugio nacional de vida silvestre Caño Negro, Costa Rica. Rev. Biol. Trop. 45, 861–866.

Mueller, C.A., Eme, J., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y. 2015. Embryonic critical windows: changes in incubation temperature alter survival, hatching phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). J. Comp. Physiol. B 185, 315–331.

Mukaida, N., Harada, A., Matsushima, K. 1988. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine Growth Factor Rev. 9, 9e23.

Nilsson, G.E. 2007. Gill remodeling in fish-a new fashion or an ancient secret? J. Exp. Biol. 210, 2403-2409.

Ogilvie, M., Yu, X., Nicolas-Metral, V., Pulido, S.M., Liu, C., Ruegg, U.T., Noguchi, C.T. 2000. Erythropoietin stimulates proliferation and interferes with differentiation of myoblasts. J Biol Chem 275, 39754–61.

Oliveira, R.D., Lopes, J.M., Sanches, J.R., Kalinin, A.L., Glass, M.L., Rantin, F.T. 2004. Cardiorespiratory response of the facultative airbreathing fish jeju, *Hoplerythrinus unitaeniatus* (Teleostei, Erythrinidae) exposed to graded ambient hypoxia. Comp. Biochem. Physiol. 139A, 479–485.

Paffett-Lugassy, N., Hsia, N., Fraenkel, P.G., Paw, B., Leshinsky, I., Barut, B., Bahary, N., Caro, J., Handin, R., Zon, L.I. 2007. Functional conservation of erythropoietin signaling in zebrafish. Blood, 110, 2718–2726.

Pelster, B., Burggren, W.W. 1996. Disruption of hemoglobin oxygen transport does not impact oxygen- dependent physiological processes in developing embryos of zebra fish (*Danio rerio*). Circ. Res. 79, 358-362.

Pelster, B., Egg, M. 2018. Hypoxia-inducible transcription factors in fish: expression and interconnection with the circadian clock. J. Exp. Biol. 221, jeb163709.

Pelster, B., Wood, C.M., Jung, E., Val, A.L. 2018. Air-breathing behavior, oxygen concentrations, and ROS defense in the swimbladders of two erythrinid fish, the facultative air-breathing jeju, and the non-air-breathing traira during normoxia, hypoxia and hyperoxia. J. Comp. Physiol. B, Biochem. Syst. Environ. Physiol. 188(3), 437–449.

Perry, S.F., Wilson, R.J., Straus, C., Harris, M.B., Remmers, J.E. 2001. Which came first, the lung or the breath? Comp. Biochem. Physiol. A Mol. Integr. Physiol. 129, 37–47.

Pierron, F., Baudrimont, M., Gonzalez, P., Bourdineaud, J.P., Elie, P., Massabuau, J.C 2007. Common pattern of gene expression in response to hypoxia or cadmium in the gills of the European glass eel (*Anguilla anguilla*). Environ. Sci. Technol. 41, 3005–3011.

Porporato, P. E., Dhup, S., Dadhich, R. K., Copetti, T. and Sonveaux, P. 2011. Anticancer targets in the glycolytic metabolism of tumors: a comprehensive review. Front. Pharmacol. 2, 49.

Prabhakar, N.R., Semenza, G.L. 2015. Oxygen Sensing and Homeostasis. Physiology (Bethesda). 30(5), 340-348.

Rabosky, D.L., Santini, F., Eastman J., Smith, S.A., Sidlauskas, B., Chang, J., Alfaro, M.E. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Commun. 4, 1958.

Rahman, M.S., Thomas, P. 2011. Characterization of three IGFBP mRNAs in Atlantic croaker and their regulation during hypoxic stress: potential mechanisms of their upregulation by hypoxia. Am. J. Physiol. Endocrinol. Metab. 301, E637-E648.

Randall, D.J., Burggren, W.W., Farrel, A.P., Haswell, M.S. 1981. The evolution of air breathing in vertebrates. Cambridge University Press, Cambridge, England.

Reyes-Cerpa, S., Maisey, K., Reyes-López, F., Toro-Ascuy, D., Sandino, A.M., Imarai, M. 2012. Fish cytokines and immune response, new advances and contributions to fish biology, Hakan Türker, IntechOpen.

Ribatti, D., Presta, M., Vacca, A., Ria, R., Giuliani, R., Dell'Era, P., Nico, B., Roncali, L., Dammacco, F. 1999. Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood.;93, 2627–2636.

Rimoldi, S., Terova, G., Ceccuzzi, P., Marelli, S., Antonini, M., Saroglia, M. 2012. HIF-1a mRNA levels in Eurasian perch (*Perca fluviatilis*) exposed to acute and chronic hypoxia. Mol. Biol. Rep. 39, 4009–4015.

Rimoldi, S., Terova, G., Zaccone, G., Parker, T., Kuciel, M., Dabrowski, K. 2016. The effect of hypoxia and hyperoxia on growth and expression of hypoxia-related genes and proteins in spotted gar *Lepisosteus oculatus* larvae and juveniles. J. Exp. Zool. B Mol. Dev. Evol. 326(4), 250-267.

Rombough, P., Drader, H. 2009. Hemoglobin enhances oxygen uptake in larval zebrafish (*Danio rerio*) but only under conditions of extreme hypoxia. J. Exp. Biol. 212, 778-784.

Rudneva, I.I. 2014. Biomarkers for Stress in Fish Embryos and Larvae. CRS Press: Boca Ratón, FL, USA. p. 206.

Rudneva, I.I., Skuratovskaya, E.N., Kuzminova, N.S., Kovyrshina, T.B. 2010. Age composition and antioxidant enzyme activities in blood of Black Sea teleosts. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 151(2), 229–239.

Rytkönen, K.T., Williams, T.A., Renshaw, G.M., Primmer, C.R., Nikinmaa, M. 2011. Molecular Evolution of the Metazoan PHD–HIF Oxygen-Sensing System, Mol. Biol. Evol. 28(6), 1913–1926.

Rytkönen, K.T., Akbarzadeh, A., Miandare, H.K., Kamei, H., Duan, C., Leder, E.H., Williams, T.A., Nikinmaa, M. 2013. Subfunctionalization of cyprinid hypoxia-inducible factors for roles in development and oxygen sensing. Evolution 67, 873-882.

Santhakumar, K., Judson, E.C., Elks, P.M., McKee, S., Elworthy, S., van Rooijen, E., Walmsley, S.S., Renshaw, S., Cross, S.S., van Eeden, F.J.M. 2012. A zebrafish model to study and therapeutically manipulate hypoxia signaling in tumorigenesis. Cancer Res. 72, 4017-4027.

Schofield, C.J., Ratcliffe, P.J. 2004. Oxygen sensing by HIF hydroxylases. Nat Rev Mol. Cell. Biol. 5(5), 343-354.

Semenza, G.L. 1999. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol. 15, 551–578.

Semenza, G.L. 2000. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 88(4), 1474-80.

Semenza, G.L. 2010. Oxygen homeostasis. WIREs Mechanisms of Disiese 2(3), 336-361.

Shah, Y.M., Xie, L. 2014. Hypoxia-inducible factors link iron homeostasis and erythropoiesis. Gastroenterology 146, 630-642.

Shartau, R.B., Brauner, C.J. 2014. Acid–base and ion balance in fishes with bimodal respiration. J. Fish Biol. 84, 682–704.

Shen, R.J., Jiang, X.Y., Pu, J.W., Zou, S.M. 2010. Hif-1a and -2a genes in a hypoxia-sensitive teleost species *Megalobrama amblycephala*: cDNA cloning, expression and different responses to hypoxia. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 157, 273-280.

Smatresk, N.J., Cameron, J.N. 1982. Respiration and acid-base physiology of the spotted far, a bimodal breather: III. Response to a transfer from fresh water to 50% sea water, and control of Ventilation. J. Exp. Biol. 96, 295-306.

Sollid, J., Rissanen, E., Tranberg, H.K., Thorstensen, T., Vuori, K.A.M., Nikinmaa, M., Nilsson, G.E. 2006. Hif-1 and iNOS levels in crucian carp gills during hypoxia-induced transformation. J. Comp. Physiol. B 176, 359-369.

Stroud, R.K., Bouck, G.R., Nebeker, A.V. 1975. Pathology of acute chronic exposure of salmonid fishes to supersaturated water. In: Adams WA.(Ed). Chemistry and Physics of Aquous Gas Solutions. The Electrochemical Society, Princeton, NJ, USA.

Sun, J.S., Zhao, L., Sun, L. 2008. Interleukin-8 of *Cynoglossus semilaevis* is a chemoattractant with immunoregulatory property. Fish Shellfish Immunol. 30, 1362e7.

Tan, T., Yu, R.M.K., Wu, R.S.S., Kong, R.Y.C. 2017. Overexpression and knockdown of hypoxia-inducible factor 1 disrupt the expression of steroidogenic enzyme genes and early embryonic development in zebrafish. Gene Regul, Syst. Biol. 11, 1177625017713193.

Terova, G., Rimoldi, S., Corà, S., Bernardini, G., Gornati, R., Saroglia, M. 2008. Acute and chronic hypoxia affects HIF-1a levels in sea bass (*Dicentrarchus labrax*). Aquaculture 279, 150–159.

Thomas, P., Rahman, M. 2009. Biomarkers of hypoxia exposure and reproductive function in Atlantic croaker: a review with some preliminary findings from the northern Gulf of Mexico hypoxic zone. J. Exp. Mar. Biol. Ecol. 381, Suppl. S38-S50.

Timmons, M.B., Ebeling, J.M. 2010. Recirculating Aquaculture. Cayuga Aqua Ventures. Ithaca, NY, USA, pp. 976.

Uchida, T., Rossignol, F., Matthay, M.A., Mounier, R., Couette, S., Clottes, E., Clerici, C. 2004. Prolonged hypoxia differentially regulates hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha expression in lung epithelial cells: implication of natural antisense HIF-1alpha. J. Biol. Chem. 279(15), 14871-8.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., Rozen, S. G. 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40(15), 1–12.

Val, A.L., Almeida-Val, V.M.F. 1995. Fishes of the Amazon and their environment. Springer, Berlin.

Vizzini, A., Vazzana, M., Cammarata, M., Parrinello, N., 2007. Peritoneal cavity phagocytes from the teleost sea bass express a glucocorticoid receptor (cloned and sequenced) involved in genomic modulation of the in vitro chemiluminescence response to zymosan. Gen. Comp. Endocrinol. 150, 114–123.

Vuori, K.A.M., Soitamo, A., Vuorinen, P.J., Nikinmaa, M. 2004. Baltic salmon (*Salmo salar*) yolk-sac fry mortality is associated with disturbances in the function of hypoxia-inducible transcription factor (Hif-1) and consecutive gene expression. Aquat. Toxicol. 68, 301-313.

Weitkamo, D.E., Katz, M. 1980. A review of dissolved gas supersaturation literature. Trans Am Fish Soc, 109, 659-702.

Whitehouse, L.M., Manzon, R.G. 2019. Hypoxia alters the expression of hif-1a mRNA and downstream HIF-1 response genes in embryonic and larval lake whitefish (*Coregonus clupeaformis*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 230,81-90.

Wood, C.M., Kajimura, M., Sloman, K.A., Scott, G.R., Walsh, P.J., Almeida-Val, V. M.F., Val, A.L. 2007. Rapid regulation of Na+ fluxes and ammonia excretion in response to acute environmental hypoxia in the Amazonian oscar, *Astronotus ocellatus*. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R2048.

Wu, H., Lee, S.H., Gao, J., Liu, X., Iruela-Arispe, M.L. 1999. Inactivation of erythropoietin leads to defects in cardiac morphogenesis. Development 126, 3597–605.

Zinkernagel, A.S., Johnson, R.S., Nizet, V. 2007. Hypoxia indicible factor (HIF) function in innate immunity and infection. J. Mol. Med. 85, 1339-1346.

FIGURE CAPTIONS

Figure 1. A schematic of the experimental design for assessing gene expression in the air-breathing tropical gar *Atractosteus tropicus* in response to hypoxia and hyperoxia. Three developmental periods were employed: Period 1= fertilization to hatch; Period 2= 1-6 days post hatch (dph) and Period 3 = 7-12 dph.

Figure 2. Relative expression levels of a) *hif-1α*, b) *fih-1*, c) *nhe1*, d) *epo*, e) *gr* and f) *il8*, all normalized to the expression of *ef1*, at the end of each developmental period in the early development of *Atractosteus tropicus*. Lowercase letters indicate differences between experimental groups within each developmental period ($P<0.001$). Capital letters show differences across development for each experimental group ($P<0.001$).

Figure 3. Heat map summarizing gene expression in the developing tropical gar in response to hypoxia and hyperoxia administered according to the critical windows design depicted in Figure 1. Black, green, and red boxes indicate control, upregulated and downregulated gene expression, respectively.

Table 1. Oligonucleotides for the hypoxia/hyperoxia response of *Atractosteus tropicus*.

Oligonucleotide	Forward (5'-3')	Reverse (3'-5')	Fragment size (pb)	Accession number	Reference
Elongation factor (*ef1*)	CCTGCAGGACGTCTACAAGATCG	GACCTCAGTGGTCACGTTGGA	121	KT351350	Jiménez-Martínez *et al.*, (2019)
Hypoxia inducible factor 1 alfa (*hif-1α*)	GAGCAGAACTCGGAGCGTAG	TTTCCATGTGGCGGACTTGA	93	MW810042	Current study
Hypoxia inducible factor 1 inhibitor (*fih-1*)	CAACGAGGAACCAGTTGTGC	CAGGAACTTGTGGGTTTGCG	133	MW810043	Current study
Na^+/H^+ exchanger 1 (*nhe1*)	AGACGTACAGCTCGAAGTCG	TTCATGACGATGCCCTGGTC	142	MW810044	Current study
Erithropoyetin (e*po*)	CTTCAACGAGTGGGAGAGCA	CCTGGGTCAGTAGCCTCAAC	77	MW810045	Current study
Glucocorticoid receptor (*gr*)	GGCTGATCCTAGCACCTCTTC	TTCATCTGAACACACCAGGCA	82	MW810046	Current study
Interleukin 8 (*il8*)	ATATTCACTGGTGGGCGGAG	GTGCGGCCTGAGATTGTTTT	369	MW965456	Current study

Graphical abstract

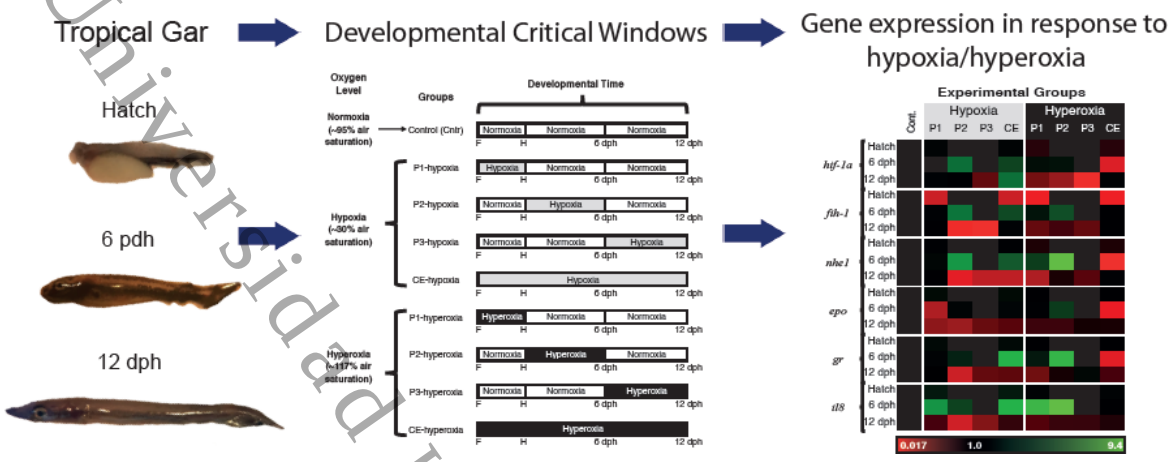


Figure 1

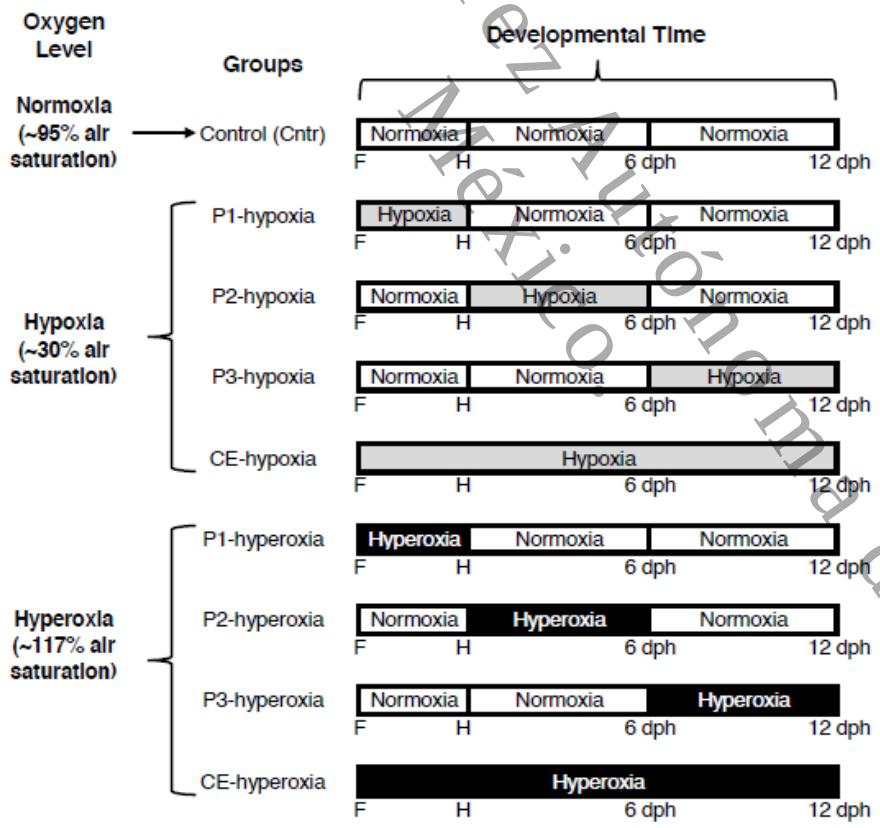


Figure 2

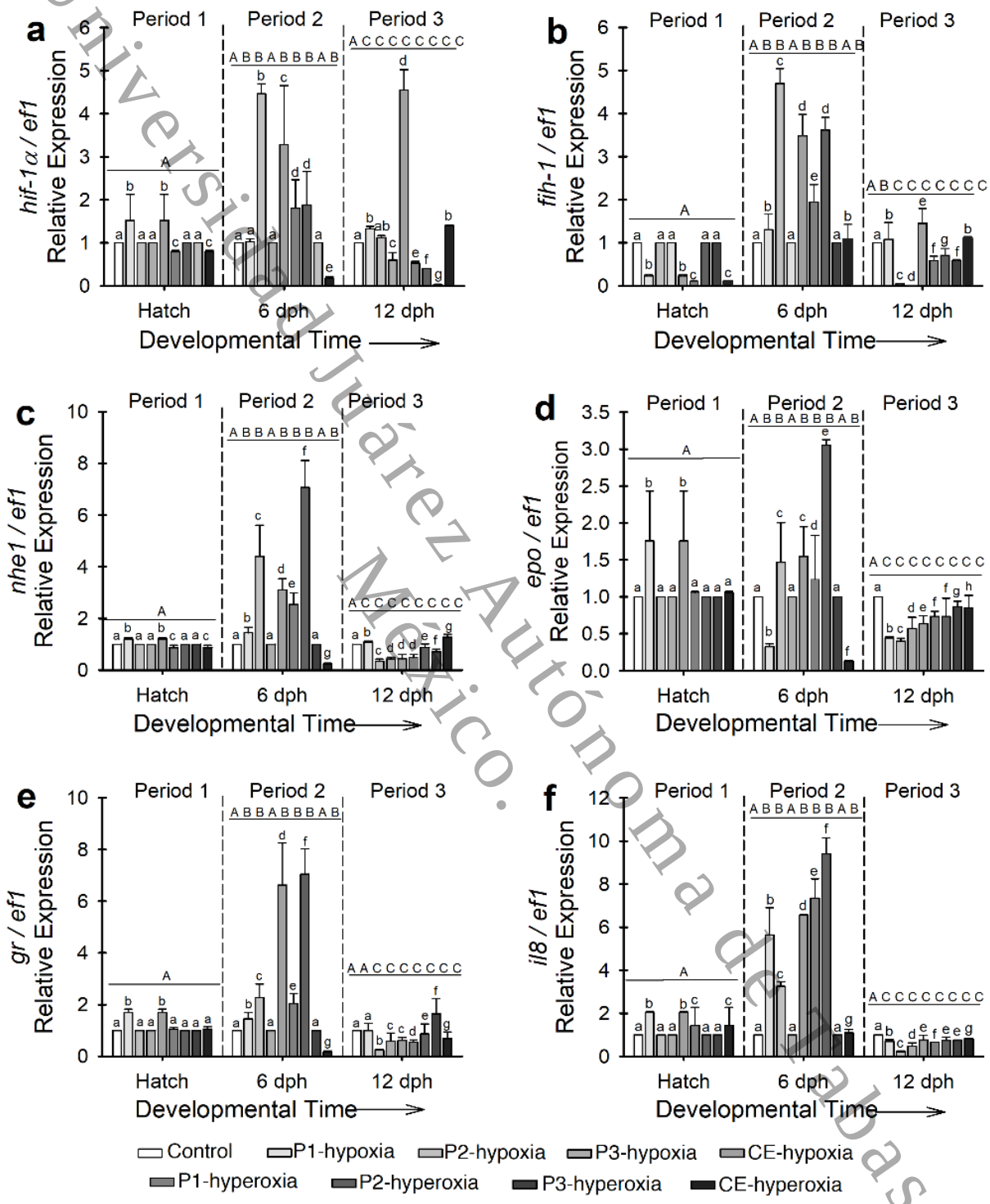
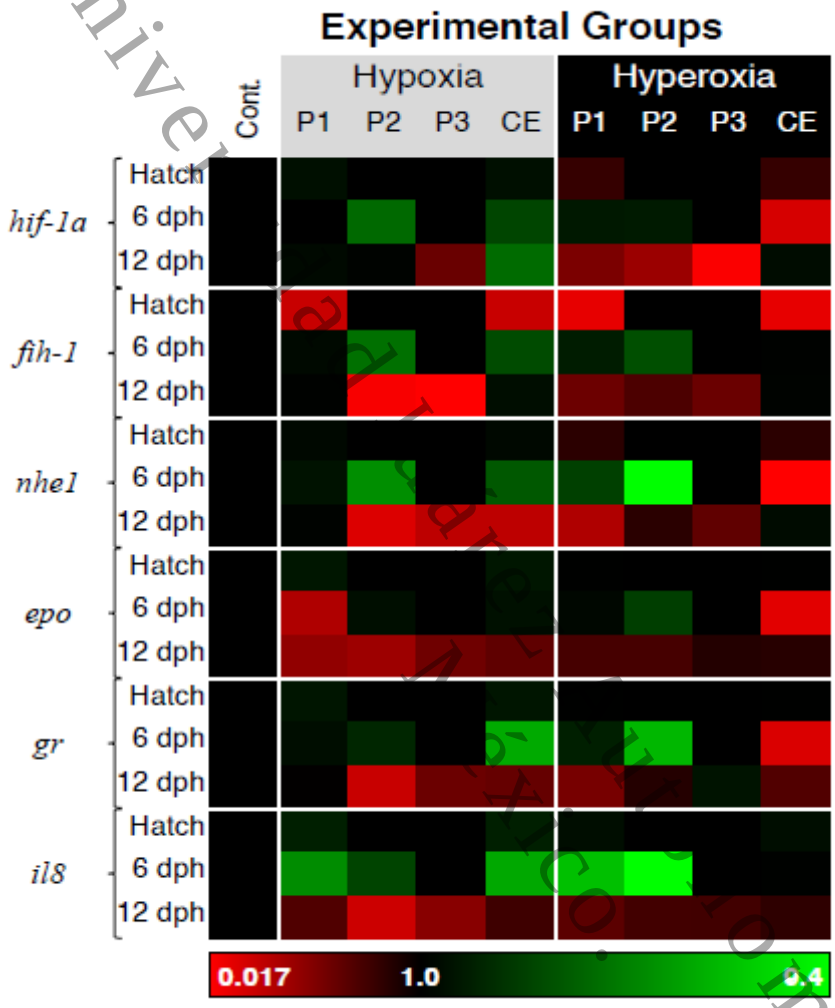


Figure 3



Capítulo 3

Dinámica Cardio-Respiratoria en el Desarrollo Temprano del Pejelagarto *Atractosteus tropicus* a los Cambios en Disponibilidad de Oxígeno, Salinidad y Temperatura en el Ambiente

RESUMEN

La transición a la respiración aérea en larvas de peces es un fenómeno muy poco conocido. En el presente Capítulo se evaluó la fisiología cardio-respiratoria de las etapas tempranas del pejelagarto en función de la actividad forzada, disponibilidad de oxígeno, la salinidad y la temperatura. El estudio se llevó a cabo durante tres periodos del desarrollo temprano: 1) fertilización a eclosión, 2) 1-6 días post eclosión (dpe) y 3) 7-12 dpe. El ritmo cardiaco (f_H), la ventilación branquial (f_G) y la ventilación aérea (f_{AB}) de los peces incrementaron con respecto al tiempo de desarrollo y se vieron afectados por los cuatro factores, el tiempo de desarrollo y el tiempo de exposición. El inicio de las características fisiológicas se modificó por la exposición a cambios en la temperatura y disponibilidad de oxígeno para f_H y f_G durante la incubación, mientras que la salinidad solo modificó el inicio de f_{AB}. El presente Capítulo contribuye al conocimiento sobre las alteraciones cardio-respiratorias y del desarrollo en las etapas tempranas de los peces con respiración bimodal.

Palabras clave: *respiración aérea, tasas metabólicas, ventanas críticas, heterokairy, Atractosteus tropicus*

INTRODUCCIÓN

Las etapas tempranas de desarrollo en los peces son las más vulnerables a las presiones selectivas impuestas por el ambiente (Burggren y Baggato, 2014; Rudneva, 2014). Dentro del desarrollo temprano existen periodos sensibles en los que el fenotipo puede verse afectado por una gran variedad de fatores (Burggren y Mueller, 2015; Burggren y Reyna, 2011; Mueller *et al.*, 2015). De igual importancia es el entendimiento del cambio en las trayectorias de desarrollo de los peces en función del ambiente, lo cual, cuando sucede dentro de poblaciones de una misma especie, o individuos de una misma población, se denomina heterokairy (Spicer y Burggren, 2003). Existen pocos estudios que relacionen ambas características, particularmente desde un enfoque multifactorial (*e.g.*, diferentes dosis de un estresor en diferentes tiempos de desarrollo), el cual puede proveer un mayor entendimiento de las interacciones de los individuos con su ambiente (Burggren y Mueller, 2015; Mueller 2015).

El caso de los peces es interesante ya que, como otros organismos acuáticos, tienen una limitante en su ambiente, ya que pueden experimentar niveles de oxígeno menores que los animales terrestres (Eddy y Handy, 2012; Jonz *et al*., 2016). Para compensar esta limitante los peces han desarrollado una serie de adaptaciones, incluyendo modificaciones en las branquias o la piel y la capacidad de respirar oxígeno atmoférico (Damsgaard *et al*., 2020; Eddy y Handy, 2012; Garduño *et al*., 2020; Jonz *et al*., 2016). La capacidad de respirar oxígeno atmosférico ha surgido en múltiples ocasiones en ambos Holosteii y Teleosteii, y se han modificado diferentes estructuras como la vejiga gaseosa, la piel, el estómago, los intestinos o como cámaras adaptadas en la cabeza (*e.g.*, Affonso y Rantin, 2005; Belaõ *et al*., 2011; Graham, 1997; Johnson *et al*., 2010; Lefevre *et al*., 2011; Lefevre *et al*., 2016; Little 2009; Lopes *et al*., 2010; Milsom, 2012; Perry *et al*., 2001, Randall *et al*., 1981; Shartau y Brauner, 2014).

La mayoría de los estudios realizados en peces con respiración aérea se han enfocado en etapas adultas (Little, 2009; Milsom, 2012; Perry *et al*., 2001; Randall, 1994; Randall *et al*., 1981; Randall e Ip, 2006; Shartau y Brauner, 2014). De acuerdo con Lefevré et al. (2014) existe una necesidad urgente de estudiar la fisiología (especialmente respiratoria) de los peces que respiran aire, en particular de aquellos empleados en proyectos de

acuacultura. En contraste, muy pocos estudios se han llevado a cabo en etapas tempranas. Algunas investigaciones describen las respuestas metabólicas en función del ambiente, incluyendo el efecto de la disponibilidad de oxígeno, del desarrollo, y los costos de este proceso (Blank, 2009; Liem, 1981; Méndez-Sánchez *et al*., 2014; Mueller *et al*., 2011a-b).

En el caso particular de los Lepisosteidos existen diversos estudios en jóvenes y adultos que describen su morfología y fisiología (Boudreux *et al*., 2007; Burleson *et al*., 1998; De Roth, 1973; Hill *et al*., 1973; Landolt y Hill, 1975; McCormack, 1967; Potter, 1927; Rahn *et al*., 1971; Renfro, 1967; Saksena, 1967; Smatresk *et al*., 1986; Smatresk y Cameron, 1982; Schwarz y Allen, 2014; Wiston, 1967; Zaccone *et al*., 2011; Zawodny, 1975). Sin embargo, el único estudio que describe la fisiología cardio-respiratoriaen etapas tempranas deun Lepisosteido (*Atractosteus* tropicus) pertenece a Burggren *et al.* (2016), donde se describe que el ritmo cardiaco (f_H) y la ventilación branquial (f_G) incrementan rápidamente durante los primeros 5 días después de la eclosión; el f_H permanece constante hasta los 30 días, mientras que la f_G disminuye con la edad y la respiración aérea (f_{AB}) incrementa significativamente a los 15 días post eclosión. El f_H y la f_G incrementan después de actividad forzada mientras que se mantiene constante f_{AB}. Del mismo modo, el f_H y la f_G incrementan conforme incrementa la temperatura del ambiente en todas las edades menores a los 30 días posteclosión. Además, el f_H y la f_G se ven afectadas por la baja disponibilidad de oxígeno. Por un lado, los peces de menos de 5 días muestran un incremento de f_G al incrementar la hipoxia. Por otro lado, peces de15 y 30 días muestran una disminución de la f_G y un incremento de f_{AB} al disminuir el nivel de oxígeno. El f_H también incrementa al exponerse a los peces a estrés hipóxico.

Con relación a estudios sobre ventanas críticas en peces y su relación con su fisiología existen muy pocos estudios. Por ejemplo, Eme et al. (2015) evaluaron el f_H y el consumo de oxígeno (MO_2) en *Coregonus clupeaformis* durante tres periodos sensibles en la incubación y en función de cambios en la temperatura. La disminución de la temperatura incrementa el tiempo de cada ventana, mientras que temperaturas más altas lo reducen. Del mismo modo, los cambios en la temperatura de incubación modificaron el f_H y MO_2 se observaron incrementos y decrementos en esta característica. De esta manera, los autores sugieren que el desarrollo embrionario entre la fertilización y la organogénesis

representa una ventana crítica de plasticidad fenotípica en embriones y peces recién eclosionados. Un ejemplo más sugiere que los cambios de temperatura en el desarrollo temprano de los peces pueden alterar el tiempo para la eclosión y la masa seca de los peces al eclosionar (Mueller *et al*., 2015). Así mismo, la tasa de conversión del vitelo disminuye y el costo de desarrollo incrementa al incrementar la temperatura. El periodo de organogénesis a los primeros movimientos de las aletas se ve afectado por la relación entre el desarrollo y la temperatura, lo cual sugiere que este intervalo de tiempo puede representar una ventana crítica donde sucede el mayor impacto en los procesos energéticos.

Con base en lo anterior, el objetivo de este Capítulo es evaluar el f_H, la f_G y el f_{AB} y su tiempo de inicio en función de la disponibilidad de oxígeno, la salinidad y la temperatura, durante tres periodos importantes en el desarrollo temprano del pejelagarto *Atractosteus ttropicus*: 1) fertilización a eclosión, 2) 1-6 días post eclosión (dep) y 3) 7-12 dpe. Los resultados de este Capítulo serán de utilidad para entender la interacción que existe entre la respuesta cardio-respiratoria de los peces ante diferentes condiciones ambientales, el tiempo de desarrollo y el tiempo de exposición a cada estresor.

MATERIALES Y MÉTODOS

Declaración ética

Los organismos se utilizaron y manipularon de acuerdo con lo establecido por la NOM-062-ZOO-1999 de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, que estipula las especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio

Adquisición y manutención de organismos

Los embriones y las larvas se obtuvieron de tres desoves inducidos artificialmente en mayo de 2017, mayo de 2018 y mayo de 2019 en el Laboratorio de Acuicultura Tropical de la Universidad Juárez Autónoma de Tabasco. Cada hembra se anestesió con 200 mg L-1 de metanosulfonato de tricaína MS-222® (Agent Chemical Laboratories) y posteriormente se inyectó con 35 de un análogo del liberador de hormona leutinizante (LNRHa). Cada hembra se introdujo con cinco machos en tanques de reproducción de 2000 L con sustrato artificial que permitió la adhesión de los huevos.

Se realizaron tres experimentos para determinar las ventanas críticas de desarrollo y el tiempo de inicio del ritmo cardiaco, la ventilación branquial y la ventilación aérea. El sexo de los peces no pudo determinarse, por lo que los datos se reportan asumiendo una mezcla de machos y hembras. Inmediatamente después de la fertilización los huevos se transfirieron a los tanques experimentales para su incubación y desarrollo. Para cada experimento, los huevos se creó un grupo control y ocho tratamientos experimentales. Cada experimento se realizó por triplicado y se utilizaron 50 huevos fertilizados por réplica (1350 huevos fertilizados por experimento).

Los tanques experimentales contuvieron agua libre de cloro con pH de 8.0 y a temperatura ambiente de 27°C-28°C (con excepción del primer experimento). Se mantuvieron en normoxia a 95% de saturación de oxígeno (excepto en el segundo experimento), en salinidad de 0.0 ppt (excepto el tercer experimento) y con un fotoperiodo de 12 horas luz y 12 horas oscuridad. Después de la absorción del vitelo se alimentó a saciedad a las larvas de pejelagarto con nauplios de *Artemia* sp. cada cuatro horas desde las 8:00 horas y hasta las 20:00 horas. Se realizaron recambios de agua del 50% en cada

tanque (con los requerimientos de cada experimento) cada dos días y las heces y nauplios muertos se removieron por sifoneo una hora después de cada alimentación.

Periodos de desarrollo y diseño experimental

Se examinaron tres periodos en el desarrollo del pejelagarto, basados en lo descrito por Aguilera et al. (2002), los cuales se identificaron como: 1) tiempo de la fertilización a la eclosión (60-70 horas), 2) completa absorción del vitelo (~del día 1 post eclosión (dpe) a los ~6 dpe) y 3) la etapa prejuvenil (~7-12 dpe). Los embriones y larvas en los tres periodos de desarrollo se expusieron a condiciones control, a seis tratamientos en condiciones experimentales descritas más adelante (y posteriormente regresados a condiciones control) y a dos tratamientos de exposición continua para cada experimento. Los detalles del protocolo se muestran en la Figura 1.

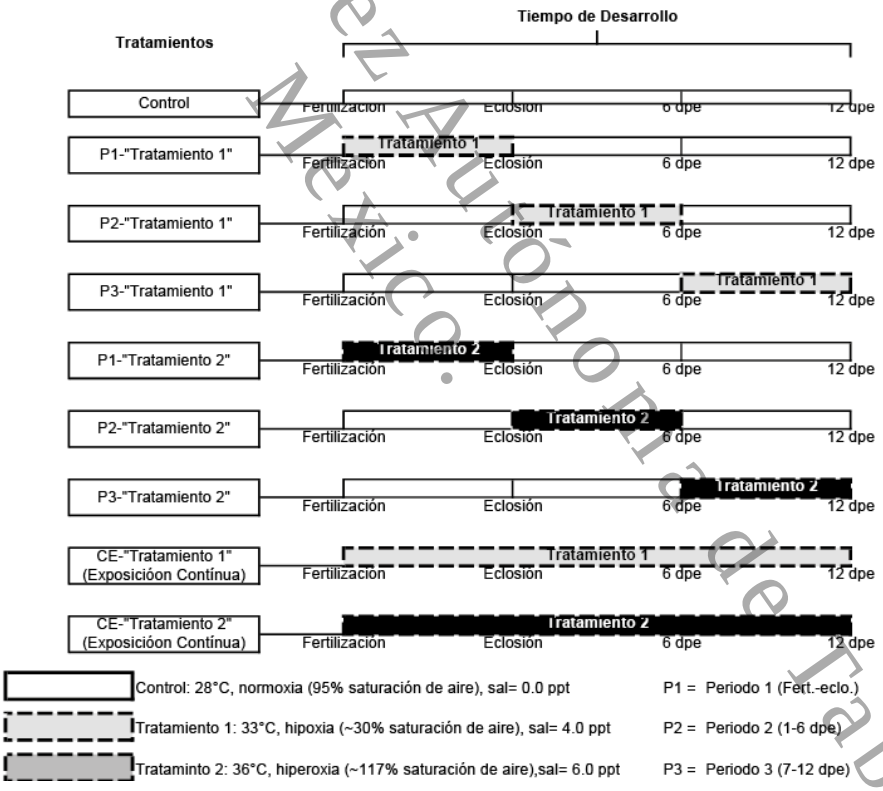


Figura 1. Diseño experimental empleado para evaluar las tasas metabólicas en el desarrollo temprano del pejelagarto *Atractosteus tropicus* durante tres periodos importantes: 1) fertilización a eclosión, 2) 1-6 dpe, 3) 7-12 dpe. Las cajas blancas indican condiciones Control (28°C, ~95% saturación de aire, no salinidad) para cada experimento. Las cajas grises denotan condiciones del tratamiento 1 (33°C, ~30% saturación de oxígeno o salinidad de 4.0 ppt). Las cajas negras indican condiciones del tratamiento 2 (36°C, ~117% saturación de oxígeno, salinidad de 6.00ppt).

El primer experimento consistió en un desafío de temperatura exponiendo a los embriones a 33°C (Tratamiento 1) y 36°C (Tratamiento 2), la cual se encuentra cerca del límite superior de tolerancia (38°C con base en Burggren et al. 2016; Figura 1). En el segundo experimento se expuso a los peces a hipoxia (~30% saturación de aire; Tratamiento 1) e hiperoxia (~117% saturación de aire; Tratamiento 2;) como se observa en la Figura 1. Para el tercer experimento se expuso a los individuos a salinidades de 4.0 ppt (Tratamiento 1) y 6.0 ppt (Tratamiento 2) como se aprecia en la Figura 1. Los cambios entre las condiciones de los tratamientos ocurrieron gradualmente en periodos cortos de ~3 horas.

Preparación de tratamientos

Temperatura. La temperatura se reguló con calentadores de inmersión conectados a termostatos Jhonson Controls A419. La temperatura de los tanques experimentales se mantuvo constante a 33±0.5°C o 36±0.5°C. Para asegurar la dispersión del calor, los calentadores se colocaron sobre las piedras de aireación. Los embriones y larvas se expusieron a condiciones control (Grupo Control), a condiciones agudas o crónicas durante el primer (P1-33°C, P1-36°C respectivamente), segundo (P2-33°C, P2-36°C) y tercer (P3-33°C, P3-36°C) periodo de desarrollo y con exposición continua a ambas temperaturas (CE-33°C y CE-36°C) como se muestra en la Figura 1.

Hipoxia e hiperoxia. La hipoxia (~30% saturación de aire, Tratamiento 1) se generó agregando nitrógeno directamente en los tanques y se dispersó con una piedra de aireación. La hiperoxia se fijó a ~117% saturación de aire (Tratamiento 2) y se produjo con una torre de agua contra corriente con una piedra de aireación que liberó oxígeno en el fondo de la torre. El agua se envió de la torre a los tanques mediante una bomba y regresó a la torre a través de un tubo de ventilación lo suficientemente alto en el centro de los tanques para mantener un flujo y una saturación de oxígeno constante dentro de los tanques. La torre promovió una alta concentración de oxígeno en el agua simulando la función de un sistema de aireación tipo tubo-U excluyendo el mecanismo de reciclaje de gas (Timmons y Ebeling, 2010). Ambas saturaciones de oxígeno se midieron

constantemente con dos sondas multiparamétricas YSI Pro2030. Se utilizó un grupo control a lo largo del experimento y los embriones y larvas se expusieron a ambas condiciones durante el primer (P1-hipoxia, P1-hiperoxia), segundo (P2-hipoxia, P2-hiperoxia) y tercer (P3-hipoxia, P3-hiperoxia) periodo de desarrollo y dos tratamientos de exposición continua a ambas condiciones (CE-hipoxia, CE-hiperoxia) como se muestra en la Figura 1.

Salinidad. La salinidad en el agua del grupo control se midió en 0.0 ppt. Los tratamientos experimentales se fijaron en salinidades de 4.0 ppt (Tratamiento 1) y 6.0 ppt (Tratamiento 2), utilizando cristales de sal de mar industrial (Grupo Industrial Roche) que se disolvieron mecánicamente en el agua de los tanques. Las concentraciones se validaron con una sonda multiparamétrica YSI Pro2030. Los tratamientos para este experimento se definieron como un grupo control, seis tratamientos correspondientes a la exposición a ambas condiciones durante cada periodo de desarrollo (P1-4.0, P1-6-0, P2-4.0, P2-6.0, P3-4.0, y P3-6.0) y dos tratamientos expuestos durante todo el experimento (CE-4.0, CE-6.0) como se muestra en la Figura 1.

Variables fisiológicas

En el presente estudio se evaluó el ritmo cardiaco, la tasa de ventilación branquial y la tasa de respiración aérea en función de la actividad forzada, temperatura, la saturación de oxígeno y la salinidad al final de cada periodo de desarrollo. Se utilizaron 20 organismos por tratamiento. Cualquier organismo que mostró actividad constante fue reemplazado.

El ritmo cardiaco, la ventilación branquial y la ventilación aérea se calcularon en rutina y después de un minuto de actividad forzada. La actividad forzada se obtuvo a través de movimientos mecánicos ligeros por ~1 minuto utilizando un tubo de vidrio.

Ritmo cardiaco. El ritmo cardiaco (f_H) se determinó visualmente bajo un microscopio estereoscópico (Stemi 305 Lab)- Los peces se colocaron sobre cámaras plásticas transparentes armadas sobre un vidrio de reloj colocado en una base transparente (para permitir la iluminación) y con un espejo inclinado debajo del vidrio para observar la parte ventral de los peces. El f_H se calculó como el número de latidos del corazón (observables

a través de la piel) de cada pez en dos intervalos consecutivos de 15 segundos, promediando los latidos y multiplicando por cuatro para ajustar el f_H a latidos por minuto.

Ventilación branquial. La tasa de ventilación branquial (f_G) se determinó visualmente a través de los movimientos del opérculo de los peces utilizando un microscopio estereoscópico (Stemi 305 Lab). Las larvas se colocaron individualmente en cámaras plásticas transparentes de tamaño apropiado (1-6 cm de diámetro, 2 cm de profundidad) de acuerdo con el tamaño de los peces en cada etapa. La f_G se calculó contando el número de movimientos operculares (latidos) en dos intervalos consecutivos de 15 segundos, promediados y multiplicados por cuatro para ajustar a latidos por minuto.

Ventilación aérea. Para calcular la tasa de ventilación aérea (f_{AB}) se colocó a las larvas en las cámaras plásticas descritas en la sección anterior. Las larvas y mantuvieron en las cajas durante una hora, tiempo que fueron grabadas en video para su posterior análisis. Para calcular la f_{AB} se registraron las respiraciones aéreas (caracterizadas por la liberación de una burbuja de aire por el opérculo) en dos periodos consecutivos de 15 minutos, promediados y multiplicados por cuatro para obtener la f_{AB} en respiraciones por hora.

Eventos fisiológicos en el desarrollo

En el presente estudio se cuantificó el tiempo de ocurrencia de tres eventos fisiológicos relevantes en el desarrollo del pejelagarto: el inicio de los latidos del corazón, el inicio de la ventilación branquial y el inicio de la ventilación aérea. Dichos marcadores se obtuvieron de grupo de 30 individuos de cada tratamiento utilizados para registrar el tiempo de ocurrencia de los eventos morfológicos descritos en el Capítulo 1. Se consideró el tiempo de ocurrencia para cada evento cuando el 100% de los individuos presentaron las características fisiológicas correspondientes.

Coeficiente de sensibilidad térmica

El coeficiente de sensibilidad térmica Q_{10} se calculó para los eventos fisiológicos, así como para f_H, f_G y f_{AB} entre el grupo control del experimento de temperatura (28°C) y los tratamientos de exposición continua a 33°C y 36°C con la fórmula $Q_{10}=(R_2/R_1)^{(10/T_2-T_1)}$,

donde Q_{10}, es el factor por el cual el tiempo de un proceso dado incrementa con el aumento de temperatura; R_1 es el valor del proceso dado a la temperatura 1 (donde $T_1 < T_2$), R_2 es el valor del proceso dado a la temperatura 2 (donde $T_2 > T_1$); T_1 es la temperatura en que R_1 es medida, y T_2 es la temperatura en que se mide R_2.

Análisis estadísticos

Se realizaron pruebas de normalidad (Kolmogorov-Smirnov) y homoscedasticidad (Levene) al resto de los datos generados en este trabajo. Los datos que no cumplieron con dichos postulados se normalizaron previamente a ser analizados. Las variables fisiológicas se analizaron mediante un análisis de varianza de tres vías (factores tiempo, tratamientos y actividad). Los datos de los eventos fisiológicos y de sensibilidad térmica se analizaron con un análisis de varianza de una vía. A todos los análisis se les realizó una prueba de comparación múltiple de Holm-Sidak para denotar diferencias significativas. Todos los análisis se realizaron con un nivel de significancia del 95% en el programa SigmaPlot versión 11.0.

RESULTADOS

Características fisiológicas

Se observaron diferencias significativas en el ritmo cardiaco (f_H), la ventilación branquial (f_G) y la ventilación aérea (f_{AB}) de los peces en función de los tres factores ambientales y la actividad forzada. Así mismo, los valores de cada tasa mostraron diferencias con respecto al tiempo de desarrollo y el tiempo de exposición. Los detalles se describen a continuación.

Ritmo cardiaco (f_H). El f_H de los peces en el experimento de temperatura mostró diferencias significativas al evaluarlo en rutina y después de actividad forzada a lo largo del experimento (*P>0.001*), excepto aquellos del grupo experimental P3-36°C al momento de la eclosión (Figura 2a). En este periodo los individuos de los grupos CE-33°Cy CE-36°C mostraron mayor f_H (~160-180 latidos min^{-1} en rutina y ~180-200 latidos min^{-1} después de actividad forzada) con respecto al Control y el resto de los tratamientos incubados a 28°C, mientras que los tratamientos P1-33°C y P1-36°C exhibieron menor f_H (~120-130 latidos min-^{1} en rutina y ~140 latidos min^{-1} después de actividad forzada; *P>0.001*; Figura 2a). Durante 1-6 dpe los peces del grupo P1-33°C registraron el mayor f_H en rutina (~160 latidos min^{-1}) comparado con el control y el resto de los grupos experimentales (~150 latidos min^{-1}; *P>0.001*; Figura 2a). En el periodo 7-12 dpe no se observaron diferencias significativas en f_H (*P<0.05,* Figura 2a).

En el experimento de disponibilidad de oxígeno los peces del grupo P1-hipoxia mostraron diferencias en el f_H con respecto al control durante los periodos fertilización-eclosión y 1-6 dph (~120 y ~125 latidos min^{-1} respectivamente (*P>0.001*; Figura 2b). Los peces del grupo CE-hipoxia exhibieron f_H reducido a lo largo del experimento, independientemente de la actividad forzada (*P>0.001*; Figura 2b).

En el experimento de salinidad, los peces incubados a 4.0 ppt de salinidad mostraron menor f_H que el Control y el resto de los grupos (~100 y ~107 latidos min^{-1}, respectivamente; *P>0.001*; Figura 2c). Durante 1-6 dpe los peces del grupo P1-6.0 mostraron menor f_H que el Control y el resto de los grupos (~140 contra ~ 150 latidos min^{-1}, *P>0.001*; Figura 2c). Al final del experimento los grupos P3-4.0 y P3-6.0 mostraron

mayor f_H que el Control y el resto de los grupos (~155 y ~149 latidos min^{-1}, *P>0.001*, Figura 2c).

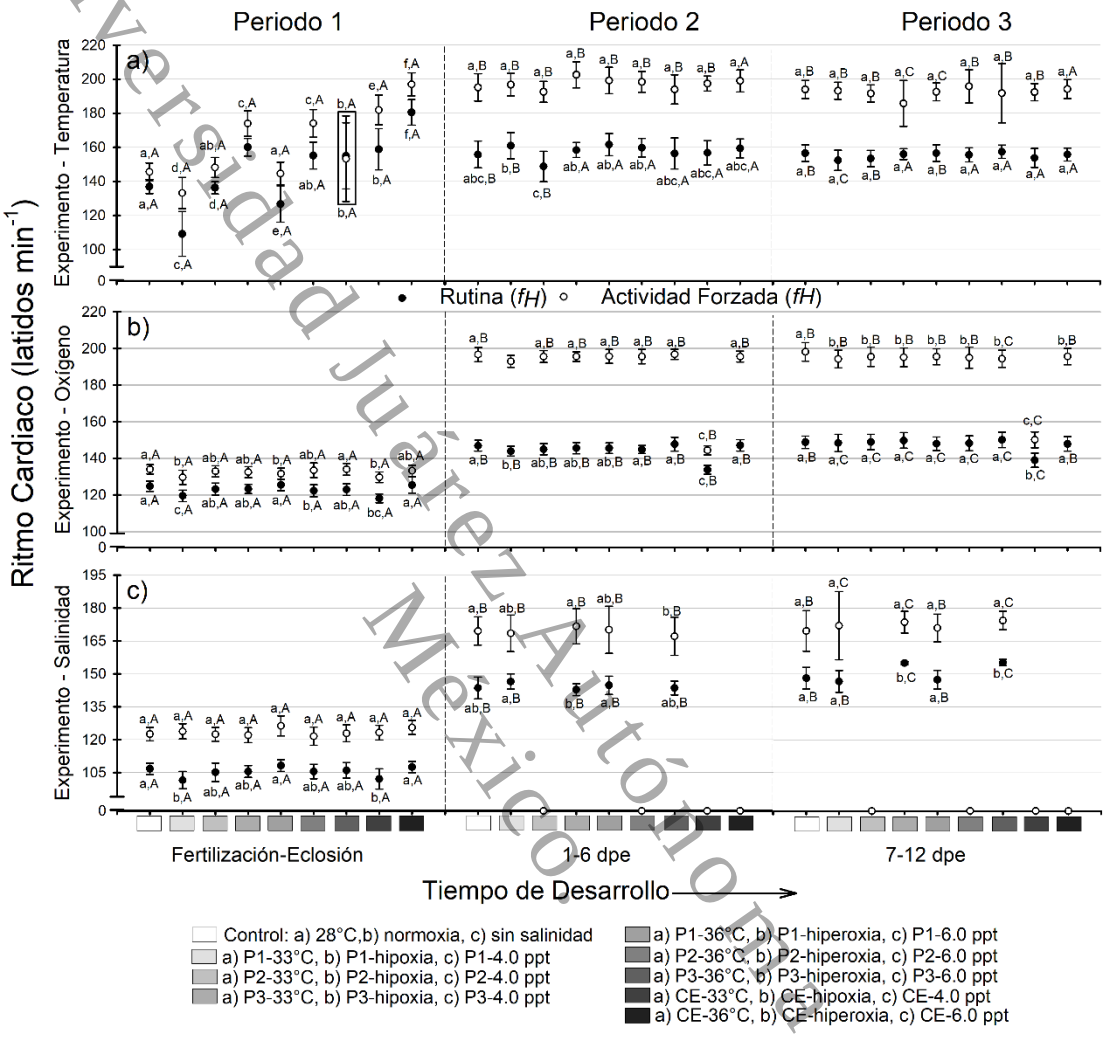


Figura 2. Ritmo cardiaco de *Atractosteus tropicus* expuesto a: a) temperatura (28°C, 33°C y 36°C), b) disponibilidad de oxígeno (hipoxia e hiperoxia) y c) salinidad (4.0 y 6.0 ppt) durante tres periodos de desarrollo (ver el texto para descripción). Letras minúsculas denotan diferencias en el ritmo cardiaco entre los diferentes grupos de peces encada periodo de desarrollo. Letras mayúsculas indican diferencias significativas a través del desarrollo para cada grupo experimental. Las cajas negras indican grupos de peces en los que no se observaron diferencias entre la tasa metabólica en rutina y después de habilidad forzada. n = 20 individuos por grupo.

La Figura 3a-c muestra la interacción del tiempo de desarrollo, los diferentes tratamientos y el ritmo cardiaco en *A. tropicus*. En ella se observa un incremento acelerado de esta tasa metabólica durante los primeros días después de la fertilización especialmente en los tratamientos con grupos de peces a mayor temperatura. Del mismo modo, puede

apreciarse una meseta para el ritmo cardiaco en rutina y después de actividad forzada a partir de ~5 dpe para los tres experimentos. En la figura 3c se aprecian periodos de bradicardia de~50% en los grupos de peces que perecieron.

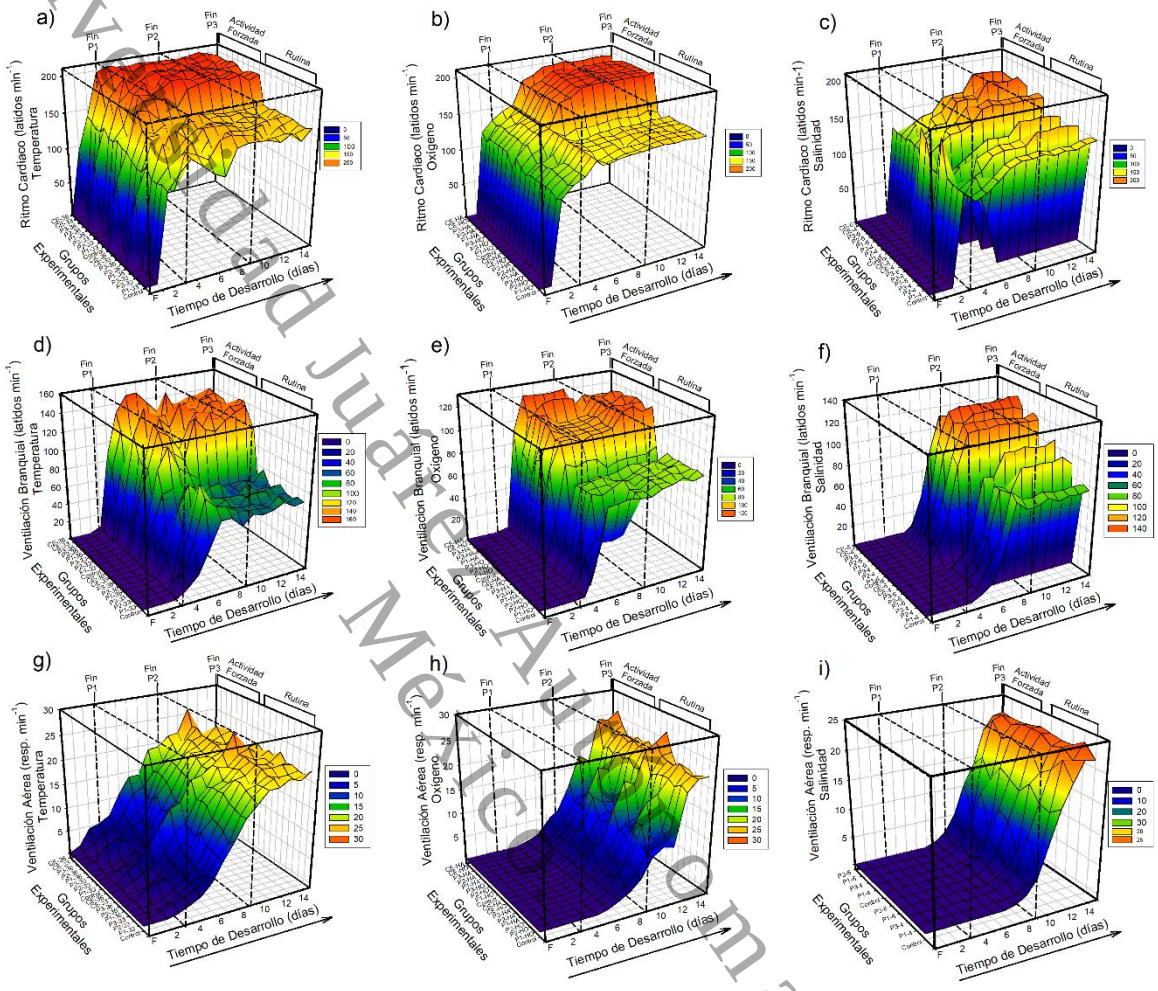


Figura 3. Interacciones entre el tiempo de desarrollo, los tratamientos empleados y el ritmo cardiaco (a-c), la ventilación branquial (d-f) y la ventilación aérea (g-i) en rutina y después de actividad forzada, a lo largo de los tres experimentos.

Ventilación branquial (f_G). En el experimento de temperatura, se observó un reflejo en la movilidad de los opérculos al momento de la eclosión, lo cual se consideró para sensibilidad térmica y no como ventilación branquial *per* se debido a la falta de madurez de las branquias. La f_G de los peces del Control y de los grupos P2-36°C y CE-33°C se registró menor (~70 latidos min^{-1}) que en el resto de los tratamientos a los 6 pde (~60 latidos min^{-1}; *P>0.001;* Figura 4a). Para el final del experimento (12 dpe) los peces del grupo CE-33°C mostraron la mayor f_G (~80 latidos min^{-1}) y los del grupo P2-36°C la

menor (~60 latidos min^{-1}; *P>0.001*; Figura 4a). A lo largo del experimento se observaron diferencias entre f_G en rutina y después de actividad forzada para todos los grupos de peces (*P>0.001*; Figura 4a).

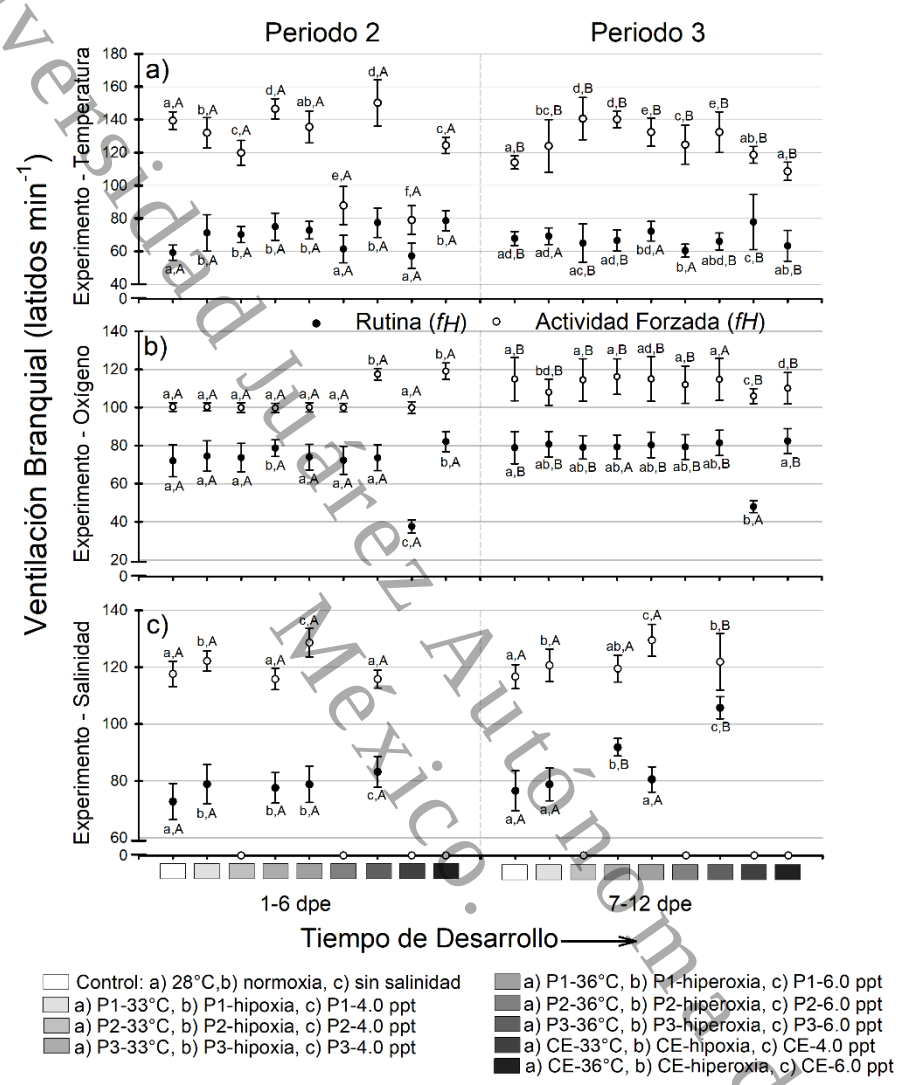


Figura 4. Ventilación branquial de *Atractosteus tropicus* expuesto a: a) temperatura (28°C, 33°C y 36°C), b) disponibilidad de oxígeno (hipoxia e hiperoxia) y c) salinidad (4.0 y 6.0 ppt) durante tres periodos de desarrollo (ver el texto para descripción). Letras minúsculas denotan diferencias en el ritmo cardiaco entre los diferentes grupos de peces encada periodo de desarrollo. Letras mayúsculas indican diferencias significativas a través del desarrollo para cada grupo experimental. n = 20 individuos por grupo.

En el experimento de disponibilidad de oxígeno, la f_G de los peces de los grupos P3-hipoxia y CE-hiperoxia se observó significativamente mayor que el Control y el resto de los grupos (~80 contra ~70 latidos min^{-1}, respectivamente, *P>0.001*, Figura 4b). En

contraste, los peces del grupo CE-hipoxia exhibieron la menor f_G a los 6 (~40 latidos min^{-1}) y 12 dpe (~50 latidos min^{-1}), comparado con el Control y el resto de los grupos (~70-80 latidos min^{-1}) en cada periodo (*P>0.001*; Figura 4b). Todos los grupos exhibieron diferencias significativas en los valores de f_G en rutina y después de actividad forzada (*P>0.001*; Figura 4b).

En el experimento de salinidad, para el periodo 1-6 dpe, los peces del grupo P3-6.0 mostraron la mayor f_G (~85 latidos min$_{-1}$) en comparación con el Control y el resto de los grupos (~70-80 latidos min-1; *P>0.001*; Figura 4c). Para el periodo 7-12 dpe los peces de los grupos P1-6.0 y CE-6.0 mostraron la mayor f_G (~90 y ~105 latidos min^{-1}) comparada con el Control y el resto de los grupos (~80 latidos min^{-1}; *P>0.001*; Figura 4c). Todos los grupos mostraron diferencias entre f_G en rutina y después de actividad forzada (*P>0.001*; Figura 4c).

La Figura 3d-f muestra la interacción del tiempo de desarrollo, los diferentes tratamientos y la ventilación branquial del pejelagarto. Obsérvese la alta variación que existe durante 1-6 dpe en el experimento de temperatura y, en contraste, las mesetas que se forman en los experimentos de disponibilidad de oxígeno y salinidad. Así mismo, puede observarse la aceleración en la ocurrencia de esta tasa metabólica en los grupos expuestos a hipoxia.

Ventilación aérea (f_{AB}). Ningún grupo de ningún experimento mostró diferencias significativas entre f_{AB} en rutina y después de actividad forzada (*P>0.001*; Figura 5). En todos los grupos, y en todos los experimentos, la f_{AB} incrementó con respecto al tiempo (*P>0.001*; Figura 5). En el experimento de temperatura, para el periodo 1-6 dpe, los peces de los grupos CE-33°C y CE-36°C mostraron mayor f_{AB} (~9 y ~14 bocanadas min$_{-1}$, respectivamente) comparado con Control y el resto de los grupos (~5 bocanadas min$_{-1}$; *P>0.001*; Figura 5a). Para el periodo 7-12 dpe, los peces del grupo CE-36°C exhibieron la mayor fAB (~26 bocanadas min^{-1}), mientras que el Control mostró la menor (~20 bocanadas min^{-1}; *P>0.001*; Figura 5a).

En el experimento de disponibilidad de oxígeno, durante el periodo 1-6 dpe los peces de los grupos P2-hipoxia y CE-hipoxia mostraron la f_{AB} más alta de todos los grupos (~7contra ~5 bocanadas min^{-1}; *P>0.001*; Figura 5b). Al final del experimento (12 dpe) los

peces del grupo CE-hipoxia mostraron la f_{AB} más alta (~26 bocanadas min-1; *P>0.001*; Figura 5b).

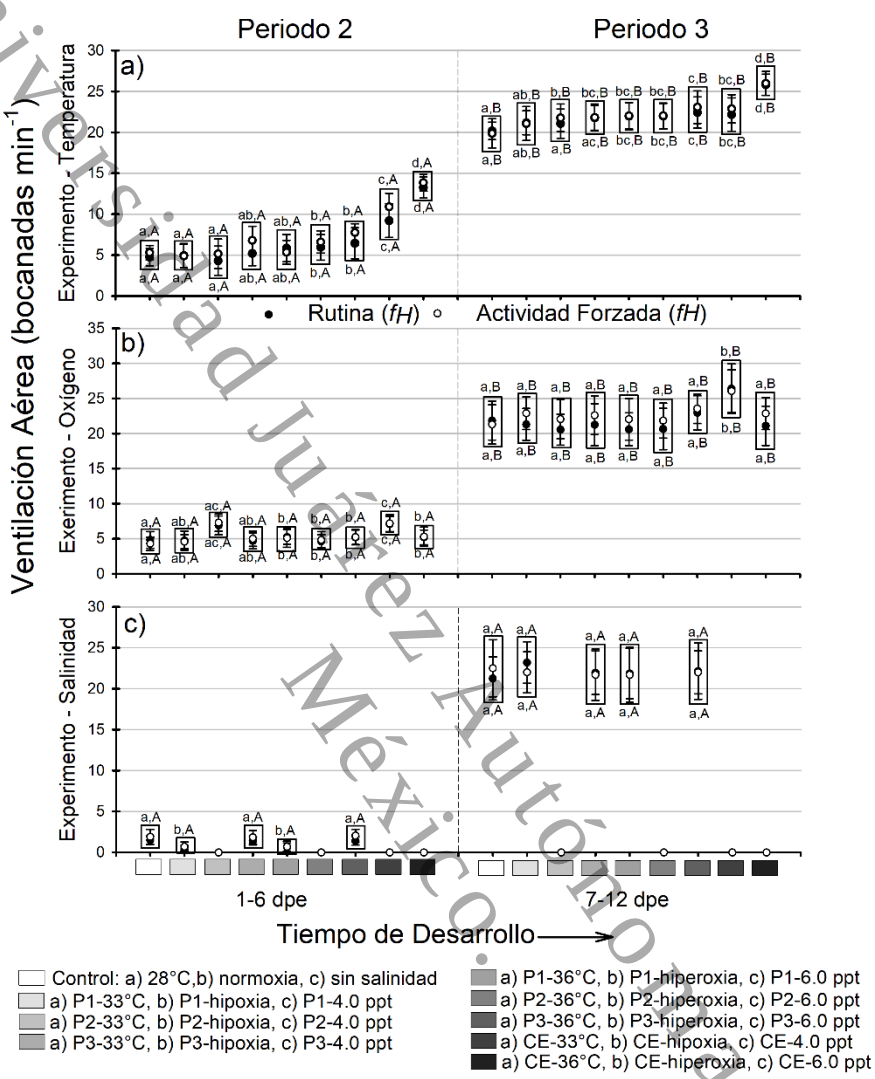


Figura 4. Ventilación branquial de *Atractosteus tropicus* expuesto a: a) temperatura (28°C, 33°C y 36°C), b) disponibilidad de oxígeno (hipoxia e hiperoxia) y c) salinidad (4.0 y 6.0 ppt) durante tres periodos de desarrollo (ver el texto para descripción). Letras minúsculas denotan diferencias en el ritmo cardiaco entre los diferentes grupos de peces encada periodo de desarrollo. Letras mayúsculas indican diferencias significativas a través del desarrollo para cada grupo experimental. Las cajas negras indican grupos de peces en los que no se observaron diferencias entre la tasa metabólica en rutina y después de habilidad forzada. n = 20 individuos por grupo.

En el experimento de salinidad, pada los 6 dpe, los peces de los grupos P1-4.0 y P1-6.0 mostraron la f_{AB} más baja de todos los grupos (~1 bocanada min^{-1}; *P>0.001*; Figura 5a). Para los 12 dpe no se observaron diferencias significativas entre ninguno de los grupos sobrevivientes (*P<0.05*; Figura 5c).

La Figura 3g-i muestra la interacción del tiempo de desarrollo, los tratamientos utilizados y la respiración aérea de los pejelagartos. En general, se observa mayor variación en la ventilación aérea de los peces en el tratamiento de temperatura, especialmente cuando la edad de desarrollo sobrepasa la edad cronológica. En la Figura 3h se observan los incrementos en la frecuencia de respiración aérea de los peces expuestos a hipoxia, a partir de los ~7 dpf.

Sensibilidad térmica

Para el f_H no se registró sensibilidad térmica entre los intervalos de temperatura ni entre los periodos de desarrollo (*P<0.05*; Tabla 1). Para la f_G se observó sensibilidad térmica durante el periodo de fertilización a eclosión, en el intervalo de 28°C a 36°C (*P>0.001*; Tabla 1). Del mismo modo, la f_{AB} mostró sensibilidad térmica a los 6 dpe en el intervalo de 28°C a 36°C (*P>0.001*; Tabla 1).

Tabla 1. Sensibilidad térmica para f_H, f_G y f_{AB} en el experimento de temperatura.

Tasa	Perido	Q_{10}		
		28-33°C	28-36°C	33-36°C
Ritmo Cardiaco (f_H)	Fert-Eclosión	1.076 ± 0.009 [a]	1.248 ± 0.012 [b]	1.04 ± 0.007 [a]
	1-6pde	1.004 ± 0.008 [a]	1.005 ± 0.004 [a]	1.02 ± 0.012 [a]
	7-12 dpe	0.991 ± 0.006 [a]	1.004 ± 0.002 [a]	0.997 ± 0.007 [a]
Ventilación Branquial (f_G)	Fert-Eclosión	1.322 ± 0.097 [a]	**2.342 ± 0.137** [b,]	1.205 ± 0.049 [a]
	1-6pde	0.983 ± 0.018 [a]	1.261 ± 0.028 [c]	1.103 ± 0.012 [b]
	7-12 dpe	1.068 ± 0.026 [a]	0.949 ± 0.029 [b]	0.945 ± 0.014 [b]
Ventilación Aérea (f_{AB})	Fert-Eclosión	-	-	-
	1-6pde	1.410 ± 0.052 [a]	**2.349 ± 0.099** [c]	1.126 ± 0.022 [b]
	7-12 dpe	1.052 ± 0.014 [a]	1.231 ± 0.036 [b]	1.047 ± 0.008 [a]

[a,b,c] indican diferencias entre intervalos para cada periodo de desarrollo.

Tiempo de ocurrencia de las variables fisiológicas

Ritmo cardiaco (f_H). El inicio de los latidos del corazón comenzó a las 36 y 24 horas post fertilización (hpf) en los grupos incubados a 33°C y 36°C respectivamente, comparado con el Control y el resto de los grupos (~48 hpf; *P>0.001*; Tabla 2). La hipoxia aceleró el tiempo de inicio de f_H por 10 h comparado con el Control y el resto de los grupos (~40 y ~50 h respectivamente; *P>0.001*; Tabla 2), mientras que la hiperoxia no tuvo efecto (*P<0.05*; Tabla 2). La salinidad no promovió diferenciasen el tiempo de ocurrencia de esta variable (*P<0.05*; Tabla 2).

Ventilación branquial (f_G). Las temperaturas de 33°C y 36°C durante la incubación y de exposición continua aceleraron el inicio de la f_G (~72 y ~60 hpf, respectivamente) con respecto del Control y el resto de los grupos (~96 hpf; *P>0.001*; Tabla 2). Del mismo modo, la hipoxia adelantó el inicio de f_G (~96 hpf) con respecto del Control (~102 hpf; *P>0.001*; Tabla 2), mientras que la hiperoxia no modificó esta característica (*P<0.05*; Tabla 2). La salinidad tampoco modificó el inicio de la f_G en ningún grupo de peces (~144 hpf; *P<0.05*; Tabla 2).

Ventilación aérea (f_{AB}). La alta temperatura modificó el inicio de f_{AB}; los grupos de peces mostraron diferentes tiempos de inicio de esta variable (*P>0.001*; Tabla 2): CE-36°C (~96 hpf), P1-36°C (~108 hpf), CE-33°C (~124 hpf), P1-33°C (~128 hpf), P2-36°C (~144 hpf), P2-33°C (~148 hpf) y el control, P3-33°C y P3-36°C mostraron el mayor tiempo (~152-154 hpf; Tabla 2). La hipoxia durante la incubación y de manera continua aceleraron el tiempo para *fAB* (~130 y ~122 hpf) comparado con el Control y el resto de los grupos, incluyendo aquellos expuestos a hiperoxia (~144 hpf; *P>0.001*; Tabla 2). En contraste, la salinidad durante la incubación retardó el inicio de f_{AB} (~212 hpf) comparado con el resto de los grupos (~192 hpf *P>0.001*; Tabla 2).

Tabla 2. Tiempo de inicio de f_H, f_G y f_{AB} para cada grupo experimental de cada experimento.

Evento	Experimento	Control	Tratamiento 1 (T1): 33 °C, Hipoxia, o Salinidad = 4.0 ppt				Tratamiento 2 (T2): 36 °C, Hiperoxia, o Salinidad = 6.0 ppt			
			P1-T1	P2- T1	P3- T1	CE- T1	P1- T2	P2- T2	P3- T2	CE- T2
Inicio de f_H	Temperatura	48 ± 2^{a}	36 ± 1^{b}	48 ± 2^{a}	48 ± 2^{a}	36 ± 2^{b}	24 ± 1^{c}	48 ± 2^{a}	48 ± 1^{a}	24 ± 1^{c}
	Oxígeno	50 ± 3^{a}	40 ± 2^{b}	50 ± 2^{a}	50 ± 3^{a}	40 ± 1^{b}	50 ± 3^{a}	50 ± 2^{a}	50 ± 3^{a}	50 ± 2^{a}
	Salinidad	48 ± 2^{a}	47 ± 1^{a}	48 ± 2^{a}	48 ± 3^{a}	48 ± 1^{a}	47 ± 3^{a}	48 ± 2^{a}	48 ± 2^{a}	48 ± 3^{a}
Inicio de f_G	Temperatura	96 ± 3^{a}	72 ± 2^{b}	96 ± 2^{a}	96 ± 2^{a}	73 ± 1^{b}	60 ± 1^{c}	96 ± 3^{a}	96 ± 2^{a}	60 ± 1^{c}
	Oxígeno	102 ± 1^{a}	96 ± 1^{b}	96 ± 2^{b}	102 ± 3^{a}	96 ± 2^{b}	102 ± 2^{a}	102 ± 2^{a}	102 ± 3^{a}	102 ± 2^{a}
	Salinidad	144 ± 3^{a}	144 ± 1^{a}	-	142 ± 2^{a}	-	144 ± 2^{a}	-	144 ± 1^{a}	-
Inicio de f_{AB}	Temperatura	156 ± 2^{a}	128 ± 2^{c}	148 ± 2^{b}	156 ± 2^{a}	124 ± 1^{c}	108 ± 2^{d}	144 ± 1^{b}	152 ± 2^{a}	96 ± 1^{e}
	Oxígen	144 ± 3^{a}	130 ± 2^{b}	144 ± 3^{a}	144 ± 2^{a}	122 ± 1^{b}	144 ± 2^{a}	144 ± 3^{a}	144 ± 3^{a}	144 ± 3^{a}
	Salinidad	192 ± 2^{a}	208 ± 3^{b}	-	193 ± 2^{a}	-	216 ± 3^{c}	-	192 ± 3^{a}	-

$^{a, b, c, d, e}$ indican diferencias significativas entre los grupos experimentales.
Control = 28°C, normoxia (~95% saturación de aíre), salinidad 0.0 ppt.
T1 = Tratamiento 1 (33°C, hipoxia ~30% saturación de aire o salinidad 4.0 ppt).
T2 = Tratamiento 2 (36°C, hiperoxia ~117% saturación de aire o salinidad 6.0 ppt).

DISCUSIÓN

La respiración aérea en los peces promueve la obtención de oxígeno para crear ATP a través de la fosforilación oxidativa para mantener las demandas energéticas del metabolismo (Damsgaard *et al.*, 2020). El surgimiento de un órgano de respiración aérea en los peces conlleva una serie de cambios en la regulación de sus sistemas cardiovascular y respiratorio para explotar eficientemente el nuevo medio rico en oxígeno (Damsgaard *et al.*, 2020: Graham, 1997; Bayley *et al.*, 2019; Milsom, 2012; Olson, 1994; Wright y Turko, 2016). En peces con respiración aérea se ha descrito una reducción del área superficie de las branquias y menor cantidad de ionocitos con respecto a los peces con respiración acuática (Hulbert *et al.*, 1978). Otra característica importante es que el ritmo cardiaco aumenta significativamente durante la respiración aérea. Este fenómeno puede incrementar la eficiencia del intercambio gaseoso relacionando las tasas de perfusión y ventilación (McKenzie *et al.*, 2007; Piiper y Scheid, 1975). Sin embargo, la respiración aérea incrementa el riesgo de depredación en los peces (Damsgaard *et al.*, 2020). En el caso del pejelagarto, donde la ventilación aérea comienza tan pronto en el desarrollo como a los 2.5 dpe (Burggren *et al.*, 2016), el riesgo es relativamente menor, ya que los desoves ocurren en áreas con vegetación, la cual puede utilizarse como refugio (Chapman y McKenzie, 2009; Shigles *et al.*, 2005).

Las larvas de peces que respiran aire muestran cierto grado de similitud con relación a su plasticidad morfológica, fisiológica, bioquímica, molecular y de comportamiento en función de diversos factores. Aunado a lo anterior, el conocimiento de los periodos sensibles en el desarrollo es importante para entender las respuestas de los peces ante los cambios en el ambiente. En el presente Capítulo se describen las posibles ventanas críticas para la respuesta fisiológica del pejelagarto a los cambios en el ambiente donde, esencialmente, los periodos más sensibles son de la fertilización a la eclosión y de 1-6 dpe. Por el contrario, el periodo más resiliente en la mayoría de las tasas metabólicas fue d 7-12 dpe, con excepción del experimento de salinidad, donde los peces expuestos durante este periodo a 4.0 y 6.0 ppt mostraron un incremento en la ventilación branquial. A continuación, se presenta el rol de cada estresor del presente estudio sobre las tasas metabólicas del pejelagarto en sus etapas tempranas.

Rol de la temperatura

La temperatura es un factor importante que acelera el desarrollo de los organismos (Mueller *et al*., 2015). En el presente Capítulo se muestra que el periodo de incubación puede representar una venta crítica para el inicio de los latidos del corazón y de a ventilación branquial, mientras que los periodos de incubación y 1-6 dpe representan una posible ventana crítica para el desarrollo de la ventilación aérea, todos en función del incremento en la temperatura. Sin embargo, se requiere de periodos de tiempo más cortos para determinar de manera específica el periodo en que el inicio de las tasas metabólicas es alterado. Con este respecto, Eme *et al.* (2015) y Mueller *et al.* (2015) describen ventanas críticas en el desarrollo embrionario de *Coregonus clupeaformis* en función de la temperatura. Sus hallazgos muestran que el periodo de organogénesis y primer aleteo son los más sensibles y que sus afectaciones pueden promover alteraciones en la vida adulta de los peces.

En el presente estudio, el ritmo cardiaco y la ventilación aérea incrementaron al exponer a los peces a diferentes temperaturas, lo cual es consistente con lo descrito por Burggren *et al.* (2016). En el caso de la ventilación branquial, en el presente estudio se observó que, a pesar de que las branquias de los peces no son maduras al momento de la eclosión, presentan un reflejo que es afectado por la temperatura durante este periodo (Tabla 1). Por otro lado, los valores de sensibilidad térmica (Q_{10}) para etapas posteriores permanece por debajo de ~1.5, lo cual es consistente con los datos de Burggren *et al*. (2016). Sin embargo, en Burggren *et al*. (2016), la mayor sensibilidad térmica se encontró en los intervalos de temperatura más bajos evaluados por los autores (20°C-25°C y 25°C a 28°C). Por lo tanto, es necesario conocer el efecto de la exposición continua a temperaturas menores a las utilizadas en este estudio para evaluar las tasas metabólicas desde una perspectiva de ventanas críticas. Del mismo modo, los resultados de la evaluación del ritmo cardiaco en este estudio son consistentes con Burggren *et al*. (2016), ya que no se observó sensibilidad térmica en ningún intervalo de temperatura, el ritmo cardiaco se mantuvo muy cercano al límite máximo reportado previamente. Esto puede deberse a que puede existir un límite superior en que los latidos del corazón puedan mantener un llenado constante de sus cámaras y ejecutar sus funciones de manera

adecuada, ya que se conoce que el corazón de las larvas de peces late a un pico máximo de ~200 latidos min^{-1} a temperaturas mayores a los 30°C (4).

La relación entre la perfusión y la ventilación es un componente esencial en el intercambio gaseoso. Consistente con lo descrito por Burggren *et al.* (2016), los cambios en el ritmo cardiaco son menores que los que ocurren en la ventilación branquial al incrementar la temperatura. Sin embargo, el ritmo cardiaco y la ventilación branquial no reflejan por si solas el volumen de irrigación y perfusión branquial, debido a que existe una compleja relación especie-específica de la función cardiaca en los peces (Farrel y Jones, 1992, Korsmeyer *et al.*,1997; Shukla, 2009).

Por otro lado, el inicio del ritmo cardiaco y las ventilaciones branquial y aérea, se aceleró por acción de la temperatura. En este caso, es importante considerar que la temperatura acelera el desarrollo de los peces (Mueller *et al.*, 2015). Por esta razón, se debe tener en cuenta que la aceleración del inicio de las tasas metabólicas función de la temperatura ocurre en tiempo cronológico, sin embargo, para el tiempo de desarrollo puede mantenerse constante. Esto se sugiere debido a que no se observó un cambio en la secuencia de aparición de los eventos (tanto fisiológicos como anatómicos en el Caítulo 1) como sucede en otros organismos (Tills *et al.*, 2010).

Rol de la disponibilidad de oxígeno

La baja disponibilidad de oxígeno tiene la capacidad de acelerar o retrasar el tiempo de inicio de las tasas metabólicas (Méndez-Sánchez y Burggren 2014). En el presente estudio, la aceleración en la ocurrencia de las tasas metabólicas ocurrió en función de la hipoxia., especialmente durante el periodo de la fertilización a la eclosión, el cual puede considerarse una ventana crítica para el inicio del ritmo cardiaco y las ventilaciones branquial y aérea. Esta aceleración en el tiempo de inicio puede representar un mecanismo de respuesta de los embriones para compensar la demanda de oxígeno necesarias para el metabolismo (Eme *et al.*, 2015; Mueller *et al.*, 2015). Esta suposición es apoyada por el hecho de que la hiperoxia no promovió efecto alguno sobre el inicio de las tasas metabólicas, comparado con el Control.

La exposición a la hipoxia en peces de respiración acuática se asocia con una reducción en el ritmo cardiaco e hiper ventilación branquial para incrementar la eficiencia

del intercambio gaseoso en las branquias (Perry *et al.*, 2009). Por otro lado, la respuesta fisiológica en los peces de respiración aérea puede presentar una desventaja, ya que el órgano de respiración aérea transporta el oxígeno hacia las venas centrales (Bayley *et al.*, 2019; Olson, 1994), creando una posible pérdida significativa de oxígeno a través de las branquias, debido a la hiper ventilación y bradicardia (Piiper y Scheid, 1975). Por otro lado, el ritmo cardiaco también puede aumentar en función de la hipoxia (Monteiro *et al.*, 2018; Skals e*t al.*, 2006). Al exponer a la especie *Synbramchus mamoratus* (respiradora de aire facultativo) a bajas concentraciones de oxígeno se observa un incremento en el gasto cardiaco, derivado el aumento del ritmo cardiaco y el volumen sistólico (Skals *et al.*, 2006). Estos incrementos ocurren al mismo tiempo que aumenta la presión sanguínea, lo cual sugiere que el sistema venoso y arterial tienen una función importante en el llenado del corazón y el volumen sistólico (Skals *et al.*, 2006). Sin embargo, estos postulados difieren con los resultados del presente estudio, ya que la hiper ventilación se observó solo en peces expuestos a hiperoxia de manera continua para los 6 dpe, y una hipoventilación y bradicardia fueron evidentes en el grupo expuesto a hipoxia durante todo el experimento. Con base en lo anterior, es posible que algunas especies como el pejelagarto presenten menores alteraciones ya que son respiradores de aire obligados, y sus hábitats naturales (Hill *et al.*, 1973; Rimoldi *et al.*, 2016; Smatresk y Cameron, 1982), así como las condiciones de cautiverio de los peces en el presente estudio, se encuentran en niveles bajos de oxígeno. En contraste, la baja disponibilidad de oxígeno promueve un incremento en el ritmo cardiaco del pejelagarto en diferentes tiempos de desarrollo (2.5-30 dpe, Burggren *et al.*, 2016). Estos datos difieren de los obtenidos en este Capítulo puesto que el trabajo realizado por Burggren *et al.* (2016), los cambios en la disponibilidad de oxígeno fueron agudos y rápidos, alcanzado niveles muy bajos. Por lo tanto, es necesario realizar estudios con niveles de oxígeno menores a los utilizados en esta tesis y determinar cuál es el efecto de dicha condición sobre la respuesta cardio respiratoria del pejelagarto.

Una disminución progresiva en la disponibilidad de oxígeno promueve diferentes respuestas en diferentes tiempos de desarrollo del pejelagarto (Burggren *et al.* 2016). Por ejemplo, peces de menos de 10 dpe muestran in incremento en la ventilación branquial. Estas características se han descrito para varias especies de peces (Jonz *et al.*, 2005; Porteus *et al.*, 2014,2015, Shakarchi *et al.*, 2013). Por otro lado, en pejelagartos de 15 y

30 dpe se observa un incremento inicial en la ventilación branquial, que disminuye significativamente al disminuir la disponibilidad de oxígeno. En el caso de los peces del grupo GE-hipoxia en del presente Capítulo, la disminución en el ritmo cardiaco y en la ventilación branquial, aunados al ligero incremento en la ventilación aérea, sugieren que son parte de un mecanismo para disminuir la pérdida de oxígeno por las branquias y, al mismo tiempo, mantener el balance ácido-base en las larvas (Burggren *et al.*, 2016; Burleson *et al.*, 1998). Esto sucede debido a que la sangre oxigenada en el órgano de respiración aérea llega directamente al corazón y es bombeada hacia las branquias, promoviendo un gradiente contrario de difusión de oxígeno, es decir, de las branquias hacia el ambiente (Burggren*et al.*, 1986, Smatresk y Cameron, 1982). En algunos peces con respiración bimodal, la sangre se bombea del corazón a los arcos branquiales posteriores a través de una derivación especial (Graham, 1997; Johansen, 1970; Randall *et al.*, 1981). Los arcos branquiales posteriores en estas especies exhiben una disminución en el área superficie para disminuir la pérdida de oxígeno. Sin embargo, estas estructuras no se observan en *L. oculatus* (Smatresk y Cameron, 1982), por lo que puede asumirse que el caso del *A. tropicus* es similar. A pesar de que el pejelagarto carece de estas derivaciones, esta especie muestra una disminución en las estructuras branquiales de las larvas (Burggren *et al.*, 2016).

Rol de la salinidad

La salinidad en el presente estudio no tuvo efecto alguno para el inicio del ritmo cardiaco ni la ventilación branquial. En contraste, el tiempo de ocurrencia de la ventilación aérea aumentó significativamente en peces incubados a 4.0 y 6.0 ppt. Por lo tanto, la exposición a la salinidad durante el periodo de fertilización-eclosión reveló una posible ventana crítica para el desarrollo de la ventilación aérea.

El ritmo cardiaco del pejelagarto en el presente estudio presentó sólo dos alteraciones:1) esta tasa disminuyó en peces incubados en salinidades de 4.0 ppt al momento de la eclosión y a los 6 pde, y 2) el ritmo cardiaco aumentó en peces expuestos a salinidades de 4.0 y 6.0 ppt durante 7-12 dpe. Consistente para los resultados al momento de la eclosión, no se observaron diferencias significativas en el rito cardiaco para *Fundulus grandis* incubado en salinidades entre 0.4 y 30.0 ppt (Brown *et al.*, 2012). Además, esta

especie mostró un incremento en la excreción de urea y amonio en salinidades de hasta 15.0 ppt. Esta excreción se regula por los intercambiadores Na^+/H^+ y, consistente con los resultados del estudio en *A. tropicus*, la actividad de los intercambiadores incrementa en peces expuestos a mayor salinidad (Capítulo 2). Por otro lado, en los grupos de peces que perecieron se observó una bradicardia de ~50% (Figura 5c). Esto puede deberse a que los peces no pusieron compensar las demandas de iono- y osmorregulación, ya que las branquias son el principal órgano para ambos procesos y, en el periodo en que murieron los peces, estas estructuras no estaban maduras (Brauner y Rombough, 2012; Kwan *et al.*, 2019; Melo *et al.*, 2019; Tresguerres *et al.*, 2020). Las branquias de los peces en sus etapas tempranas tienen una función más relacionada con la iono-regulación (Rombough, 2007), ya que obtienen oxigeno del medio a través de difusión por la piel y, de esta manera, cuando el área superficie es alta se desarrolla un sistema cardiovascular eficiente antes de que sea requerido para satisfacer las necesidades respiratorias de los peces (Pelster y Burggren, 1996; Kranenbarg *et al.*, 2000). Incluso algunas especies iono-regulan a través de células ricas en mitocondriones localizadas en la membrana basolateral del saco vitelino y su función cesa al madurar las branquias (Burnett *et al.*, 2007; Katoh *et al.*, 2000). En el caso del presente estudio en el pejelagarto, la salinidad incrementó la ventilación branquial en los peces incubados en salinidades de 4.0 y 6.0 ppt, a los 6 y 12 pde. En contraste, disminuyó la tasa de ventilación aérea en los mismos tratamientos exclusivamente a los 6 dpe.

Del mismo modo, la disminución en la ventilación branquial en presencia de salinidad puede incrementar la presión parcial de CO_2 en el plasma, producir acidosis y promover una disrupción en el balance ácido-base (Smatresk y Cameron, 1982). Por otro lado, existe una relación entre la respiración aérea y la salinidad en *L. oculatus* y *A. spatula* (Schwarz y Allen, 2014; Smatresk y Cameron, 1982). Esta relación se caracteriza por un incremento en la tasa de ventilación aérea al exponer a los peces a mayor salinidad. A pesar de que esta característica se describió en peces juveniles, la respuesta es consistente con los resultados de esta investigación. Con base en lo anterior, se sugiere estudiar esta interacción en etapas tempranas del pejelagarto y ampliar el panorama sobre la respuesta respiratoria de los peces con respiración bimodal a los cambios en salinidad.

Rol de la actividad forzada

En el presente estudio, la actividad forzada incrementó significativamente el ritmo cardiaco y la ventilación branquial de los peces, y no tuvo efecto alguno sobre la ventilación aérea, en ningún tratamiento ni en ningún tiempo de desarrollo. Estos resultados coinciden con el trabajo de Burggren *et al.,* (2016). En el caso del ritmo cardiaco, los valores de esta tasa se encontraron por debajo o cerca de los ~200 latidos por minuto, consistente por lo descrito por (Barrionuevo y Burggren, 1999). Del mismo modo, el incremento en el ritmo cardiaco en cualquier periodo de desarrollo del presente estudio fue mayor con respecto a los valores después de someter a los peces a los otros factores. Consistente con esto, los efectos cardiovasculares de la actividad forzada en *Lepisosteus oculatus* fueron relativamente bajos (Burleson *et al.,* 1998).

El incremento en la ventilación branquial de los peces en todas las edades del presente estudio (en función de la actividad forzada), incluso los latidos que se apreciaron al momento de la eclosión, sugieren que existe un control reflejo para la ventilación branquial, incluso a horas de la eclosión. Esta información es consistente con los resultados de Burggren *et al.* (2016). De esta manera, los reflejos ayudan a optimizar el intercambio de gases, especialmente porque el inicio de la respiración aérea ocurre después del inicio de la ventilación branquial y, de esta manera se involucran las branquias y la vejiga gaseosa para satisfacer las demandas de oxígeno en los peces (Burggren *et al.*, 2016).

La actividad forzada aumenta la frecuencia de ventilación aérea en adultos de *L. oculatus* (Burleson *et al.*, 1998). En el caso de las etapas tempranas del desarrollo del pejelagarto en este estudio, la ventilación aérea no se ve afectada por este factor, a pesar de que las otras tasas se modifican de manera significativa. Para esta característica existen tres posibles especulaciones sugeridas por Burggren *et al*. (2016). La primera sugiere que, durante las etapas tempranas, la vejiga gaseosa no funciona como un órgano para la respiración, sino que provee la flotabilidad a las larvas; así mismo, puede suceder que los reflejos que controlan la respiración aérea ocurran después que aquellos de la ventilación branquial. La segunda sugiere que los Lepisosteidos anteponen el balance ácido-base al consumo de oxígeno al experimentar condiciones ambientales adversas (Burggren *et al.*, 2016; Burleson *et al.*, 1998). De esta manera, la hiperventilación branquial promovida por

la actividad forzada en las larvas del pejelagarto puede contribuir a la hipocapnia, lo cual incrementaría el pH de la sangre. Con base en lo anterior, el incremento en la obtención de oxígeno atmosférico promovería una disrupción en el balance ácido-base de las larvas. La tercera especulación sugiere que, al no haber efecto de la actividad sobre la respiración aérea, la respuesta no esté relacionada con procesos cardio respiratorios, sino con comportamientos de evasión de depredadores.

CONCLUSIÓN

Las respuestas cardio respiratorias del pejelagarto en el presente estudio indican que incluso al momento de la eclosión existen reflejos fisiológicos, lo cual sugiere la presencia de receptores sensoriales y las estructuras que modulan los procesos fisiológicos clave para la ventilación y circulación. Además, los factores utilizados en el presente estudio mostraron que los periodos de fertilización a eclosión y 1-6 dpe son los más sensibles y pueden representar ventanas críticas para el ritmo cardiaco, la ventilación branquial y la ventilación aérea. Desde otra perspectiva, el periodo que resultó ser el más resiliente a los cambios en el ambiente (7-12 dpe), también resultó en afectaciones para los peces expuestos a mayor salinidad durante este periodo, específicamente para el caso de la ventilación branquial, la cual está fuertemente ligada a la iono- y osmorregulación. El presente Capítulo muestra cómo se puede alterar el sistema cardio respiratorio de los peces con respiración bimodal y las interacciones entre metabolismo, desarrollo y ambiente.

REFERENCIAS

Affonso, E.G., Rantin, F.T. 2005. Respiratory responses of the airbreathing fish *Hoplosternum littorale* to hypoxia and hydrogen sulfide. Comparative Biocheistry. and Physiology, 141C, 275–280.

Aguilera, C., Mendoza, R., Rodríguez, G., Márquez, G. 2002. Morphological description of alligator gar and tropical gar larvae, with an emphasis on growth indicators. Transactions of the American Fisheries Society, 131(5), 899-909.

Barrionuevo, W.R., Burggren, W.W. 1999. O_2 consumption and heart rate in developing zebrafish (*Danio rerio*): influence of temperature and ambient O_2. American Journal of Physiology, Regulative Integrative and Comparative Physiology, 276: R505–R513.

Bayley, M., Damsgaard, C., Thomsen, M., Malte, H., Wang, T. 2019. Learning to air-breathe: the first steps. Physiology,.34:14-29.

Belaõ, T.C., Leite, C.A.C., Florindo, L.H., Kalinin, A.L., Rantin, F.T. 2011. Cardiorespiratory responses to hypoxia in the African catfish, *Clarias gariepinus* (Burchell 1822), an air-breathing fish. Journal of Comparative Physiology B, 181, 905–916.

Blank, T.M. 2009. Cardio-respiratory ontogeny and the transition to bimodal respiration in an air-breathing fish, the blue gourami (*Trichogaster trichopterus*): morphological and physiological development in normoxia and hypoxia. In: Biological Sciences. Denton, TX: University of North Texas.

Boudreux, P.J., Ferrara, A.M., Fontenot, Q.C. 2007. Acute toxicity of ammonia to spotted gar, *Lepisosteus oculatus*, alligator gar, *Atractosteus spatula*, and paddlefish, *Polyodon spathula*. Journal of the World Aquaculture Society, 38, 322–325.

Brauner, C. J., Rombough, P. J. 2012. Ontogeny and paleophysiology of the gill: new insights from larval and air-breathing fish. Respiratory Physiology & Neurobiology, 184(3), 293-300.

Brown, C.A., Galves, F., Green, C.C. 2012. Embryonic development and metabolic cost in Gulf killifish *Fundulus grandis* exposed to varying environmental salinities. Fish Physiology and Biochemistry, 38, 1071-1082.

Burggren, W.W., Bagatto, B. 2008. Cardiovascular anatomy and physiology. In Fish Larval Physiology; Finn, R.N., Kapoor, B.J., Eds.Science Publishers: Enfield, NH, USA, pp. 119–161.

Burggren, W.W., Bautista, G.M., Coop, S.C., Couturier, G.M., Delgadillo, S.P., García, R.M., González, C.A.A. 2016. Developmental cardiorespiratory physiology of the air-breathing tropical gar, *Atractosteus tropicus*. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 311(4), R689-R701.

Burggren, W.W., Johansen, K., McMahon, B.R. 1986. Respiration in primitive fishes. En: The Biology of Primitive Fishes, edited by Foreman RE, Gorbman A, Dodd JM, y Olsson R. New York: Plenum, pp. 217–252.

Burggren, W.W., Mueller, C.A. 2015. Developmental critical windows and sensitive periods as three-dimensional constructs in time and space. Physiological and Biochemical Zoology, 88(2), 91-102.

Burggren, W.W., Reyna, K.S. 2011. Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. Respiratory Physiology & Neurobiology, 178(1), 13-21.

Burleson, M., Shipman, B., Smatresk, N. 1998. Ventilation and acid-base recovery following exhausting activity in an air-breathing fish. Journal of Experimental Biology, 201, 1359–1368.

Burnett, K.G., Bain, L.J., Baldwin, W.S. *et al*. 2007. *Fundulus* as the premier teleost model in environmental biology: opportunities for new insights using genomics. Comparative Biochemistry and Physiology D, 2:257–286

Chapman, L.J., McKenzie, D.J. 2009. Behavioral responses and ecological consequences. En: Richards JG, Farrell AP, Brauner CJ, eds. Fish Physiology. Cambridge, MA: Elsevier; pp. 25-77.

Damsgaard C., Baliga, V.B., Bates, E., Burggren, W., McKenzie, D.J., Taylor, E., Wright, P.A. 2020. Evolutionary and cardio-respiratory physiology of air-breathing and amphibious fishes. Acta Physiologica, 228(3),e13406.

De Roth, G. 1973. Effects of temperature and light on aerial breathing behavior of the spotted gar, *Lepisosteus oculatus*. Ohio Journal of Science, 73, 35–41.

Eme, J., Mueller, C.A., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y. 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (*Coregonus clupeaformis*) embryos and hatchlings. Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 179,71-80.

Eddy, F. B., Handy, R. D. 2012. Ecological and environmental physiology of fishes. OUP Oxford.

Farrell, A.P., Jones, D.R. 1992. The Heart, editado por Hoar WS, Randall DJ. San Diego, CA: Academic Press, p. 1–88.

Garduño-Paz, M.V., Méndez Sánchez, J.F., Burggren, W., García Martínez, J.L.A. 2020. Metabolic rate and hypoxia tolerance in *Girardinichthys multiradiatus* (Pisces: Goodeidae), an endemic fish at high altitude in tropical Mexico. Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 239,110576.

Graham JB. 1997. Air-Breathing Fishes: Evolution, Diversity and Adaptation. San Diego, CA: Academic Press.

Hill, L.G., Schnell, G.D., Echelle, A.A. 1973. Effect of dissolved oxygen concentration on locomotory reactions of the spotted gar, *Lepisosteus oculatus* (Pisces: Lepisosteidae). Copeia, 1, 119–124.

Hulbert, W.C., Moon, T.W., Hochachka, P.W. 1978. The erythrinid gill: correlations of structure, function, and metabolism. Canadian Journal of Zoology, 56, 814-819.

Johansen, K. 1970. Air Breathing in Fishes. En: Fish Physiology, editado por Hoar W.S., Randall, D.J. New York: Academic Press.

Johnson, D.W., Christie, M.R., Moye, J. 2010. Quantifying evolutionary potential of marine fish larvae: heritability, selection, and evolutionary constraints. Evolution, 64, 2614–2628.

Jonz MG, Buck LT, Perry SF, Schwerte T, Zaccone G. 2016. Sensing and surviving hypoxia in vertebrates. Annals of the New York Academy of Sciences. 1365(1),43-58.

Jonz, M.G., Nurse, C.A. 2005. Development of oxygen sensing in the gills of zebrafish. Journal of Experimental Biology, 208: 1537–1549.

Katoh, F., Shimizu, A., Uchida, K., Kaneko, T. 2000. Shift of chloride cell distribution during early life stages in seawater- adapted killifish, *Fundulus heteroclitus*. Zoological Sciences, 17,11–18

Kranenbarg, S., Muller, M., Gielen, J.L.W., Verhagen, J.H.G. 2000. Physical constraints on body size in teleost embryos. Journal of Theoretical Biology, 204,113–133.

Korsmeyer, K.E., Lai, N.C., Shadwick, R.E., Graham, J.B. 1997. Heart rate and stroke volume contribution to cardiac output in swimming yellowfin tuna: response to exercise and temperature. Journal of Experimental Biology, 200,, 1975–1986.

Kwan, G.T.;Wexler, J.B.;Wegner, N.C.; Tresguerres, M. 2019. Ontogenetic changes in cutaneous and branchial ionocytes and morphology in yellowfin tuna (*Thunnus albacar*es) larvae. Journal of Comparative Physiology B, 189, 81–95.

Landolt, J.C., Hill, L.G. 1975. Observations of the gross structure and dimensions of the gills of three species of gars (Lepisosteidae). Copeia 3: 47–75.

Lefevre, S., Bayley, M., McKenzie, D. J. 2016. Measuring oxygen uptake in fishes with bimodal respiration. Journal of Fish Biology, 88(1), 206-231.

Lefevre, S., Huong do, T.T., Wang, T., Phuong, N.T., Bayley, M. 2011. Hypoxia tolerance and partitioning of bimodal respiration in the striped catfish (*Pangasianodon hypophthalmus*). Comparative Biochemistry and Physiology A Molecluar and Integrative Physiology, 158(2), 207–214.

Lefevre, S., Wang, T., Jensen, A., Cong, N.V., Huong, D.T., Phuong, N.T., Bayley, M. 2014. Air-breathing fishes in aquaculture. What can we learn from physiology? Journal of Fish Biology, 84,705–731.

Liem, K.F. 1981. Larvae of air-breathing fishes as countercurrent flow devices in hypoxic environments. Science, 211, 1177–1189.

Little, C. 2009. The Colonisation of Land: Origins and Adaptations of Terrestrial Animals. Cambridge University Press: Cambridge, UK, p. 290.

Lopes, J.M., Boijink, C.L., Florindo, L.H., Leite, C.A.C., Kalinin, A.L., Milsom, W.K., Rantin, F.T. 2010. Hypoxic cardiorespiratory reflexes in the facultative air-breathing fish jeju (*Hoplerythrinus unitaeniatus*): role of branchial O2 chemoreceptors. Journal of Comparative Physiology, 180B, 797–811.

McCormack, B.T. 1967. Aerial respiration in the Florida spotted gar. Quartertly Journal of Florida Academy of Sciciences, 30, 68–72.

McKenzie, D.J., Campbell, H.A., Taylor, E.W., Micheli, M., Rantin, F.T., Abe, A.S. 2007. The autonomic control and functional significance of the changes in heart rate associated with air breathing in the jeju, *Hoplerythrinus unitaeniatus*. Journal of Experimental Biology, 210,4224-4232.

Melo, L.H.; Martins, Y.S.; Melo, R.M.C.; Prado, P.S.; Luz, R.K.; Bazzoli, N.; Rizzo, E. Low salinity negatively affects early larval development of Nile tilapia, *Oreochromis niloticus*: Insights from skeletal muscle and molecular biomarkers. Zygote, 27, 375–381.

Mendez-Sanchez, J.F., Burggren, W.W. 2014. Environmental modulation of the onset of air breathing and survival of the Siamese fighting fish Betta splendens and the three spot gourami Trichopodus trichopterus. Journal of Fish Biology, 84, 794–807.

Milsom, W.K. 2012. New insights into gill chemoreception: receptor distribution and roles in water and air breathing fish. Respiration Physiology and Neurobiology, 184, 326–339.

Monteiro, D.A., Taylor, E.W., Sartori, M.R., Cruz, A.L., Rantin, F.T., Leite, C.A.C. 2018. Cardiorespiratory interactions previously identified as mammalian are present in the primitive lungfish. Scientific Advances, 4,eaaq0800.

Mueller, C.A., Joss, J.M., Seymour, R.S. 2011a. Effects of environmental oxygen on development and respiration of Australian lungfish (*Neoceratodus forsteri*) embryos. Journal of Comparative Physiology B Biochemistry, Systems and Environmental Physiology, 181, 941–952.

Mueller, C.A., Joss, J.M., Seymour, R.S. 2011b. The energy cost of embryonic development in fishes and amphibians, with emphasis on new data from the Australian lungfish, *Neoceratodus forsteri*. Journal of Comparative Physiology B Biochemistry, Systems and Environmental Physiology, 181, 43–52.

Mueller, C.A., Eme, J., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y. 2015. Embryonic critical windows: changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (*Coregonus clupeaformis*). Joural of Comparative Physiology B, 185(3),315-31.

Olson, K.R. 1994. Circulatory anatomy in bimodally breathing fish. American Zoology, ;34:280-288.

Pelster, B., Burggren, W.W. 1996. Disruption of hemoglobin oxygen transport does not impact oxygen-dependent Physiological processes in developing embryos of zebra fish (*Danio rerio*). Circulatory Respiration, 79, 358–362

Perry, S.F., Wilson, R.J., Straus, C., Harris, M.B., Remmers, J.E. 2001. Which came first, the lung or the breath? Comparative Biochememistry and Physiology Molecular Integrative Physiology 129, 37–47.

Perry, S.F., Jonz, M.G., Gilmour, K.M. 2009. Oxygen sensing and the hypoxic ventilatory response. En: Richards JG, Farrell AP, Brauner CJ, eds. Fish Physiology. Cambridge, MA: Academic Press;

2009:193-253.

Piiper, J., Scheid, P. 1975. Gas transport efficacy of gills, lungs and skin: theory and experimental data. Respiration Physiology, 23,209-221.

Porteus, C.S., Abdallah, S.J., Pollack, J., Kumai, Y., Kwong, R.W., Yew, H.M., Milsom, W.K., Perry, S.F. 2014. The role of hydrogen sulphide in the control of breathing in hypoxic zebrafish (*Danio rerio*). Journal of Physiology, 592, 3075–3088.

Porteus ,C.S., Pollack, J., Tzaneva, V., Kwong, R.W., Kumai, Y., Abdallah, S.J., Zaccone, G., Lauriano, E.R., Milsom, W.K., Perry, S.F. 2015. A role for nitric oxide in the control of breathing in zebrafish (*Danio rerio*). Journal of Experimental Biology, 218: 3746–3753.

Potter, G.E. 1927. Respiratory function of the swim bladder in *Lepisosteus*. Journa of Experimental Zoology, 49, 45–65.

Rahn, H., Rahn, K.B., Howell, B.J., Gans, C., Tenney, S.M. 1971. Air breathing of the garfish (*Lepisosteus osseus*). Respiration Physiology, 11, 285–307.

Randall, D.J. 1994. Cardio-respiratory modeling in fishes and the consequences of the evolution of airbreathing. Cardioscience, 5, 167–171.

Randall, D.J., Burggren, W.W., Farrell, A.P., Haswell, M.S. 1981. The Evolution of Air Breathing in Vertebrates. Cambridge, UK: Cambridge University Press.

Randall, D.J., Ip, Y.K. 2006. Ammonia as a respiratory gas in water and airbreathing fishes. Respiratory Physiology and Neurobiology, 154, 216–225.

Renfro, J.L. 1967. Factors influencing aerial breathing and metabolism in gars (Tesis Doctoral). Norman, Oklahoma, USA: University of Oklahoma.

Rimoldi, S., Terova, G., Zaccone, G., Parker, T., Kuciel, M., Dabrowski, K. 2016. The effect of hypoxia and hyperoxia on growth and expression of hypoxia-related genes and proteins in spotted gar *Lepisosteus oculatus* larvae and juveniles. Journal of Experimental Zoology B Molecular and Developmetal Evolution, 326(4),250-67.

Rombough, P. 2007. The functional ontogeny of the teleost gill: which comes first, gas or ion exchange? Comparative Biochemistry and Physiology A, 148,732–742.

Rudneva, I. 2014. Biomarkers for Stress in Fish Embryos and Larvae; CRS Press: Boca Raton, FL, USA; p. 206.

Saksena, V.P. 1963. Effects of temperature, light, feeding, and activity on the rate of aerial breathing of gar (*Lepisosteus*). Norman, OK: University of Oklahoma.

Schwarz, D.E., Allen, P.J. 2014. Effects of salinity on growth and ion regulation of juvenile alligator gar *Atractosteus spatula*. Comparative Biochemistry and Physiology A Molecular and Integrative Physiology, 169, 44–50.

Shakarchi, K., Zachar, P.C., Jonz, M.G. 2013. Serotonergic and cholinergic elements of the hypoxic ventilatory response in developing zebrafish. Journal of Expwerimental Biology, 216, 869–880.

Shartau, R.B., Brauner, C.J. 2014. Acid-base and ion balance in fishes with bimodal respiration. Journal of Fish Biology 84, 682–704.

Shingles, A., McKenzie, D.J., Claireaux, G., Domenici, P. 2005. Reflex cardioventilatory responses to hypoxia in the flathead gray mullet (*Mugil cephalus*) and their behavioral modulation by perceived threat of predation and water turbidity. Physiological and Biochemical Zoology, 78,744-755.

Shukla, A.N. 2009. Physiology of Fishes. New Delhi, India: Discovery Publishing House.

Skals, M., Skovgaard, N., Taylor, E.W., Leite, C.A.C., Abe, A.S., Wang, T. 2006. Cardiovascular changes under normoxic and hypoxic conditions in the air-breathing teleost *Synbranchus marmoratus*: importance of the venous system. Journalof Experimental Biology, 209,4167-4173.

Smatresk, N.J. 1982. Respiration and acid-base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. Journal of Experimental Biology, 96, 263–280.

Smatresk, N.J., Burleson, M.L., Azizi, S.Q. 1986. Chemoreflexive responses to hypoxia and NaCN in longnose gar: evidence for two chemoreceptor loci. American Journal of Physiology Regululatory Integrative and Comparative Physiology, 251, R116–R125.

Smatresk, N.J.; Cameron, J.N. 1982. Respiration and acid-base physiology of the spotted gar, a bimodal breather: III. Response to a transfer from fresh water to 50% sea water, and control of ventilation. Journal of Experimental Biology, 96, 295–306.

Spicer, J.I., Burggren, W.W. 2003. Development of physiological regulatory systems: altering the timing of crucial events. Zoology, 106(2), 91-99.

Tills, O.; Spicer, J.I.; Rundle, S.D. 2010. Salinity-induced heterokairy in an upper-estuarine population of the snail *Radix balthica* (Mollusca: Pulmonata). Aquatic Biology, 9, 95–105.

Timmons, M.B., Ebeling, J.M. 2010. Recirculating Aquaculture. Cayuga Aqua Ventures. Ithaca, NY, USA, pp. 976.

Tresguerres, M.; Clifford, A.M.; Harter, T.S.; Roa, J.N.; Thies, A.B.; Yee, D.P.; Brauner, C.J. 2020. Evolutionary links between intra-and extracellular acid–base regulation in fish and other aquatic animals. Journal of Experimental Zoology A Ecologica and Integrative Physiology, 333, 449–465.

Winston, W.D. 1967. Effects of temperature and light on the rate of aerial breathing of the alligator gar, *Lepiososteus spatula* (Tesis Doctoral). Norman, OK: University of Oklahoma.

Wright, P.A., Turko, A.J.2016. Amphibious fishes: evolution and phenotypic plasticity. Journal of Experimental Biology, 219:2245-2259.

Zaccone, D., Grimes, A.C., Farrell, A.P., Dabrowski, K., Marino, F.M. 2011. Morphology, innervation and its phylogenetic step in the heart of the longnose gar *Lepisosteus osseus*. Acta Zoologica (Stockholm) 93, 1–9.

Zawodny, J.R. 1975. Osmoregulation in the Florida spotted gar, *Lepisosteus oculatus*. Miami, FL: University of Miami.

Discusión General

Las etapas tempranas de desarrollo son las más vulnerables en el ciclo de vida de los peces (Burggren y Bagatto, Rudneva, 2014). Durante ellas, el fenotipo y las trayectorias de desarrollo pueden modificarse por una gran variedad de factores, tanto intrínsecos como extrínsecos a los organismos (Burggren y Reyna, 2011; Hutchins, 2011; Burggren, 2019). Estas alteraciones pueden afectar directamente las características de los organismos en la vida adulta (Vehaskari et al., 2011; Morgan et al., 1998; Sallout y Walker, 2003). El estudio de las ventanas críticas de desarrollo, desde un punto de vista multifactorial, provee un mejor entendimiento de las interacciones de los diferentes factores con el fenotipo de los organismos en función del tiempo de exposición y la dosis de cada factor (Burggren y Mueller, 2015; Mueller *et al.*, 2016). En la presente Tesis se evaluaron aspectos morfológicos, fisiológicos y moleculares en tres periodos de la ontogenia temprana del pejelagarto *Atractosteus tropicus* en función de la temperatura, la disponibilidad de oxígeno y la salinidad.

Lefevré *et al.* (2014) ha denotado la gran necesidad de estudiar los aspectos fisiológicos de los peces en sus etapas tempranas, especialmente de aquellos que son utilizados en acuacultura. De esta manera, esta Tesis provee información clave para el entendimiento de diversos procesos durante el desarrollo temprano de los peces. Desde un punto de vista meramente fisiológico, se ha sugerido la importancia de utilizar como modelos peces con aletas radiadas y que respiren aire para estudiar la evolución de la respiración aérea, ya que son grupos que han evolucionado recientemente, comparado con los peces con aletas lobuladas que divergieron hace millones de años (Damsgaard *et al.*, 2020). Los peces con aletas radiadas proveen un mejor entendimiento de las adaptaciones iniciales asociadas con la evolución de la respiración aérea y permiten comparar si diferentes grupos en la filogenia de los peces y conocer las diferentes adaptaciones (morfológicas o fisiológicas) para resolver los mismos desafíos impuestos por la respiración aérea y la terrestrialidad (Damsgaard *et al.*, 2020).

El conjunto de datos de la presente investigación sugiere que los periodos de fertilización a eclosión y 1-6 dpe pueden considerarse ventanas críticas de desarrollo para la supervivencia, el crecimiento, la expresión de genes relacionados con la respuesta a la

hipoxia, las tasas metabólicas y el tiempo de inicio de los eventos (fisiológicos y morfológicos) clave en el desarrollo temprano del pejelagarto. Existen diversos estudios que han relacionado los parámetros evaluados en esta Tesis (citados en los Capítulos 1-3). Sin embargo, no existen estudios que relacionen de manera conjunta un contexto general de las respuestas de los organismos ante el ambiente cambiante.

En contraste, el último periodo en que se evaluaron las diferentes características en este trabajo se mostró como el más resiliente, debido a que se observaron mínimos o nulos cambios en respuesta al ambiente en el pejelagarto. Al ser un periodo de transición entre la lecitoexotrofia y la exotrofia (Comabella *et al.*, 2014), se esperaría que durante este periodo se observaran alteraciones en las diferentes características. Sin embargo, solo la ventilación branquial de los peces expuestos a 4.0 y 6.0 ppt de salinidad mostraron un incremento durante los 7-12 dpe. Esta observación está más relacionada con el iono y osmorregulación de los peces. Estos procesos son de gran importancia en los peces que respiran aire debido a las complicaciones que de ellos surgen, incluso desde el momento de la incubación. Los embriones de los peces mantienen un balance iónico y osmótico a través de células especializadas en la piel (Burnett *et al.*, 2007; Katoh *et al.*, 2000). Esta característica disminuye en funcionalidad a medida que se desarrollan las branquias. Estos órganos, aparte de la iono- y osmorregulación se encargan de la captación de oxígeno en el medio acuático (Brauner y Rombough, 2012; Kwan *et al.*, 2019; Melo *et al.*, 2019; Tresguerres *et al.*, 2020). Sin embargo, en peces con respiración aérea puede surgir una limitante, ya que, en condiciones de hipoxia, hiperoxia e hipertónicas, un incremento en la ventilación branquial conllevaría una pérdida de oxigeno por las branquias (Kranenbarg et al., 2000; Pelster y Burggren, 1996; Rombough, 2007), promovería una acidosis en el pH sanguíneo (Pester y Burggren, 1996) y, por consiguiente, una disminución de la capacidad de la hemoglobina para transportar oxígeno (efecto Root). Esta condición se discute detalladamente en los Capítulos 2 y 3.

Por otro lado, al conocer los diferentes aspectos de la biología básica de los peces en desarrollo, en este caso del pejelagarto, ofrece un panorama amplio para conocer y entender los mecanismos por los cuales estos peces son tan exitosos en su hábitat. Los sitios de distribución del pejelagarto pueden contener una alta cantidad de sólidos disueltos, que pueden incrementar la dureza y salinidad del agua. Al mismo tiempo,

pueden ser sitios con baja disponibilidad de oxígeno y, al encontrarse en zonas tropicales, la temperatura del agua se encuentra por encima de los ~28-20°C (Márquez-Couturier *et al.*, 2015; Miller *et al.*, 2005; Mora *et al.*,1997). Es por ello por lo que, futuras investigaciones deberán enfocarse en diferentes aspectos, con base en diferentes criterios. Primero, es necesario determinar el efecto de los estresores durante periodos más cortos de desarrollo. Por ejemplo, dividir el periodo de incubación para conocer cual es la etapa más sensible y puede afectar la vida adulta de los peces, tal como lo mencionan Mueller *et al.* (2015). En segundo lugar, es necesario evaluar más aspectos sobre la fisiología de los peces en desarrollo, tal como la eficiencia en la absorción del vitelo, el costo de desarrollo y la pérdida del equilibro homeostático, etc. (Eme *et al.*, 2015) para dilucidar y entender de mejor manera los procesos que ocurren en las etapas tempranas de los peces, su interacción con el ambiente y las posibles asociaciones de estas adaptaciones con el entendimiento de la transición a la terrestrialidad (Damsgaard *et al.* 2020). En tercer lugar, es de gran importancia evaluar la interacción que tienen más de un factor ambiental sobre el fenotipo de los peces. Muy pocos trabajos conjuntan la interacción entre dos o más factores con las respuestas funcionales de los peces (Cominassi *et al.*, 2019; Serafin *et al.*, 2019; Simning *et al*., 2019; Sswat *et al.*, 2018). Finalmente, una vez teniendo el conocimiento, es posible aplicarlo de diferentes maneras y con diferentes objetivos. Por ejemplo, esta Tesis menciona algunas condiciones importantes para el larvicultivo del pejelagarto que, desde un punto de vista productivo, puede incrementar la eficiencia de los peces en los sistemas en que se desarrollan. De igual manera, este documento contiene información que puede ser útil para la conservación de la especie a través de la conservación de sus hábitats naturales ya que, tomando en cuenta el cambio climático, una disminución en la temperatura viene acompañada de una disminución en la disponibilidad de oxígeno (Breitburg *et al.*, 2018; Diaz, 2001) y probablemente in incremento de la salinidad y, en este trabajo se han reportado las alteraciones a las que está sujeta el pejelagarto en su ontogenia temprana.

REFERENCIAS

Brauner, C. J., Rombough, P. J. 2012. Ontogeny and paleophysiology of the gill: new insights from larval and air-breathing fish. Respiratory Physiology & Neurobiology, 184(3), 293-300.Burggren, W. W., Mueller, C. A. (2015). Developmental critical windows and sensitive periods as three-dimensional constructs in time and space. Physiological and Biochemical Zoology, 88(*2*), 91-102.

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., *et al*. (2018). Declining oxygen in the global ocean and coastal waters. Science, 359(6371).

Burggren, W.W. 2019. Phenotypic switching resulting from developmental plasticity: fixed or reversible? Frontiers in Physiology, 10, 1634.

Burggren, W.W., Bagatto, B. 2008. Cardiovascular anatomy and physiology. In Fish Larval Physiology; Finn, R.N., Kapoor, B.J., Eds.Science Publishers: Enfield, NH, USA, pp. 119–161.

Burggren, W.W., Reyna, K.S. 2011. Developmental trajectories, critical windows and phenotypic alteration during cardio-respiratory development. Respiratory Physiology & Neurobiology, 178(1), 13-21.

Burnett, K.G., Bain, L.J., Baldwin, W.S. *et al*. 2007. *Fundulus* as the premier teleost model in environmental biology: opportunities for new insights using genomics. Comparative Biochemistry and Physiology D, 2:257–286

Comabella, Y., Hurtado, A., Canabal, J., García-Galano, T. 2014. Effect of temperature on hatching and growth of Cuban gar (*Atractosteus tristoechus*) larvae. Ecosistemas y Recursos Agropecuarios, 1(1), 19-32.

Cominassi L, Moyano M, Claireaux G, Howald S, Mark FC, Zambonino-Infante JL, Le Bayon N, Peck MA. 2019. Combined effects of ocean acidification and temperature on larval and juvenile growth, development and swimming performance of European sea bass (*Dicentrarchus labrax*). PLoS One,14(9):e0221283.

Damsgaard C., Baliga, V.B., Bates, E., Burggren, W., McKenzie, D.J., Taylor, E., Wright, P.A. 2020. Evolutionary and cardio-respiratory physiology of air-breathing and amphibious fishes. Acta Physiologica, 228(3),e13406.

Diaz, R. J. 2001. Overview of hypoxia around the world. Journal of Environmental Quality, 30(2), 275-281.

Eme, J., Mueller, C. A., Manzon, R. G., Somers, C. M., Boreham, D. R., Wilson, J. Y. 2015. Critical windows in embryonic development: Shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (Coregonus clupeaformis) embryos and hatchlings. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 179, 71-80.

Hutchings, J.A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. Heredity, 106(3), 421-437.

Katoh, F., Shimizu, A., Uchida, K., Kaneko, T. 2000. Shift of chloride cell distribution during early life stages in seawater- adapted killifish, *Fundulus heteroclitus*. Zoological Sciences, 17,11–18.

Kranenbarg, S., Muller, M., Gielen, J.L.W., Verhagen, J.H.G. 2000. Physical constraints on body size in teleost embryos. Journal of Theoretical Biology, 204,113–133.

Kwan, G. T., Wexler, J. B., Wegner, N. C., Tresguerres, M. 2019. Ontogenetic changes in cutaneous and branchial ionocytes and morphology in yellowfin tuna *(Thunnus albacares*) larvae. Journal of Comparative Physiology B, 189(1), 81-95.

Lefevre, S., Wang, T., Jensen, A., Cong, N.V., Huong, D.T., Phuong, N.T., Bayley, M. 2014. Air-breathing fishes in aquaculture. What can we learn from physiology? Journal of Fish Biology, 84,705–731.

Márquez-Couturier, G., Vázquez-Navarrete, C. J., Contreras-Sánchez, W. M., Álvarez-González, C. A. 2015. Acuicultura tropical sustentable: Una estrategia para la producción y conservación del pejelagarto (*Atractosteus tropicus*) en Tabasco, México. Colección José Narciso Rovirosa (2ª Ed. Vol. 3). México: Universidad Juárez Autónoma de Tabasco.

Melo, L. H., Martins, Y. S., Melo, R. M. C., Prado, P. S., Luz, R. K., Bazzoli, N., Rizzo, E. 2019. Low salinity negatively affects early larval development of Nile tilapia, *Oreochromis niloticus*: insights from skeletal muscle and molecular biomarkers. Zygote, 27(6), 375-381.

Miller, R. R., Minckley, W. L., Norris, S. M. 2005. Freshwater fishes of Mexico (No. QL 629. M54 2005).

Mora, M., Cabrera-Peña, J., Galeano, G. 1997. Reproducción y alimentación del gaspar *Atractosteus tropicus* (Pisces: Lepisosteidae) en el refugio nacional de vida Silvestre Caño Negro, Costa Rica. Revista de Biología Tropical, 45(2), 861-866.

Morgan, S. M., Huryn, V. M. B., Downes, S. R., Mercer, A. R. 1998. The effects of queenlessness on the maturation of the honey bee olfactory system. *Behavioural Brain Research*, 91(*1-2*), 115-126.

Mueller, C. A., Willis, E., Burggren, W. W. 2016. Salt sensitivity of the morphometry of *Artemia franciscana* during development: a demonstration of 3D critical windows. Journal of Experimental Biology, 219(*4*), 571-581.

Pelster, B., Burggren, W.W. 1996. Disruption of hemoglobin oxygen transport does not impact oxygen-dependent Physiological processes in developing embryos of zebra fish (*Danio rerio*). Circulatory Respiration, 79, 358–362.

Rombough, P. 2007. The functional ontogeny of the teleost gill: which comes first, gas or ion exchange? Comparative Biochemistry and Physiology A, 148,732–742.

Rudneva, I. 2014. Biomarkers for Stress in Fish Embryos and Larvae; CRS Press: Boca Raton, FL, USA; p. 206.

Sallout, B., Walker, M. (2003). The fetal origin of adult diseases. Journal of Obstetrics and Gynaecology, 23(*5*), 555-560.

Serafin J, Guffey SC, Bosker T, Griffitt RJ, De Guise S, Perkins C, Szuter M, Sepúlveda MS. Combined effects of salinity, temperature, hypoxia, and Deepwater Horizon oil on *Fundulus grandis* larvae. Ecotoxicological and Environmental Safety, 181:106-113.

Simning D, Sepulveda M, De Guise S, Bosker T, Griffitt RJ. 2019. The combined effects of salinity, hypoxia, and oil exposure on survival and gene expression in developing sheepshead minnows, *Cyprinodon variegatus*. Aquatic Toxicology, 214:105234.

Sswat M, Stiasny MH, Jutfelt F, Riebesell U, Clemmesen C. 2018. Growth performance and survival of larval Atlantic herring, under the combined effects of elevated temperatures and CO_2. PLoS One, 13(1):e0191947.

Vehaskari, V. M., Aviles, D. H., Manning, J. 2001. Prenatal programming of adult hypertension in the rat. Kidney International, 59(1), 238-245.

Conclusión

El presente trabajo muestra las diferentes respuestas funcionales de los embriones y larvas del pejelagarto ante los cambios en la temperatura, la disponibilidad de oxígeno y la salinidad durante su ontogenia temprana. Del mismo modo, sugiere que los periodos de desarrollo que comprenden desde la fertilización y hasta los 6 pde pueden representar ventanas críticas para la supervivencia, el crecimiento, el tiempo de ocurrencia de eventos morfológicos y fisiológicos clave y las tasas metabólicas en función de los tres factores, así como para la expresión de genes relacionados con la respuesta a la variación en la disponibilidad de oxígeno. Este trabajo provee información útil sobre la biología temprana de un pez con respiración bimodal, su relación con el tiempo de desarrollo y los cambios en el ambiente, lo cual contribuye al conocimiento y entendimiento de los mecanismos y adaptaciones de esta especie que favorecen su prevalencia. También, este escrito provee información que puede asociarse a la evolución de la transición de la respiración acuática a la respiración aérea, desde un punto de vista fisiológico. Por otro lado, los resultados obtenidos en la presente Tesis pueden ser utilizados para eficientizar el larvicultivo del pejelagarto en sistemas de producción, ya que provee datos sobre supervivencia y crecimiento que podrían beneficiar a dicho sector. Finalmente, se sugiere que el pejelagarto es un excelente modelo para el estudio de las etapas tempranas de los peces, debido a su rápido desarrollo y metamorfosis.