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

.

Impacto de la reducción de salinidad mediante diferentes tasas de aclimatación en la salud de postlarvas del camarón blanco del Pacifico *expuestos* al estrés agudo: Respuestas fisiológicas, metabólica e histológicas

T E S I S

QUE PARA OBTENER EL GRADO DE
DOCTOR EN CIENCIAS EN OCEANOGRAFÍA COSTERA

PRESENTA

VITALINA MAGALHÃES BRAGA DE SOUZA

ENSENADA, BAJA CALIFORNIA, ENERO 2025

FACULTAD DE CIENCIAS MARINAS
INSTITUTO DE INVESTIGACIONES OCEANOLÓGICAS
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VITALINA MAGALHÃES BRAGA DE SOUZA

Aprobada por:

Dr. Mario Alberto Galaviz Espinoza
Director de tesis.

Dr. André Luiz Braga de Souza
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Dr. Adriana Gisel González Silvera
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Dr. Miguel Arturo Cabanilla Gámez
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Dr. Samuel Sánchez Serrano
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Dr. Ernesto Larios Soriano
Sinodal

Asunto: Voto aprobatorio sobre trabajo
de tesis de grado de Doctorado

Dra. Ivone Giffard Mena
Coordinadora de Investigación y
Posgrado, F.C.M.
Presente

Estimada Dra. Giffard:

Me dirijo a usted en mi calidad de **Director de tesis** encargado de revisar la tesis de Doctorado presentada por la estudiante **Vitalina Magalhães Braga de Souza,** como parte de los requisitos para obtener el grado de **Doctor en Ciencias** en Oceanografía Costera.

Tras llevar a cabo una revisión minuciosa y exhaustiva del trabajo mencionado, es mi deber informarle que he emitido mi **Voto Aprobatorio** sobre la tesis titulada:

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El trabajo exhibe una sólida base teórica, una metodología rigurosa y una presentación coherente de los hallazgos. Las referencias bibliográficas están actualizadas y pertinentes, y las figuras y tablas son claras y respaldan eficazmente los argumentos del texto. La sección de conclusiones proporciona un resumen sólido de los resultados y sus implicaciones, la referencias y citas están actualizadas y son pertinentes.

Ensenada, B. C., a 14 de enero de 2025

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Director de tesis

c.c.p. Expediente

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Dr. Ernesto Larios Soriano
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PREFACIO

"La vida es una serie de cambios naturales y espontáneos. No los resistas; eso solo crea tristeza. Deja que la realidad sea la realidad. Deja que las cosas fluyan naturalmente hacia adelante de la manera que les guste." ***– Lao-Tsé***

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INDICE GENERAL

LISTA DE TABLAS

LISTA DE FIGURAS

Figura 5: Cambios histológicos en las branquias de postlarvas de *Penaeus vannamei* expuestas a diferentes tratamientos de reducción de salinidad en el rango de 5–1 g/L, durante los períodos de aclimatación (LW24h) y no aclimatación. En LW24H: (i) En el tratamiento control (CON5), las branquias exhibieron filamentos saludables y preservados, sin signos de desorganización ni alteraciones celulares, con epitelio funcional; (j) En el tratamiento 2aChoque (choque osmótico), se observaron daños severos, como desorganización de las láminas, pérdida de integridad, infiltración de hemocitos, necrosis en algunas áreas y vacuolización indicativa de edema epitelial; (k) En el tratamiento 2bRápida (reducción rápida de salinidad), se identificaron daños moderados, incluyendo infiltración de hemocitos y áreas aisladas con vacuolización; (l) En el tratamiento 2cGradual (reducción gradual de salinidad), se observó engrosamiento de las láminas, pequeñas áreas con necrosis e infiltración discreta de hemocitos, pero la mayoría de las láminas permanecieron saludables, con daños menos intensos en comparación con los otros tratamientos. En LW24H+: (m) En el tratamiento control (CON5), las branquias permanecieron saludables, sin signos de desorganización o cambios estructurales; (n) En el tratamiento 2aChoque, los daños severos persistieron, con desorganización de las láminas, vacuolización epitelial y sin signos de recuperación estructural; (o) En el tratamiento 2bRápida, hubo una ligera mejora, pero aún se observaron vacuolización significativa e infiltración de células inflamatorias, con desorganización celular; (p) En el tratamiento 2cGradual, la recuperación estructural fue evidente, con láminas preservadas, ausencia de necrosis significativa, infiltración mínima de hemocitos y sin signos de inflamación, destacándose como el tratamiento más efectivo para minimizar el daño histológico.

Figura 6: Cambios histológicos en el hepatopáncreas de postlarvas de *Penaeus vannamei* expuestas a diferentes tratamientos de reducción de salinidad en el rango de 35-5 g/L durante los períodos de aclimatación (LW24H) y no aclimatación (LW24H+). En LW24H: (a) En el tratamiento control (CON35), la arquitectura típica del hepatopáncreas se mantuvo preservada, con túbulos bien organizados, lumen en forma de estrella y tipos celulares distintos, como Blasenzellen (B), Fibrillenzellen (F) y Restzellen (R); (b) En el tratamiento 1aShock (reducción abrupta de salinidad), se observaron lesiones graves, incluyendo dilatación significativa de los túbulos, deformidades del lumen, marcada vacuolización de las células epiteliales, colapso estructural y necrosis; (c) En el tratamiento 1bRápida (reducción rápida de salinidad), el tejido mostró daño moderado, con lumen anormal, túbulos dilatados, vacuolización pronunciada y desorganización de los túbulos, pero con menor gravedad en comparación con el tratamiento 1aShock; (d) En el tratamiento 1cGradual (reducción gradual de salinidad), el hepatopancreas permaneció relativamente preservado, con lumen regular, túbulos organizados y daño mínimo, como vacuolización discreta. En LW24H+: (e) En el tratamiento control (CON35), la estructura del hepatopáncreas permaneció completamente preservada, manteniendo su organización y funcionalidad; (f) En el tratamiento 1aShock (reducción abrupta de salinidad),

Figura 7: Cambios histológicos en el hepatopáncreas de postlarvas de *Penaeus vannamei* expuestas a diferentes tratamientos de reducción de salinidad en el rango de 35-5 g/L durante los períodos de aclimatación (LW24H) y no aclimatación (LW24H+). En LW24H: (a) En el tratamiento control (CON35), la arquitectura típica del hepatopáncreas se mantuvo preservada, con túbulos bien organizados, lumen en forma de estrella y tipos celulares distintos, como Blasenzellen (B), Fibrillenzellen (F) y Restzellen (R); (b) En el tratamiento 1aShock (reducción abrupta de salinidad), se observaron lesiones graves, incluyendo dilatación significativa de los túbulos, deformidades del lumen, marcada vacuolización de las células epiteliales, colapso estructural y necrosis; (c) En el tratamiento 1bRápida (reducción rápida de salinidad), el tejido mostró daño moderado, con lumen anormal, túbulos dilatados, vacuolización pronunciada y desorganización de los túbulos, pero con menor gravedad en comparación con el tratamiento 1aShock; (d) En el tratamiento 1cGradual (reducción gradual de salinidad), el hepatopancreas permaneció relativamente preservado, con

Resumen

Penaeus vannamei es una especie eurihalina, tolerando salinidades de 0,5 a 40 g/L, pero requiere protocolos eficientes de aclimatación para soportar cambios bruscos de salinidad, esenciales para su transición de sistemas larvarios de alta salinidad a cultivo en baja salinidad. Este estudio se dividió en dos capítulos para investigar los efectos de la aclimatación y las tasas de reducción de salinidad en la salud de las postlarvas (PL). En el primero se investigó el impacto de dos tasas de reducción de salinidad (CON-SAL y VAR-SAL) en la supervivencia y las alteraciones histológicas de las PL (PL21) durante la reducción de salinidad de 35 a 1 g/L en 24 horas. Para probar esta hipótesis, postlarvas (PL de 21 días) fueron aclimatadas a la baja salinidad mediante su disminución de 35 a 1 g/L en 24 horas según dos tratamientos de tasa de reducción de la salinidad en nueve tanques de 5 L: Grupo CON-SAL, tasa constante de 1,45 g/L/h; VAR-SAL, utilizando 0,46%/h de 35 a 5 g/L, 0,25g/L/h de 5 a 2 g/L, y 0,13 g/L/h de 2 a 1 g/L. Además, un grupo de control (SW) se mantuvo a 35 g/L pero con una tasa de intercambio de agua salada de 40%/h. Todos los grupos se evaluaron por triplicado. Al final de la aclimatación (A24h) y 24 horas más tarde en el periodo de no aclimatación ($A24h^{+}$), se determinó la supervivencia y se recogieron PL para su posterior análisis histológico de las branquias y el hepatopáncreas siguiendo el método H&EA. La supervivencia fue superior al 80% al final de la aclimatación (A24h), pero se redujo significativamente en el período de no aclimatación (A24h+), especialmente en el grupo VAR-SAL (65,47%). Histológicamente, las branquias y el hepatopáncreas presentaron daños significativos. En el grupo VAR-SAL, se observó vacuolización, desorganización estructural y pérdida de células B. Después de 24 horas adicionales (A24h+), se observó cierta recuperación estructural en el hepatopáncreas, especialmente en el grupo CON-SAL. El tratamiento CON-SAL demostró ser el protocolo más adecuado para la aclimatación de postlarvas de *Penaeus vannamei*, reduciendo los daños histológicos y favoreciendo la recuperación estructural del hepatopáncreas. Extender el período de recuperación más allá de 24 horas es clave para optimizar la salud y la supervivencia de las postlarvas, mejorando la sostenibilidad de la acuicultura en condiciones de baja salinidad. En el segundo, se investigaron los efectos críticos de dos intervalos de reducción de salinidad (35-5 y 5-1 g/L) en postlarvas. Para el bioensayo, las PLs18 fueron distribuidas en 24 tanques de 16 L y aclimatadas a baja salinidad mediante reducciones en dos rangos: 35-5 g/L y 5-1 g/L, según diferentes tasas de reducción de salinidad realizadas por triplicado durante 24 horas: Grupo 1 (35-5 g/L): *1^{a}Shock* (sin aclimatación), *1^{b}Rápida*, a una tasa constante de 0.46% en 4 horas, *1^{c}Gradual*, a una tasa de 1.45 g/L en 22 horas. Grupo 2 (5-1 g/L): *2^{a}Shock* (sin aclimatación), *2^{b}Rápida*, a una tasa constante de 1.45 g/L en 4 horas, *2^{c}Gradual,* con tasas de 0.25 y 0.13 g/L en 22 horas, reduciendo con agua dulce cada hora. Dos grupos control (CON35 y CON5) se mantuvieron con una tasa de cambio del 40%/h. Después de 24 horas de aclimatación, todos los tratamientos fueran mantenidos por más 24 horas con salinidad final de 5 o 1 g/L sin aclimatación (LW24H+), con excepción de CON35. Al final de LW24H, se determinó la supervivencia, y las PLs se recolectaron para evaluar la tasa de consumo de oxígeno (OCR) y realizar análisis histológico de branquias y hepatopáncreas utilizando el método de hematoxilina y eosina. En el intervalo de

35-5 g/L, la supervivencia fue superior al 80% en todos los tratamientos (Choque, Rápido y Gradual). En el intervalo de 5-1 g/L, los tratamientos Choque y Rápido presentaron menor supervivencia y mayor consumo de oxígeno (8,2 mg O2/g/min). Los análisis histológicos mostraron daños severos en las branquias (vacuolización, infiltración hemocítica) y el hepatopáncreas (necrosis, túbulos dilatados) en los tratamientos Choque y Rápido, sin recuperación después de 24 horas (A24h+). Los resultados indican que la reducción gradual de salinidad en el intervalo 35-5 g/L es la más adecuada para minimizar daños histológicos y promover la supervivencia, mientras que el intervalo 5-1 g/L causa impactos negativos significativos. De esta manera, estos trabajos subrayan la importancia de una gestión adecuada durante la transición de las postlarvas desde los sistemas de larvario de alta salinidad hasta los sistemas de cultivo de baja salinidad. Los resultados obtenidos no solo aportan conocimiento sobre los mecanismos fisiológicos de osmorregulación, sino que también abren nuevas oportunidades para mejorar las estrategias de manejo en la acuicultura. Este estudio refuerza la importancia de protocolos optimizados para minimizar los efectos del estrés ambiental y promover la sostenibilidad en el cultivo de *P. vannamei* en condiciones de baja salinidad.

Abstract

Penaeus vannamei is a euryhaline species, tolerating salinities from 0.5 to 40 g/L, but requires efficient acclimation protocols to withstand abrupt salinity changes, which are essential for its transition from high-salinity larval systems to low-salinity grow-out systems. This study was divided into two chapters to investigate the effects of acclimation and salinity reduction rates on the health of postlarvae (PL). In the first chapter, the impact of two salinity reduction rates (CON-SAL and VAR-SAL) on the survival and histological alterations of PL (PL21) during salinity reduction from 35 to 1 g/L over 24 hours was investigated. To test this hypothesis, postlarvae (21-day-old PL) were acclimated to low salinity by reducing salinity from 35 to 1 g/L over 24 hours according to two salinity reduction rate treatments in nine 5-L tanks: the CON-SAL group, with a constant rate of 1.45 g/L/h, and the VAR-SAL group, with rates of 0.46 g/L/h from 35 to 5 g/L, 0.25 g/L/h from 5 to 2 g/L, and 0.13 g/L/h from 2 to 1 g/L. Additionally, a control group (SW) was maintained at 35 g/L with a seawater exchange rate of 40%/h. All groups were evaluated in triplicate. At the end of the acclimation period (A24h) and 24 hours later during the post-acclimation period (A24h+), survival was determined, and PL were collected for subsequent histological analysis of the gills and hepatopancreas using the H&E method. Survival was above 80% at the end of acclimation (A24h) but significantly decreased during the post-acclimation period (A24h+), particularly in the VAR-SAL group (65.47%). Histologically, the gills and hepatopancreas exhibited significant damage. In the VAR-SAL group, vacuolization, structural disorganization, and loss of B cells were observed. After an additional 24 hours (A24h+), some structural recovery in the hepatopancreas was observed, especially in the CON-SAL group. The CON-SAL treatment proved to be the most suitable protocol for the acclimation of *P. vannamei* postlarvae, minimizing histological damage and favoring structural recovery of the hepatopancreas. Extending the recovery period beyond 24 hours is key to optimizing the health and survival of postlarvae, improving the sustainability of aquaculture under low salinity conditions. In the second chapter, the critical effects of two salinity reduction ranges (35-5 g/L and 5-1 g/L) on postlarvae were investigated. For the bioassay, 18-day-old PL were distributed into 24 16-L tanks and acclimated to low salinity by reductions within two ranges: 35-5 g/L and 5-1 g/L, according to different salinity reduction rates conducted in triplicate over 24 hours. Group 1 (35-5 g/L) included: *1^{a}Shock* (no acclimation), *1^{b}Fast*, with a constant rate of 0.46 g/L in 4 hours, and *1^{c}Gradual*, with a rate of 1.45 g/L over 22 hours. Group 2 (5-1 g/L) included: *2^{a}Shock* (no acclimation), *2^{b}Fast*, with a constant rate of 1.45 g/L over 4 hours, and *2^{c}Gradual*, with rates of 0.25 and 0.13 g/L over 22 hours, reducing salinity with freshwater every hour. Two control groups (CON35 and CON5) were maintained with a water exchange rate of 40%/h. After 24 hours of acclimation, all treatments were maintained for an additional 24 hours at final salinities of 5 or 1 g/L without acclimation (LW24H+), except for CON35. At the end of LW24H, survival was determined, and PL were collected to assess the oxygen consumption rate (OCR) and conduct histological analysis of the gills and hepatopancreas using the hematoxylin and eosin method. In the 35-5 g/L range, survival was above 80% in all treatments (Shock, Fast, and Gradual). In the 5-1 g/L range, the Shock and Fast treatments showed lower survival and higher oxygen consumption (8.2 mg

O2/g/min). Histological analyses revealed severe damage to the gills (vacuolization, hemocytic infiltration) and hepatopancreas (necrosis, dilated tubules) in the Shock and Fast treatments, with no recovery after 24 hours (A24h+). The results indicate that gradual salinity reduction in the 35-5 g/L range is the most suitable for minimizing histological damage and promoting survival, whereas the 5-1 g/L range causes significant negative impacts.These findings underscore the importance of proper management during the transition of postlarvae from high-salinity larval systems to low-salinity grow-out systems. The results obtained not only contribute to the understanding of the physiological mechanisms of osmoregulation but also open new opportunities to improve management strategies in aquaculture. This study reinforces the importance of optimized protocols to minimize the effects of environmental stress and promote sustainability in the cultivation of *P. vannamei* under low salinity conditions.

CAPITULO 1

INTRODUCCIÓN GENERAL

Introducción general

La acuicultura es una de las actividades económicas de mayor crecimiento a nivel mundial, desempeñando un papel fundamental en la producción sostenible de alimentos y el desarrollo económico de regiones costeras e interiores (Khanjani et al., 2020a,b; Khanjani and Sharifinia, 2020). Dentro de este sector, el camarón blanco del Pacífico, *Penaeus vannamei*, se ha consolidado como una de las especies más importantes debido a su rápido crecimiento, alta tolerancia a un amplio rango de salinidades y notable capacidad de adaptación a diversas condiciones ambientales (McGraw y Scarpa, 2004; Cheng et al., 2006; Walker et al., 2009; Wang et al., 2019). Además, su importancia económica es significativa, reflejado en que es la especie de camarón más producida a nivel mundial, con 6.8 millones de toneladas métricas (FAO, 2024).

Los peneidos presentan una amplia gama de tolerancia a la salinidad, aunque las condiciones ideales para el crecimiento se cumplen dentro de un rango limitado. Para, el camarón blanco del Pacífico *Penaeus vannamei* el rango de tolerancia es extenso, puede crecer en salinidades de 0.5 a 50 g/L, por lo que se utiliza como especie modelo para estudiar el mecanismo de osmorregulación y tolerancia a la sal (Pante, 1990; Esparza-Leal et al., 2019; Shen et al., 2020; Saraswathy et al., 2020). Esta tolerancia a la salinidad del camarón blanco pasa por una gran variedad de salinidades a lo largo de su ciclo vital. La mayoría de las especies de penaeidos maduran y se reproducen en aguas oceánicas abiertas de alta salinidad, de 30 a 35 ppt. Posteriormente, las larvas migran a zonas de cría estuarinas de menor

salinidad, donde se metamorfosean en postlarvas y crecen rápidamente (Gunter et al., 1964; Bishop et al., 1980; Castille y Lawrence 1981; Re et al., 2004).

Además, *Penaeus vannamei* es un camarón marino nativo de la región costera occidental del hemisferio occidental y se distribuye desde Sonora, México hasta el norte de Perú y se concentra en los mares de Ecuador (Elovaara, 2003; Wang et al., 2019). Se encuentra, desde ambientes ligeramente salobres (1-2 ppt) hasta hipersalinos (40 ppt), (Stern et al. 1990), lo que la hace ideal para sistemas de cultivo tanto en zonas costeras como en regiones continentales con aguas de baja salinidad (McGraw y Scarpa, 2004). Sin embargo, esta flexibilidad osmótica, puede generar un estrés fisiológico significativo, principalmente cuando se expone a los organismos a cambios abruptos de salinidad (Chong-Robles et al., 2014; Yuan et al., 2017; Tian et al., 2020; Li et al., 2022).

Actualmente, la acuicultura ha desarrollado una tendencia a utilizar especies de agua dulce para aclimatarse al agua de mar y especies de agua de mar para aclimatarse a la baja salinidad (Nikapitiya et al., 2014). El cultivo de camarones en aguas de baja salinidad se ha convertido en una práctica cada vez más extendida a nivel mundial, siendo ejemplo de ellos paises como China, Tailandia, Vietnam, Ecuador, Brasil, México y Estados Unidos (McNevin et al., 2004). Esta pratica ha favorecido en la reducción de la incidencia de enfermedades, así como en el aprovecharmiento de terrenos agrícolas no productivos logrando fomentar el desarrollo económico regional (Roy et al., 2007; Cao et al., 2012; Van Wyk et al., 2013; Wang et al., 2016). En particular, la producción acuícola de crustáceos eurihalinos, como *Penaeus vannamei*, ha experimentado una notable expansión desde los entornos costeros hacia regiones del interior con acceso a recursos

hídricos subterráneos de baja salinidad (1–6 g/L), promoviendo el uso sostenible de agua limpia y generando beneficios económicos significativos (Li et al., 2017), siendo una estrategia que maximiza la utilización de recursos disponibles, dado su notable capacidad osmorreguladora en baja salinidad.

Además, *Penaeus vannamei* actúa como hipo-osmorregulador a salinidades más altas e hiper-osmorregulador a salinidades más bajas, dependiendo de si la salinidad ambiental está por debajo o por encima de su punto isotónico, el cual corresponde a 25 g/L (Castille y Lawrence, 1981) lo que permite un crecimiento y supervivencia satisfactorios a salinidades cercanas a la del agua dulce (Laramore et al., 2001; McGraw et al., 2002; Davis et al., 2005; Esparza-Leal et al., 2010). Estas características fisiológicas posicionan a especie como una de las especies más adaptables para sistemas de cultivo en ambientes de baja salinidad, consolidándola como un recurso clave en el desarrollo sostenible de la acuicultura a nivel mundial (Flaherty et al., 2000; Atwood et al., 2003; Green 2008; Li et al., 2017).

Los estudios han demostrado que *Penaeus vannamei* puede desarrollarse en ambientes de baja y media salinidad (Samocha et al., 1998; 2002; Ednoff, 2001), y se reporta que la mayoría de las investigaciones previas se han enfocado en la supervivencia y crecimiento del *Penaeus vannamei*, pero algunos con resultados contradictorios sobre los efectos de la salinidad, tales como Mair (1980), quién reportó juveniles sobreviviendo en salinidades entre 1‰ y 8‰ en el oeste de México, mientras que Chauvin (1983) documentó el crecimiento de postlarvas silvestres en salinidades inferiores a 2‰ en Ecuador. Wulff (1987) registró el cultivo exitoso de esta especie en agua dulce en Arizona. En condiciones de laboratorio, Olin y Fast (1987) observaron una supervivencia del 89% y 99% al transferir

directamente postlarvas de 5 a 12 días de edad desde una salinidad de 32‰ a 20‰ o 36‰, respectivamente.

Así como, el eportado por Ogle et al. (1992) que informaron que postlarvas de 8 días tuvieron menor supervivencia (19.8%) a 16‰ en comparación con las de 22 días (83.8%). Ponce-Palafox et al. (1997) confirmaron que *Penaeus vannamei* puede cultivarse eficazmente en salinidades de 5 a 35 g L^{-1}. Además, Bray et al. (1994) indicaron un crecimiento máximo en salinidades de 5‰ a 15‰, mientras que Araneda et al. (2008) reportaron un crecimiento de 0.38 g/semana con un 77% de supervivencia en agua dulce (0 ppt). En contraste, Laramore et al. (2001) probaron tratamientos con diferentes salinidades, y encontraron una baja supervivencia de postlarvas (PL) a salinidades inferiores a 2 ppt. No obstante, reconocen que los camarones *Penaeus vannamei* han sido producidos comercialmente en agua dulce (0.5 ppt).

Estudios previos han documentado la importancia de *Penaeus vannamei* para la acuicultura en baja salinidad, siendo más fuerte que otras especies como *Litopenaeus setiferus, Litopenaeus stylirostris, y Penaeus chinensis* (Castille & Lawrence, 1981; Chen et al., 1996; Su et al., 2005; Roy et al., 2007a; Jayasankar et al., 2009; Chong-Robles et al., 2013), el éxito en estos sistemas depende de un manejo adecuado de la aclimatación, especialmente en postlarvas (PLs), cuya supervivencia y desarrollo inicial son cruciales para el rendimiento del cultivo (Laramore et al., 2001; McGraw et al., 2002; Samocha et al., 2002; McGraw y Scarpa, 2004; Roy et al., 2009). Además, para transferir las PL desde los criaderos y cultivarlas con éxito en sistemas de cultivo de baja salinidad, es necesario desarrollar una técnica de aclimatación a la salinidad, para que este organismo

pueda responder a los cambios fisiológicos provocados por estas condiciones (Roy et al., 2009; Niu et al., 2011; Miandare et al., 2016).

Aunque los camarones *L. vannamei* pueden crecer con éxito en cultivos de baja salinidad, como lo reportan varios estudios (Samocha et al., 1998; Ednoff 2001, Samocha et al., 2002), en la fase de postlarvas existe controversia sobre la supervivencia, que depende principalmente del proceso de aclimatación a condiciones de baja salinidad (Laramore et al., 2001, McGraw et al., 2002, McGraw & Scarpa, 2004). Además, la supervivencia de camarones marinos aclimatados a baja salinidad, según algunos autores como Aquacop et al., 1991; Samocha et al., 1998; McGraw et al., 2002 y Saoud et al., 2003, puede verse afectada por factores como la composición iónica del agua, la salinidad final de aclimatación, la tasa de reducción de salinidad y la edad de las postlarvas, entre otros. Por ejemplo, salinidades reducidas pueden afectar la fisiología de *L. vannamei*, principalmente por debajo de 5 g/L, lo que resulta en una menor supervivencia, un crecimiento lento (Lin et al., 2012; Chen et al., 2015; Gao et al., 2016) y una baja tolerancia al estrés (Li et al., 2007).

La aclimatación a baja salinidad implica complejos procesos fisiológicos que buscan restablecer el equilibrio osmótico del organismo frente a cambios abruptos en la concentración de sales (Yamaguchi et al., 2018). En los organismos acuáticos, se han desarrollado mecanismos adaptativos osmorreguladores para que puedan sobrevivir en diferentes condiciones de salinidad. Se han publicado varios informes sobre las respuestas celulares y moleculares de las enzimas osmorreguladoras y las proteínas transportadoras de las branquias de los crustáceos en condiciones de estrés por salinidad agudo y a largo plazo (Scoot et al., 2005; Henry et al., 2006; De

la et al., 2007; Gao et al., 2012,). A nivel celular, la mayoría de los organismos reestructuran sus membranas, lo que ayuda a mantener constante la microviscosidad de la membrana en condiciones alteradas (Turner et al., 2003; Shivkamat & Roy 2005).

Las principales estrategias osmorreguladoras en crustáceos aclimatados a bajas salinidades incluyen: reducción de la permeabilidad corporal (Kirschner, 1991), síntesis de osmolitos (Gilles, 1979), producción de orina isosmótica con pérdida de sal (Lockwood, 1977) y absorción activa de iones (Freire et al., 2008; Henry et al., 2012). Estos mecanismos, realizados por la pared corporal, branquias y tejidos especializados, varían según la etapa de desarrollo (Cieluch et al., 2004).

Por ejemplo, el estrés ambiental agudo podría desencadenar alteraciones histopatológicas y ultraestructurales que se observan en diferentes órganos, lo que puede afectar negativamente la salud y el rendimiento de los camarones (Gao et al., 2012; Chiodi Boudet et al., 2015). Las branquias de los crustáceos, órgano multifuncional, desempeñan un papel esencial en la respiración y la regulación de la homeostasis, además de ser el principal punto de contacto con el ambiente externo, y proporcionan la mayor parte de la función osmorreguladora cuando los animales se transfieren de aguas de alta a baja salinidad (Charmantier, 1998; Péqueux et al., 2006; Pham et al., 2012).

Frente a condiciones adversas y cambios severos en los parámetros del entorno, tales como pH, oxígeno disuelto, salinidad y metales pesados, las branquias responden con estrés, reflejado en cambios morfológicos, inflamación, oxidación, alteraciones en el metabolismo energético y otros indicadores fisiológicos (Pequeux, 1995; Freire et al., 2008; Faleiros et al., 2010; Henry et al., 2012; Pham

et al., 2012; McNamara et al., 2012). En bajas salinidades, los ionocitos branquiales de los crustáceos hiperreguladores compensan la pérdida pasiva de iones debida al gradiente osmótico entre la hemolinfa y el medio circundante (Péqueux, 1995; Cieluch et al., 2004; Freire et al., 2008; Kaeodee et al., 2011; Leone et al., 2015).

Estudios previos, como el reportado por Fregoso-López et al. (2017) expusieron camarones *P. vannamei* a aguas de baja salinidad (1,9 g L^{-1}) bajo diferentes densidades de cultivo y con concentraciones crecientes de amoníaco, nitrito y sólidos suspendidos totales. En su estudio registraron alteraciones histológicas en las branquias, como fueron los edemas e inflamación (hemolinfa e infiltración hemocítica) durante las primeras 5 semanas de cultivo. Por otro lado, Wu et al. (2009) observaron alteraciones en las branquias, como vacuolización que provocó edema en los filamentos, en *P. vannamei* expuesto a diferentes concentraciones de cadmio (Cd) y zinc (Zn) durante un período de hasta 28 días. Así como edemas en branquias se ha reportado previamente en *Litopenaeus schmitti* cultivado a baja salinidad (8 g L^{-1}), atribuido a una reducción en la capacidad osmorreguladora y en la permeabilidad vascular (Laria-Lamela et al., 2005). Neufeld et al. (1980) indicaron que el edema en *Callinectes sapidus* expuesto a bajas salinidades podría ser consecuencia de la reducción de la capacidad osmorreguladora y el aumento del volumen celular como respuesta adaptativa rápida a cambios bruscos de salinidad.

Mientras, que el hepatopáncreas de los crustáceos es un órgano importante sensibles no sólo para la digestión, pero también es un indicador para el metabolismo, la absorción, el almacenamiento de nutrientes y las enfermedades. Además, juega un papel importante en el equilibrio iónico del organismo (Zhuang &

Ahearn, 1996), así como en la respuesta al estrés y las respuestas inmunes en varios crustáceos decápodos (Al-Mohanna & Nott, 1989; Bautista et al., 1994; Rosas et al., 1995; Jiang, 2009). Este órgano sufre modificaciones histológicas e histoquímicas en respuesta a diferentes demandas fisiológicas (muda, reproducción) y cambios ambientales como la salinidad y la contaminación (Al-Mohanna & Nott, 1989; Icely & Nott, 1992; Sousa & Petriella, 2000; Masson, 2001; Cuartas et al., 2003; Jamshidizadeh et al., 2019) y, ha sido ampliamente utilizado como un método práctico para evaluar el impacto de los factores de estrés ambiental en la acuicultura de camarones (Wu et al., 2008; Sun et al., 2015; Liu et al. 2024).

Además, estudios previos han demostrado que los cambios rápidos en la salinidad pueden generar un estrés osmótico significativo, que puede producir especies reactivas de oxígeno (ROS), y puede provocar alteraciones histológicas y funcionales en estos órganos (Paital & Chainy, 2010). Sin embargo, se reportan daños a nivel celular cuando los cambios en la salinidad superan la capacidad fisiológica de los camarones para responder al estrés osmótico (Stevens et al., 2003), lo que coincide con las altas mortalidades experimentadas en producciones acuícolas cuando los protocolos de aclimatación fallan.

El proceso de aclimatación a bajas salinidades incrementa el consumo de oxígeno debido al costo energético para mantener la homeostasis (Péqueux & Gilles, 1988; Laramore et al., 2001; Sokolova et al., 2012; Lin et al., 2012; Li et al., 2017). Este consumo es un indicador del estado fisiológico de crustáceos, reflejando la energía disponible para funciones biológicas (Rosas et al., 1996; 1999). Dado que el oxígeno es el receptor final de electrones, se asocia con la activación de enzimas como Na+/K+-ATPasa, regulación hormonal y liberación de aminoácidos en

respuesta al cambio salino (Lima et al., 1997). Aumentos en el consumo de oxígeno debido el impacto de la salinidad han sido observados en especies como *Fenneropenaeus indicus* (Kutty et al., 1971), cangrejo *Callinectes similis* (Rosas et al., 1991), *Palaemonetes pugio* (Vernberg & Piyatiratitivorakul, 1998) y *Penaeus vannamei* (Villarreal et al., 1994, Rosas et al., 2001, Zhang et al., 2009).

Bajo condiciones ambientales favorables, la energía se destina al crecimiento, pero en ambientes desfavorables se prioriza la recuperación de la homeostasis (Lucas, 1996). Además, fluctuaciones salinas inhiben el metabolismo energético, causando daño oxidativo y cambios en supervivencia, crecimiento y metabolismo (Choi et al., 2008; Ye et al., 2009; Farrae et al., 2014). La osmorregulación en bajas salinidades demanda aún más energía (Tseng & Hwang, 2008), con hasta el 50% del gasto metabólico utilizado para regular la presión osmótica, limitando así la energía disponible para el desarrollo, especialmente en postlarvas (Ern et al., 2014; Abou Anni et al., 2016; Li et al., 2017).

Además, el ajuste de la tasa respiratoria en juveniles de *Penaeus vannamei* ante cambios de salinidad depende del tiempo de aclimatación y de mecanismos fisiológicos clave como la captación activa de iones, el cambio en la permeabilidad branquial, la producción de orina diluida y la liberación de aminoácidos osmóticos (Péqueux y Gilles, 1981, Robinson, 1982, Regnault, 1987). Estos procesos, que requieren energía metabólica, permiten mantener la regulación osmótica en crustáceos expuestos a ambientes diluidos. Ajustes a corto plazo (de minutos a horas), como la activación de Na+, K+-ATPasa, y a largo plazo (de horas a días), como cambios en la permeabilidad branquial, incrementan el consumo de oxígeno para cubrir las demandas energéticas necesarias para mantener la homeostasis

(Prosser, 1973, Chen y Nan, 1995). Por ejemplo, Rosas et al., 2001 evaluaron el efecto de la salinidad (5, 10 y 30 ppt) y el tiempo de aclimatación en el consumo de oxígeno de juveniles de camarón blanco *Penaeus vannamei*, observaron que el consumo de oxígeno variaba significativamente en función de la salinidad, siendo mayor a niveles bajos de salinidad (Puig & Sanz, 1987; Rosas et al.,1999b).

Aunque diversas investigaciones han demostrado la capacidad de camarones peneidos, de tolerar amplios cambios en la salinidad ambiental (Guerin & Stickle, 1992; Rosas et al., 1997; 1999a*)*, y por consiguiente regular mecanismos como el metabolismo, la respuesta inmunitaria y la osmorregulación para adaptarse a las alteraciones en su entorno derivadas de la exposición al estrés salino (Fan et al., 2022b; Xue et al., 2022), la tolerancia o respuesta fisiológica de los camarones a la salinidad depende del rango de salinidades, las etapas de desarrollo y los procedimientos de exposición (transferencia directa o gradual) *(*Ponce-Palafox et al., 1997; Laramore et al., 2001; McGraw et al., 2002; Balbi et al., 2005; Sowers et al., 2006; Zhang et al., 2009; Jayasankar et al., 2009; Esparza-Leal et al., 2010*).*

Sin embargo, existe un conocimiento limitado sobre el tiempo requerido para que los camarones completen ajustes fisiológicos que permitan su supervivencia bajo cambios de salinidad. Estudios previos en juveniles de *Melicertus plebejus*, *Penaeus esculentus*, *Fenneropenaeus merguiensis* y *Metapenaeus bennettae* han demostrado que cuatro días son suficientes para alcanzar una regulación iónica completa (Dall & Smith, 1981). Según McGraw & Scarpa (2004) al evaluar el impacto del tiempo de aclimatación, del período de habituación y de la composición iónica final del agua dulce en la supervivencia de postlarvas del camarón blanco del Pacífico *Penaeus vannamei*, demostraron que extender el tiempo de aclimatación

de 48 a 72 horas mejora en un 27% la supervivencia de *Penaeus vannamei* en ambientes de baja salinidad, sin necesidad de un período de habituación intermedio, destacando la importancia del manejo adecuado del tiempo de aclimatación.

Mientras, en otras investigaciones con procesos de osmorregulación sugieren que la homeostasis se restablece en aproximadamente 24 horas (Bursey & Lane, 1971; Castille & Lawrence, 1981; Li et al., 2017). Así como se han reportado duraciones similares de recuperación en pruebas de tolerancia a la salinidad para otras especies de peneidos, como reportado por Ferraris et al., 198; Chen & Lin, 1994a; Saoud & Davis, 2003. Mientras que Rosas et al. (1999b) demostraron en sus estudios que las postlarvas (PL 10 a PL 14) de *L. setiferus* (Linnaeus) pueden tolerar medios diluidos de hasta 5‰ y son capaces de adaptarse en sólo 2 h a cambios en la salinidad.

En contraste, Chen y Lin (1994b) encontraron que diferentes salinidades de aclimatación pueden requerir períodos más prolongados para que los camarones mantengan la osmolalidad de la hemolinfa, debido a la naturaleza de los mecanismos fisiológicos involucrados en las pruebas de tolerancia a la salinidad. Por lo tanto, realizar un estudio de reducción rápida de salinidad en un corto período de tiempo permite evaluar la capacidad de adaptación inmediata y los límites fisiológicos de las postlarvas de *Penaeus vannamei*. Esto es crucial para desarrollar protocolos más eficientes y de menor duración, optimizando los costos y esfuerzos en la acuicultura de baja salinidad, sin comprometer la supervivencia y el rendimiento productivo.

Los resultados de estos estudios evidencian que el proceso de aclimatación de las postlarvas de *Penaeus vannamei* a condiciones de baja salinidad depende

directamente del tiempo de aclimatación, del protocolo de reducción de salinidad aplicado y el tiempo de reaclimatación. Estos hallazgos coinciden con trabajos previos que sugieren que el éxito de la aclimatación está relacionado con la capacidad osmorreguladora del camarón blanco del Pacífico (Davis et al., 2002; Gong et al., 2004; Araneda et al., 2008). De esta manera, la aclimatación a bajas salinidades requiere una gestión cuidadosa debido a la ambigüedad en los patrones de osmorregulación y a las variaciones en la tolerancia de las postlarvas a estas condiciones, para evitar el estrés osmótico que puede provocar daños en los tejidos, como en las branquias y el hepatopáncreas, que desempeñan funciones críticas en la osmorregulación y el metabolismo (Charmantier et al., 1998; Rosas et al., 2001; Lemaire et al., 2002; Li et al., 2008).

Los estudios sugieren que la tasa de aclimatación a la salinidad puede tener efectos variables sobre la fisiología y la supervivencia de los camarones (Van Wyk et al., 1999, McGraw et al., 2002). Por ejemplo, Laramore et al. (2001) al cultivar *P. vannamei* a 2 y 3 ppt no encontraron diferencias significativas en la supervivencia, pero la tasa de supervivencia fue significativamente mayor a 30 ppt. McGraw et al. (2002) evaluaron la influencia de la edad de las postlarvas (PL10, PL15 y PL20), la tasa de aclimatación y el nivel final de salinidad en la supervivencia de *Penaeus vannamei*. Los resultados mostraron que las tasas de aclimatación no afectaron la supervivencia de PL15 y PL20 (83-98%), mientras que PL10 presentó menor supervivencia a 1 ppt en comparación con 4 ppt. Además, PL15 y PL20 toleraron una rápida reducción de salinidad de 24 ppt a 1 ppt en aproximadamente 5 horas.

McGraw & Scarpa (2004) observaron un aumento en la supervivencia de la misma especie después de un período de aclimatación de 72 horas a 1 ppt. Así,

como Jayasankar et al. (2009) demostraron que postlarvas (PL15) aclimatadas gradualmente de 30 g/L a 5 g/L presentaron tasas de supervivencia superiores al 85%, mientras que a 1 g/L estas tasas fueron significativamente menores. La exposición directa a 1 g/L redujo la supervivencia al 20% en 5 horas, debido al estrés osmótico severo y a la dificultad en mantener el equilibrio iónico. Estudios adicionales, como los de Jaffer et al. (2020) destacaron que *Penaeus vannamei* mostró altas tasas de supervivencia (91.7%–95.8%) bajo salinidades de 1 a 25 ppt, siempre que la aclimatación se realizara gradualmente. Además, Abrori et al. (2022), confirman que reducciones progresivas en salinidad de 35‰ a 5‰ permiten mayores tasas de supervivencia (90%) comparadas con reducciones abruptas (80% en 10‰ y 58% en 15‰), atribuyendo estos resultados al tiempo adicional para la adaptación osmótica.

Considerando el hecho mencionado anteriormente, varios trabajos sobre la aclimatación de postlarvas de *Penaeus vannamei* disponibles en la literatura han reportado buenos resultados en protocolos a largo plazo (superiores a 24h) (Aquacop, 1991;Villalón et al., 1991; Ponce-Palafox et al., 1997; Rosas et al., 1999; Samocha et al., 2002; Davis et al., 2004). Sin embargo, aunque se han obtenido resultados prometedores a largo plazo, es importante investigar los efectos de tasas variables de reducción de salinidad en función de la supervivencia y los cambios histológicos en branquias y hepatopáncreas, con el fin de contribuir al desarrollo de protocolos para la reducción de salinidad a corto plazo, lo que podría representar un menor esfuerzo y costos para los productores.

De manera que, esta tesis se ha dividido en dos capítulos. En el primero se pretende identificar protocolos de reducción de la salinidad de 35 a 1 g/L utilizando

diferentes tasas de reducción (constante y variada) durante el periodo de 24 horas de aclimatación con el fin de minimizar el estrés y preservar la integridad histológica de los tejidos de las postlarvas. En el segundo capítulo se comparan dos rangos de reducción de la salinidad (35-5 g/L y 5-1 g/L) y diferentes protocolos (aclimatación gradual frente a rápida) para determinar qué enfoque es más seguro y eficaz para preservar la salud de los tejidos, mantener el consumo de oxígeno y la supervivencia de las postlarvas. Estudios previos han demostrado que las reducciones graduales tienden a minimizar el estrés, pero todavia no hay mucha informacion sobre como las reducciones rápidas de salinidad pueden exacerbar el daño histológico (Li et al., 2008; Chen et al., 2015).

Comprender los impactos de los diferentes protocolos de aclimatación a la salinidad no sólo puede mejorar el bienestar de los camarones, sino también promover prácticas de gestión más sostenibles en la acuicultura (Kumar et al., 2024). La identificación de métodos de aclimatación que reduzcan el estrés y el daño tisular, que consideren diferentes tasas de salinidad, rangos de tolerancia y niveles finales de salinidad adecuados permitirá a los productores adoptar prácticas que maximicen la supervivencia y el crecimiento de los camarones, incluso en entornos de baja salinidad (Roy et al., 2007). Además, una aclimatación optimizada es esencial para reducir las pérdidas económicas y mejorar la eficiencia de la cría de *Penaeus vannamei*, especialmente en zonas con acceso limitado a fuentes de agua de alta salinidad (Boyd & Thunjai, 2003). Este conocimiento es fundamental para desarrollar protocolos de manejo que minimicen el estrés durante la transferencia de las postlarvas desde criaderos de alta salinidad a sistemas de cultivo de baja salinidad (Roy et al., 2009, Chong-Robles et al., 2014).

El presente estudio tiene como hipótesis: la aclimatación de postlarvas de *Penaeus vannamei* a tasas constantes (CON-SAL) de reducción de salinidad resultará en una mayor integridad histológica y tasas de supervivencia, en comparación con tasas variables (VAR-SAL) de reducción de salinidad de 35 a 1 g/L, demostrando que los protocolos de aclimatación con tasas constantes son más efectivos para mitigar el daño tisular en las branquias y el hepatopáncreas, y facilitar la recuperación estructural durante el periodo de no aclimatación en condiciones de baja salinidad durante 24 horas de aclimatación. Así como, la reducción gradual de salinidad en el rango de 35-5 g/L será el protocolo más efectivo para la aclimatación de postlarvas de *Penaeus vannamei* a condiciones de baja salinidad, minimizando la mortalidad, el consumo de oxígeno y los daños histológicos en las branquias y el hepatopáncreas, en comparación con reducciones rápidas que generan mayor estrés osmótico y efectos histopatológicos irreversibles, especialmente en el rango extremo de 5-1 g/L, siendo considerado el rango más crítico para postlarvas de *Penaeus vannamei*.

Por lo tanto, este estudio ofrecerá valiosos conocimientos sobre la producción de *Penaeus vannamei* a bajas salinidades y puede servir de base para futuras investigaciones sobre las respuestas fisiológicas al estrés osmótico durante la fase de transición entre larvario y sistema de crecimiento. La recuperación de los tejidos tras el periodo de aclimatación proporciona información que puede aplicarse en otros estudios sobre la plasticidad adaptativa de los camarones (Calvo et al., 2011, Zhang et al., 2023). Además, el estudio se justificó por la ambigüedad observada en la literatura respecto al rendimiento de *Penaeus vannamei* en condiciones de baja salinidad. A pesar del uso creciente de esta especie en sistema de cultivo en este

tipo de ambiente en diversas regiones del mundo, existe una carencia de información precisa y consistente que permita comprender completamente su desempeño y las implicaciones asociadas. La investigación pretende colmar las lagunas de conocimiento sobre la osmorregulación y la tolerancia a salinidades extremas, con el objetivo de optimizar las prácticas acuícolas y promover el cultivo sostenible del camarón blanco en aguas continentales.

La relevancia de este trabajo radica en llenar estas lagunas de conocimiento mediante la evaluación de diferentes tasas y rangos de reducción de salinidad (35–5 g/L y 5–1 g/L) en postlarvas de *Penaeus vannamei*. Además, se busca identificar los protocolos de aclimatación más efectivos para preservar la integridad histológica y garantizar una transición exitosa de las postlarvas a entornos de baja salinidad. En conclusión, este estudio tiene como objetivo proporcionar una base científica sólida para mejorar las prácticas de manejo en la acuicultura de baja salinidad, contribuyendo al desarrollo sostenible de la producción de *Penaeus vannamei*. Los resultados obtenidos no solo aportarán conocimiento sobre los mecanismos fisiológicos de adaptación, sino que también tendrán implicaciones prácticas para optimizar la gestión de los sistemas de cultivo en diversas regiones del mundo.

CAPITULO 2

Articulo1: Penaeus vannamei **postlarval survival and histological changes in gill and hepatopancreas acclimated to low salinity in the short-term: Does dissolved salt content matter in defining their reduction rates?**

Penaeus vannamei postlarval survival and histological changes in gill and hepatopancreas acclimated to low salinity in the short-term: Does dissolved salt content matter in defining their reduction rates?

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ABSTRACT. An experimental design was used to evaluate *Penaeus vannamei* postlarvae (PL) survival and histological changes in gills and hepatopancreas and investigate the association between dissolved salt content and reduction rates for acclimation during reduction from 35 to 1 g L^{-1} over a 24-hour acclimation period (A24h). Two treatments were based on adapted protocols available in literature. The first, constant rate (CON-SAL), involved a gradual reduction of 1.45 g L^{-1} h^{-1} regardless of salt content. The second, variable rates according to salinity (VAR-SAL), began with a rapid reduction from 35 to 5 g L^{-1} in the first four hours: 1h at a 16 g L^{-1}, 2h at 9 gL^{-1}, and 3h at 5 g L^{-1} per hour. This was followed by a reduction of 0.25 g L^{-1} per hour from 5 to 2 g L^{-1} over 13 hours and, finally, a slower reduction of 0.13 g L^{-1} per hour from 2 to 1 g L^{-1} over the last eight hours. A control group was maintained at 35 $\pm$ 1 g L^{-1} with 40% h^{-1} saltwater exchange. At the end of A24h, all treatments were maintained for an additional 24 hours during a non-acclimation period (A24h^{+}). Live shrimp were counted, and samples were collected for histological analysis at the end of both periods. No statistical differences were found in survival at A24h, while VAR-SAL showed a significant reduction at A24h^{+}. Histological gill damage was observed in CON-SAL and VAR-SAL at A24h, but VAR-SAL was the only group showing progressive damage at A24h^{+}. Both treatments resulted in hepatopancreas changes, but damage was more severe in VAR-SAL at both A24h and A24h^{+}. These results support that reduction rates should be defined as a concentration function, as VAR-SAL was less effective, likely due to the rapid reduction from 35 to 5 g L^{-1}. Regardless of reduction rates, PL older than 15 days can survive salinity reductions to 1 g L^{-1} in a short period (A24h). Few studies have investigated short-term histological changes during PL acclimation to low salinity, emphasizing the significance of these findings.

Keywords: *Penaeus vannamei*: osmotic stress; histopathology; aquaculture

INTRODUCTION

Aquaculture production of euryhaline crustaceans has expanded from coastal to inland regions, where groundwater is used at around 1 to 6 g L^{-1}. *Penaeus vannamei* is one of the most successful cases of this expansion, among which the main crustaceans are reared at low salinity (FAO 2022). For example, suitable production of this species has been achieved in extreme salinities, such as 0.5 and 50 g L^{-1} (McGraw & Scarpa 2004, Cheng et al. 2006). In land, *P. vannamei* aquaculture production is based on its great osmoregulatory ability associated with ontogenetic adaptations to salinity required during its life cycle (Ogle et al. 1992), which explains why it has been considered one of the preferred crustacean species for inland aquaculture (McGraw et al. 2002). Although *P. vannamei* ability to grow at low salinity depends on postlarvae (PL) acclimation (Laramore et al. 2001, McGraw et al. 2002, McGraw & Scarpa 2004), during this life phase, shrimp is transferred and acclimated to commercial farm-water conditions. Therefore, an efficient acclimation protocol is crucial to allow shrimp to respond properly to salinity changes (Roy et al. 2009). Otherwise, production may be compromised by growth and survival reduction. Ionic water composition, final salinity content, PL age, and salinity rate reduction have been demonstrated as factors causing osmotic stress during acclimation (Aquacop 1991, Samocha et al. 1998, McGraw et al. 2002, Saoud et al. 2003).

Post-larvae exchange ions across epithelial cell membranes during acclimation to ensure that the body is protected from hypotonic effects and thus maintain the original structure (Li et al. 2008). Crustaceans show adaptive osmoregulation strategies to reduce stress during acclimation to low salinities. For example, they reduce body surface permeability (mainly gills) to salt and water to decrease osmotic gradient and actively absorb ions to maintain a hyperosmotic extracellular fluid (Freire et al. 2008, Henry et al. 2012, Rahi et al. 2018). Various studies have been performed where these strategies are observed and naturally represent an increase in oxygen consumption and metabolic activity (Laramore et al. 2001, Decamp et al. 2003, Lin et al. 2012). However, damages at cellular level

are reported when salinity changes exceed shrimp physiological capacity to respond to osmotic stress (Stevens et al. 2003), matching with the high mortalities experienced in aquaculture productions when acclimation protocols fail.

Several works about *P. vannamei* PL acclimation are available in the literature and have reported good results for long-term protocols (longer than 24 h) (Aquacop 1991, Villalón et al. 1991, Rosas et al. 1999). Although the promised results obtained for the long-term, investigating the effects of variable salinity reduction rates is important depending on survival and histological changes in gills and hepatopancreas to contribute developing protocols for salinity reduction in the short-term, which could represent less effort and costs for farmers.

Among the studies that addressed salinity reduction in the short term, McGraw et al. (2002) evaluated the influence of PL age (10, 15, and 20 days old), salinity endpoints (0, 1, 2, 4, 8, and 12 g L^{-1}), and salinity reduction rates (constant and variable) on survival during acclimation. The authors concluded that PL older than 15 days could survive salinity reductions to 1 g L^{-1} over a short period (24–48 hours), regardless of the reduction rate. However, to our knowledge, few studies have investigated histological changes during PL acclimation to low salinity in the short term. Therefore, the present study aimed to evaluate the association between dissolved salt content and reduction rates during P. vannamei PL acclimation in the short term, focusing on survival and histological changes in the gills and hepatopancreas.

MATERIALS AND METHODS

Animal origin

Twelve-day-old *P. vannamei* postlarvae (PL12; n = 10 000) were obtained from a commercial hatchery (Mariculture del Pacífico, Mazatlán, México) and reared in the Crustacean Laboratory (Facultad de Ciencias Marinas, Universidad Autónoma de Baja California, Ensenada, México) for one week to ensure that the entire batch had full branchial filament development and thus would be able to tolerate salinity reduction (Davis et al. 2002, Balbi et al. 2005). During this period, PL were kept in 9 fiberglass tanks of 1000 Liters sheltered in an enclosed greenhouse and filled with

filtered natural seawater at 35 g L^{-1} under constant aeration. In addition, temperature was maintained at 28 ± 1°C using electric heaters; total ammonia nitrogen (mg L^{-1}) was maintained below 0.5 mg L^{-1} via 100% water exchange daily and photoperiod was natural (12:12 light:dark). Shrimp were fed at 100% of estimated biomass with a commercial feed formulated for post-larvae to contain 45% of crude protein (CP) and 9% of lipids (Biogrow®, Proaqua, Mazatlán, México) five times a day (6:00, 9:00, 12:00, 15:00, and 18:00 h).

Experimental design

In the following week, 360 PL22 (0.048 ± 0.02 g) were randomly selected and distributed in 9 experimental units with a volume of 5 liters filled with seawater (35 g L^{-1}) from the system where shrimp were previously reared. Each tank was continuously aerated using an air stone connected to a blower and equipped with a heater (28 ± 1°C). An experiment was conducted to acclimate the PLs to low salinity (1 g L^{-1}) over 24 hours (A24h) using the following protocol treatments: (1) CON-SAL, a constant rate of salinity reduction at 1.45 g L^{-1} per hour, regardless of salinity level; and (2) VAR-SAL, following adaptations from Van Wyk et al. (1999) and McGraw et al. (2002). In the first four hours, salinity was rapidly reduced from 35 to 5 g L^{-1} at an initial rate of 16 g L^{-1} during the first hour, 9 g L^{-1} during the second hour, and 5 g L^{-1} during the third hour. This was followed by a slower reduction from 5 to 2 g L^{-1} at a fixed rate of 0.25 g L^{-1} per hour over 13 hours. Finally, salinity was further reduced from 2 to 1 g L^{-1} at 0.13 g L^{-1} per hour over the last 8 hours. In both treatments, salinity reduction was achieved via water exchange using municipal freshwater treated with 10 mg L^{-1} of chlorine (using 12% sodium hypochlorite). The added chlorine was removed through constant aeration for 24 hours.

In addition to the treatments, a control group (SW) was simultaneously maintained to simulate water exchange conducted in CON-SAL and VAR-SAL but using seawater (35 g L^{-1} at a 40% h^{-1} rate) to keep the same salinity level during the experimental A24h. All three experimental groups were evaluated in triplicate. No feed was provided for 24 h following the protocols described by Mousa &Taha 2003. At the end of the 24 h (A24h) of acclimation, PLs were kept for an additional 24 h

(A24h$^+$) in their corresponding tanks at the final salinity (1 g L^{-1}); likewise, the control group was kept for 24 h more. During the A24h$^+$ period, shrimp were fed according to the protocol described in section 2.1.

Water quality and survival

During A24h, water quality was monitored every hour measuring the following parameters: temperature (ºC), dissolved oxygen (mg L^{-1}) (YSI-55 multiparameter, YSI Inc., Yellow Springs, OH, USA), pH (pH meter YSI 100, Yellow Springs, OH, USA), total ammonia nitrogen (TAN, mg L^{-1}) (UNESCO 1983), and alkalinity (mg L^{-1} $CaCO_3$) (APHA 2012). Salinity was measured at each water exchange to corroborate the expected salinity reduction according to treatments using a refractometer (Atago Co. Ltd, Tokyo, Japan). In addition, the same parameters were measured at the end of A24h$^+$.

At the end of A24h and A24h$^+$, alive PLs from each tank were individually counted to calculate survival rate (%) according to the following equation:

Survival rate (%) = 100 × (number of alive PLs at the end of A24h or A24h$^+$) / (number of alive PLs at the beginning of A24h or A24h$^+$).

Histopathological and statistical analyses

Additionally, at the end of both periods (A24h or A24h$^+$), 15 whole-body shrimp from each treatment were collected for histology following the protocols described by Bell & Lightner 1988. Briefly, samples were fixed with Davidson's solution for 24 h and then immersed in a 70% ethanol solution. Later, the samples were dehydrated using a series of increasing ethanol concentrations (70, 80, 90, and 100%), cleared in a xylene solution, and embedded with paraplast. Histological sections of 5-µm thickness were obtained with the aid of a microtome (Leica HistoCore Autocut, Wetzlar, Germany), mounted on a Poly-L-lysine solution-coated slides, and stained with hematoxylin-eosin. The sections were examined under a light microscope (NikonEclipse-E200, Tokyo, Japan) coupled to a digital camera for gill and hepatopancreas image acquisition with 10, 20, 40 and 60 objectives to detect damages, cellular and structural changes. Ten tubules per replica (n = 50 per

treatment) were randomly selected and observed for B-cell number quantification, ruptured epithelial cells, dilated central tubules, and degenerated tubule lumen for each treatment (Romano et al. 2015).

Prior to the analyses, survival data on percentage were arcsine transformed (Zar 2010), but only the untransformed values are presented. In addition, the statistical assumptions of normality and homoscedasticity were evaluated by Bartlet and Leveneˈs tests, respectively and two-way analysis of variances (ANOVA) to determine statistical difference between treatments. For water parameters, two-way ANOVA was performed to identify differences among experimental groups (control, CON-SAL, and VAR-SAL) and times (A24h and A24h^{+}), which followed by Tukey's *post-hoc* comparison test when significant differences were found. While Kruskall Wallis' (non-parametric) test was used to analyze numbers of B cells, ruptured epithelial cells, dilated central tubules and degenerated tubule lumen in hepatopancreas. All statistical analyses were executed at a level of significance of $P < 0.05$.

RESULTS

Water quality and survival

No significant statistical differences were found for the selected water quality parameters throughout the experimental period. The mean ± standard deviation, minimum and maximum values of the parameters are shown (Table 1). Figure 1 shows salinity variation according to the experimental groups throughout the trial.

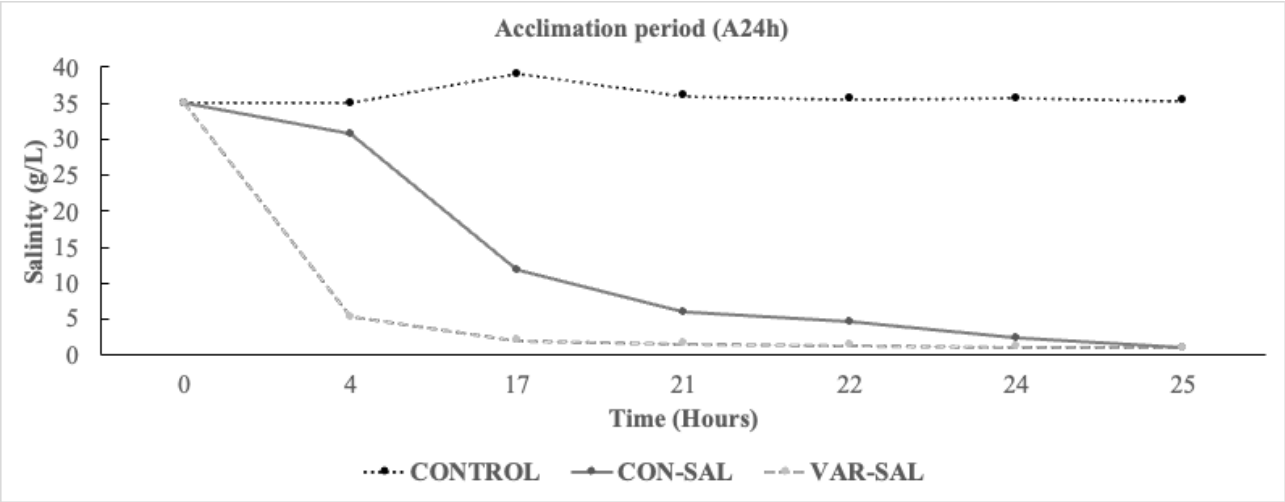

Figure 1. Salinity variation throughout the 24 h acclimatation (A24h) of *Penaeus vannamei* postlarvae to 1 g L^{-1} of salinity and the additional 24 h ($A24h^{+}$) in the control (35 g L^{-1} of salinity) and treatments with constant salinity and variable salinity reduction rates (CON-SAL and VAR-SAL, respectively).

Table 1. Mean (± standard deviation; SD), minimum (Min.), and maximum (Max) values of water quality parameters in the control (35 g L^{-1}) and the treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) throughout the experimental period ($P < 0.05$).

	CONTROL		CON-SAL		VAR-SAL	
	Mean (± SD)	Min-Max	Mean (± SD)	Min-Max	Mean (± SD)	Min-Max
Temperature (°C)	28.24 ± 0.48	27.5-29	28.00 ± 0.33	27.5-28.3	28.24 ± 0.45	27.5-29
Dissolved oxygen (mg L^{-1})	5.76 ± 0.35	5.24-6.23	5.61 ± 0.41	5.23-6.23	5.68 ± 0.36	5.20-6.22
pH	7.73 ± 0.51	7.13-8.55	7.87 ± 0.60	7.29-8.67	7.87 ± 0.55	7.2-8.52
Alkalinity (mg $CaCO_3$ L^{-1})	146.45 ± 15.64	125.3-161.1	161.00 ± 21.92	125.3-196	149.55 ± 33.02	107-196
Total ammonia nitrogen (mg L^{-1})	0.08 ± 0.19	0.0-0.5	0.11 ± 0.20	0.0-0.5	0.08 ± 0.19	0.0-0.5

At the end of A24h, survival rate was higher than 80% in all experimental groups, without showing statistical difference ($P < 0.05$) among them. Significant differences were found at the end of A24h$^+$ ($P < 0.05$) when lower survival was recorded in VAR-SAL (65.47%) in comparison with control (83.33%) and CON-SAL (81.07%) (Table 2).

Table 2. Survival (%) of *Penaeus vannamei* postlarvae in the control (35 g L^{-1}) and treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of the 24 h of acclimatation (A24h) and the additional 24 h (A24h$^+$). Different superscript letters within rows indicate significant differences ($P < 0.05$).

	Control	CON-SAL	VAR-SAL
A24h	88.67 ± 4.42	83.60 ± 4.45	84.07 ± 5.21
A24h$^+$	83.33 ± 4.16^a	81.07± 4.58^a	65.47 ± 6.27^b

Histopathology structure of postlarvae

For both A24h and A24h$^+$, control exhibited normal gill filament structure and no changes in tissue structure (Fig. 2a,d). Shrimp acclimated following a salinity reduction constant rate (CON-SAL) showed histological changes in comparison with the control characterized by the increase in the intercellular hemocytes at A24h. However, no progressive changes were observed 24 h later (A24h$^+$) (Figs. 2b,e). In the VAR-SAL treatment at A24h, several histological changes were observed including loss in normal structure of gill filaments and changes in tissue structure with increasing intercellular hemocytes and appearance of vacuoles indicating an epithelial edema shown by roughness of the gill filament surfaces. At A24h$^+$, shrimp from VAR-SAL exhibited progressive histological changes characterized by inflammation increase and appearance of high-density vacuole, indicating filament edema intensification (Fig. 2c,f).

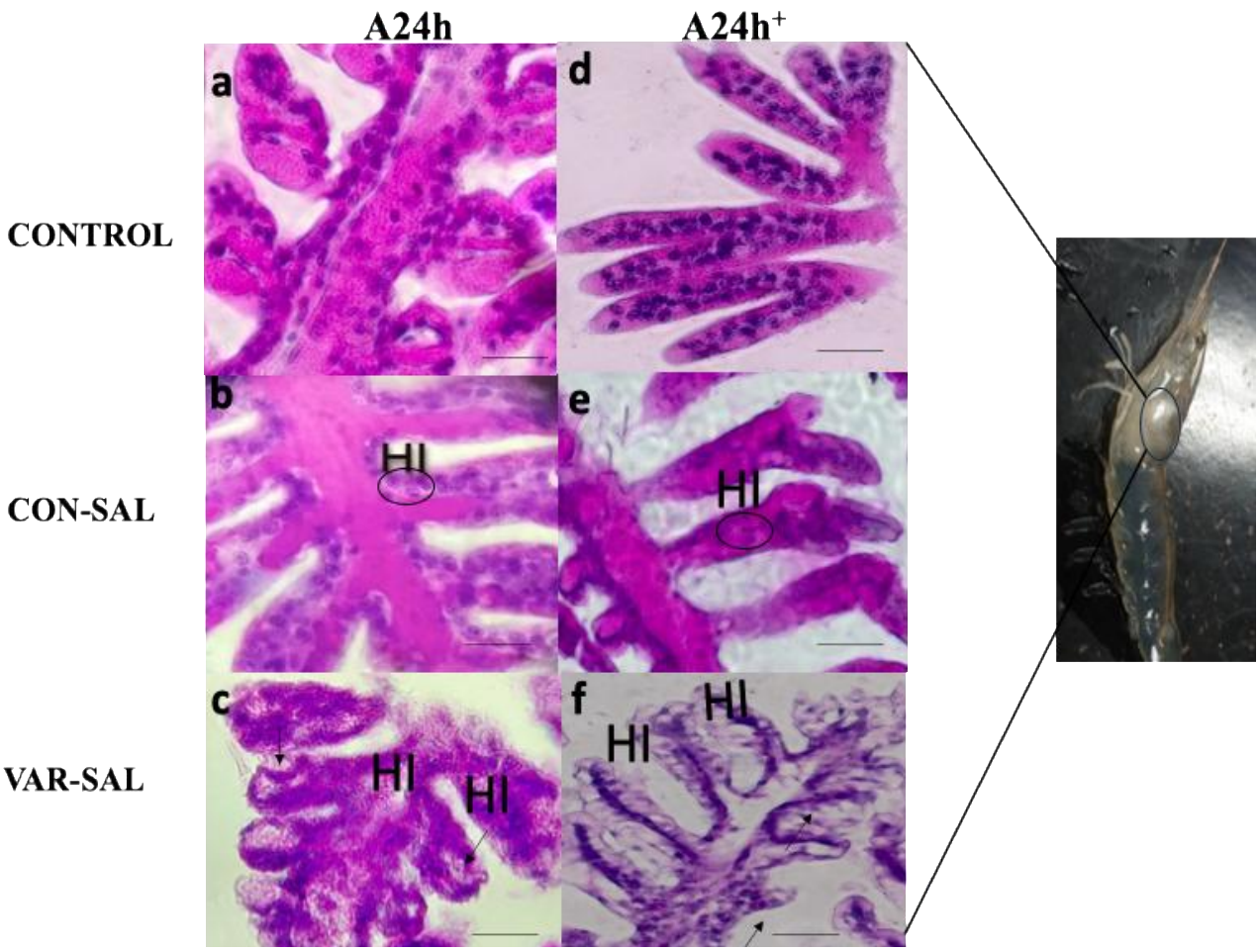

Figure 2. Histological microscopy of *Penaeus vannamei* postlarvae's gills in the control (35 g L^{-1}) and the treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of the 24 h of acclimation (A24h) to 1 g L^{-1} (a, b, and c, respectively) and of the additional 24 h ($A24h^{+}$) (d, e, and f, respectively). a) Control at A24h showed normal structure of gill filaments and no changes in tissue structure. d) Control at $A24h^{+}$ still showed no histological damages; b) CON-SAL at A24h showed an increase in amount of intercellular hemocytes (HC) in comparison with control. e) CON-SAL at $A24h^{+}$ still showed more intercellular hemocytes than control but with no increasing in comparison with CON-SAL at A24h. c) VAR-SAL at A24h showed several histological changes, such as loss of gill filament structure, increasing in hemocytic infiltration, and appearance of vacuoles resulting in a roughening of the surfaces of the gill filaments (epithelial oedema). f) VAR-SAL at $A24h^{+}$ still showed loss of gill filament structure, but with increasing in inflammation and appearance of high density of vacuoles resulting in intensification of the filament oedema. Sections of tissue were stained using Hematoxylin and Eosin (H & E), 40x. HI: hemocytic infiltration, V: vacuoles

At the end of A24h, PLs from the control exhibited typical histological features in the tubular hepatopancreas structure observed in *P. vannamei*. The tubules were closely arranged. The lumen was star-shaped and different cell types could be clearly observed under higher magnification, such as B and R cells (blassenzelen and restzellen cells) (Fig. 3a). For CON-SAL, the samples analyzed exhibited epithelium vacuolization, abnormal structures in the lumen and B cell presence (Fig. 3b). In contrast, PLs hepatopancreas structure from VAR-SAL exhibited severe histopathological damage, including ruptured epithelial cells, abnormal lumen shape formation, dilated central tubes, storage vacuoles absent and degenerated tubule lumen (Fig. 3c). At the end of A24h^{+}, no histological changes were observed for the control (Fig. 3d). For CON-SAL, the hepatopancreas structure exhibited epithelium vacuolization and increasing in B cell volume (Fig. 3e). While for PLs acclimated to VAR-SAL, abnormal lumens were observed, as well as the presence of B cells, tubes with a dilated and degraded appearance (Fig. 3f). In general, the histological changes were more severe in VAR-SAL compared with CON-SAL.

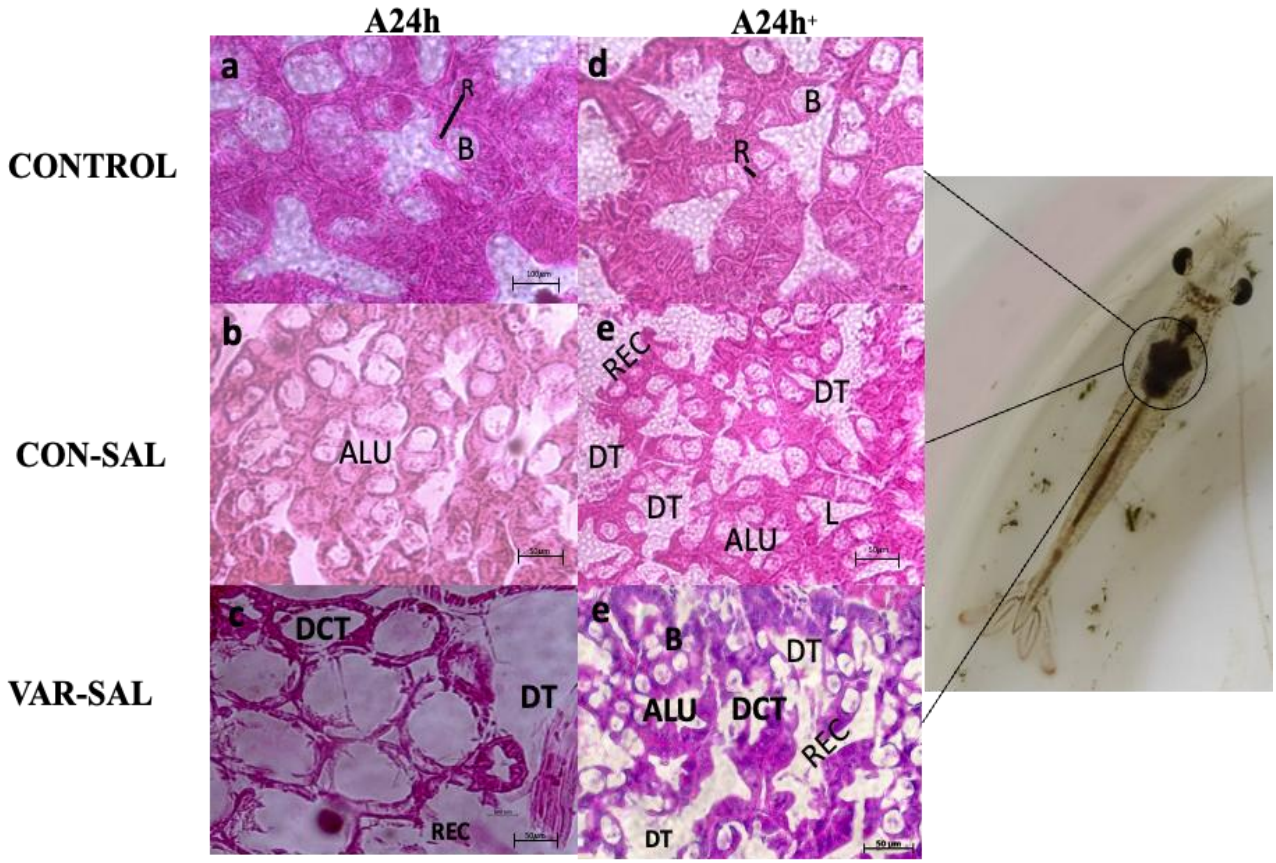

Figure 3. Histological microscopy of *Penaeus vannamei* postlarvae's hepatopancreas in the control (35 g L^{-1}) and the treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of the 24 h of acclimation (A24h) to 1 g L^{-1} (a, b, and c, respectively) and of the additional 24 h ($A24h^{+}$) (d, e, and f, respectively). a,d) Control showed a normal structure of hepatopancreas, with star shape-like lumen and presence of B and R cells in A24h and $A24h^{+}$, respectively. In 24h, b) CON-SAL showed the two types of cells (B and R cells) but exhibited vacuolization of the epithelium, abnormal structure of the lumen, and abnormal increased volume of B cells compared with control. c) VAR-SAL exhibited histological changes in the tubular glands and epithelial cells including cells (B and R cells) were not observed, tubule lumen showed dilatation, REC, DT, storage vacuoles were absent, and epithelial tissue was lost. In $A24h^{+}$, e) CON-SAL exhibited vacuolization of the epithelium, abnormal structure of the lumen, REC and DT. f) VAR-SAL was observed tubule lumen dilatation, REC, DT, but a recovery in the structure in comparison with the A24h time, exhibiting a reduction of dilatation in tubule lumen resulting in a normal appearance in some of the hepatopancreatic tubules; also, B cells were observed again. Hematoxylin-eosin. Bar 50 and 100 µm. B cell (blasenzellen cell, B), R cell (restzellen cell, R), L: star shape-like lumen, ALU: abnormal lumen, DCT: dilated central tube, REC: ruptured

epithelial cells and DT: degenerated tubule.

For A24h and A24h^{+}, VAR-SAL showed a significantly lower number of B cells and higher dilated central tubules, ruptured epithelial cells and dilated tubule lumen in comparison with CON and CON-SAL. No statistical differences were observed between CON and CON-SAL, except for B cell number at A24h^{+}, which was significantly higher in CON-SAL (Table 3).

Table 3. Mean ± standard deviation values of the histological damage of the hepatopancreas (n = 50 per experimental group) of *Penaeus vannamei* postlarvae in the control (35 g L^{-1}) and the treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of the 24 h of acclimatation (A24h) and the additional 24 h (A24h^{+}). Different superscript letters within rows indicate significant differences ($P < 0.05$). B cells: blassenzelen cells, REC: ruptured epithelial cells.

Parameter	A24h			A24h+		
	CON	CON-SAL	VAR-SAL	CON	CON-SAL	VAR-SAL
B cells	5.57 ±0.39^{b}	5.86±0.34^{ab}	3.22 ±0.55^{d}	5.67 ±0.40^{b}	6.18 ± 0.35^{a}	4.45 ± 0.55^{c}
Dilated central tubules	0.0 ± 0.0^{c}	0.24±0.24^{c}	0.96 ±0.14^{a}	0.0 ± 0.0^{c}	0.27 ±0.28^{c}	0.67 ±0.23^{b}
REC	0.0 ±0.0^{c}	0.04 ±0.06^{c}	0.40 ±0.13^{a}	0.0 ±0.0^{c}	0.03 ± 0.06^{c}	0.22 ± 0.22^{b}
Degenerated tubule lumen	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	1.12 ±0.10^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.77 ± 0.06^{c}

DISCUSSION

As expected, the salinity variation occurred according to the rates defined for each experimental group, resulting in maintenance at 35.93 ± 1.40 g L^{-1} in the acclimation control over 24 h (A24h); a reduction from 35 to 5 g L^{-1} in 21 h and from 5 to 1 g L^{-1} in 3 h in CON-SAL. In the VAR-SAL treatment, salinity reductions began with a rapid decrease in the first four hours, from 35 to 5 g L^{-1} at rates of 16 g L^{-1} during the first

hour, 9 g L^{-1} during the second hour, and 5 g L^{-1} during the third hour. This was followed by a slower reduction from 5 to 2 g L^{-1} at a fixed rate of 0.25 g L^{-1} per hour over 13 hours. Finally, salinity was further reduced from 2 to 1 g L^{-1} at 0.13 g L^{-1} per hour over the last eight hours.

This protocol was strategically implemented to simulate a rapid salinity reduction over a short period, allowing for the evaluation of the immediate adaptive capacity and physiological limits of *Penaeus vannamei* postlarvae under osmotic stress conditions that may occur in culture environments, where abrupt salinity changes require rapid physiological responses (Wu et al. 2009, Fregoso-López et al. 2017). Additionally, the variability in reduction rates provides insight into differentiated responses under progressive osmotic stress, which may not be fully observed under uniform reductions (Charmantier et al. 2001, Lemaire et al. 2002).

The protocol was adapted from Van Wyk et al. (1999) and McGraw et al. (2002), who investigated salinity tolerance thresholds in shrimp at different life stages. While their studies focused on survival, the current study expands this framework to evaluate the resilience of shrimp under rapid and gradual reductions, incorporating strategic change points (5 g L^{-1} and 2 g L^{-1}) as reported in previous research exploring histological damage and recovery (Lignot et al. 2000).

Considering that other water quality parameters showed no statistical differences and remained within recommended ranges for shrimp culture (Wyban et al. 1995, Van Wyk et al. 1999, Valencia-Castañeda et al. 2018), survival at A24h indicated that PLs tolerated the reduction from 35 to 1 g L^{-1} in 24 h, with survival rates exceeding 80% across all treatments. These results corroborate the ability of *P. vannamei* to regulate hypo-osmotically, as widely reported in the literature (Soyel & Kumlu 2003, Re et al. 2004, Díaz et al. 2004b). However, previous studies recommend gradual acclimation protocols exceeding 24 hours to minimize stress (Van Wyk et al. 1999, Jayasankar et al. 2009, Jaffer et al. 2020).

On the other hand, McGraw et al. (2002) evaluated PLs' survival during acclimation in the short-term and showed that the oldest PL (PL15 and PL20) were able of tolerating salinity reduction from 24 to 2 g L^{-1} at a rate of 4 g L^{-1} h^{-1}, and then from 2 to 1 g L^{-1} at 1 g L^{-1} h^{-1}, achieving approximately 6 h to reach the endpoint.

Survival rates were higher than 85% at 24 h from the beginning of salinity reduction. Therefore, our results in the present study agree with those previously reported by McGraw et al. (2002), indicating that PL older than 15 days can survive to salinity reduction up to 1 g L^{-1} in a short-term, e.g. 24 h. However, our study kept PLs for an additional period of 24 h, which revealed a significant survival reduction for VAR-SAL in comparison with the other experimental groups.

The histopathological analysis results in gill and hepatopancreas revealed changes that most likely explain the results recorded for survival. For A24h, PL gill filaments acclimated in the CON-SAL exhibited moderate and reparable structural changes and increased number of intercellular hemocytes in comparison with control. Nevertheless, no progressive changes were observed at A24h^{+}. On the other hand, several histological damages were observed in VAR-SAL, including vacuolization presence, hemocyte infiltration and loss in its normal structure. Moreover, damages in this treatment were progressively severe at A24h^{+}, showing an epithelial edema characterized by vacuolation. Fregoso-Lopez et al. 2017 exposed shrimp *P. vannamei* to low-salinity waters (1.9 g L^{-1}) under different stocking densities and with increasing concentrations of ammonia and nitrite as well as total suspended solids resulted in histological alterations in gills such as oedema and inflammation (haemolymph and haemocytic infiltration) during the first 5 weeks of culture. Secondly, Wu et al. (2009) observed gill alteration as vacuolation resulting in filament edema in *P. vannamei* exposed to different concentrations of cadmium (Cd) and zinc (Zn) for up to 28 days.

Edema in gills was previously reported in *Litopenaeus schmitti* reared at low salinity (8 g L^{-1}), which was attributed to a reduction in osmoregulatory capacity that affected vascular permeability (Laria-Lamela et al. 2005). In the present study, the edema observed in VAR-SAL is most likely related with the fast reduction from 35 to 5 g L^{-1} in only 5 h. Neufeld et al. (1980) reported that the edema observed in *Callinectes sapidus* exposed to low salinity could be an osmoregulatory capacity reduction consequence due to a vascular permeability decrease. Therefore, an increase in cell volume as a rapidly adaptive change to very rapid salinity change regulates ion transport across plasma membranes.

Osmoregulation represents an increase in energy requirement when shrimp is reared at salinities below its isosmotic point (28 g L^{-1}). For example, Rosas & Sanchez (1996) suggested that *Litopenaeus setiferus* required extra energy to regulate osmotic pressure and ionic concentration to maintain homeostasis when experiencing salinity change. According to the authors, the necessary energy was reallocated from other processes instead of increasing the total aerobic metabolism rate. Böer et al. (2007) reported that *Clione limacina* organisms obtain energy from the hepatopancreas lipid reserve to deal with osmotic stress. Although hepatopancreas has adaptation ability towards the short term stress factor that may clarify the appearance of swollen tubules, changes in lumen shapes and damaged cells were gradually restored as the culture period increased (Díaz et al. 2010). The hepatopancreas histological analysis has been reckoned as one of the important means for reflecting health status in shrimp (Wu et al. 2008, Sun et al. 2015).

In the present study, the control at the end of A24h and A24h+ showed the normal hepatopancreas structure previously reported for *P. vannamei* (Li et al. 2008, Chen et al. 2019, Jamshidizadeh et al. 2019). On the other hand, damages were observed in CON-SAL and VAR-SAL, being more severe in VAR-SAL, such as vacuolization and cellular disorganization in the hepatopancreas at A24h, but a histological recovery trend was observed in this treatment when comparing data on hepatopancreas damage between treatments by time; In the $24h^{+}$, a normal appearance in structure was exhibited with a less dilated structure and more presence of B compared with A24h. This finding is consistent with research demonstrating the ability of *P. vannamei* to exhibit adaptive plasticity, and that suggest that tissue damage caused by osmotic stress may be partially reversible if conditions are stabilized, as observed in the VAR-SAL treatment, which, despite the first four hours of rapid salinity reduction, with the last 20 hours of salinity reduction being performed gradually and slowly, indicating stabilized salinity conditions, contributed to some individuals showing signs of recovery after $24h^{+}$ (Li et al. 2008, Wu et al. 2009).

Similar results were reported by Chen et al. 2015 and Gao et al. 2017, that showed that the hepatopancreas of *P. vannamei*, exposed to abrupt changes in

salinity, can suffer significant structural damage (vacuolization, tubule dilation and cell necrosis), but that this damage is partially reversible if salinity is stabilized, and the organism has time to recover, as observed in the VAR-SAL treatments. However, this reversibility depends on the degree and duration of the initial stress. Extreme and abrupt changes, can induce a level of damage that, depending on the intensity, may not be completely reversible, leading to permanent tissue sequelae (Jayasankar et al. 2009, Cao et al. 2024), especially in organs that require high energy demand for osmoregulation processes. Therefore, although our study observed some signs of recovery in the A24h^{+} period, it is possible that deeper damage or repeated injuries compromise the long-term health of shrimp, especially in culture environments where other stressors are also present. Under stable salinity conditions, hepatopancreas can reestablish their structural integrity, reducing vacuolization and improving tubule organization, which suggests a potential for recovery (Calvo et al. 2011, Zhang et al. 2023), as were observed gradual acclimation protocols that maximize survival and reduce osmotic stress, allowing more effective tissue recovery, which is essential for the sustainable management of *Penaeus vannamei* (Jayasankar et al. 2009, Zhang et al. 2023).

According to Table 3, the results showed a decrease in B-cell number in VAR-SAL compared to control in A24h, which were found using the indicators that determine hepatopancreas structure damage in PLs with decreased salinity and significantly smaller than in the other treatments. On the other hand, the number of tubules with ruptured epithelial cells (REC) and dilated central tubes were significantly higher in PLs acclimated in VAR-SAL than CON-SAL and CON. Similarly, greater damage was observed significantly in VAR-SAL in terms of tubule degradation; corroborating histological images, B and R cells were not possible to identify in some fields, while no tissue loss was observed in the other groups. An increase was observed in some regions of shrimp hepatopancreas tubules in the number of B cells in VARS-SAL group during A24h^{+}. A notable reduction was observed in some indicators, such as dilated central tubules, REC and degeneration tubes compared to the same treatment for A24h. A small improvement was observed

in hepatopancreas tubes when maintained at 1g/l without acclimation as recovery in VAR-SAL hepatopancreas structure.

Structural changes were found in *P. vannamei* hepatopancreas in CON-SAL and VAR-SAL and observed in marine crustacean studies acclimated to low salinities, such as *Artemesia longinaris* (Masson 2001) and *Pleoticus muelleri* (Cuartas et al. 2003). In contrast, studies performed by Li et al. (2008), juvenile *P. vannamei* were acclimated gradually to the desired salinity by changing 2‰ per day from 22 to 3.0, 17.0 and 32.0‰. Firstly, the hepatopancreas of euryhaline shrimp *P. vannamei* was gradually acclimated at different salinities and did not exhibit histological alterations, except for an increase in B cell number in hepatopancreas tubules at 3.0‰. Secondly, B-cell increase in *P. vannamei* has been observed at low salinities, indicating that the high synthesis rate and digestive and antioxidant enzyme release accelerated nutrient mobilization in hepatopancreas tubules (Diaz et al. 2010, Liu et al. 2016). Thus, similar results were reported by Abad-Rosales et al. 2010 with juvenile *P. vannamei* exposed to Cu^{2+} concentration at salinities of 1, 5, and 10 practical salinity units (psu) for 25 days. The histological effects observed in the organism hepatopancreas grown in hypo-osmotic conditions are similar to those found by Li et al. (2008). The B number was altered at a salinity of 3 psu. B cells are the main site for digestive enzyme synthesis, which accelerate nutrient mobilization in hepatopancreas tubules when an increased energy demand is required to adapt to environmental stress. The decrease of these cell might be due to the utilization of their nutrient reserves, because of the increased energy demand for osmoregulation (Chen et al. 2020, Liu et al. 2023).

The histological changes found in the present study suggest that the organisms under stressful conditions, such as salinity challenges, often lead to an increase in reactive oxygen species (ROS), ultimately inducing oxidative stress that negatively affects crustaceans. Many crustacean species typically develop an immune response, such as salinity fluctuations to adapt to changing environments. However, if environmental stress exceeds an organism's tolerance, the production and removal of free radicals in the body become unbalanced, triggering tissue cell apoptosis, which can be detected through histological methods (Cao et al. 2021,

Frías-Espericueta et al. 2022, Huang et al. 2019). Pallavi et al. (2012) studied the decreasing salinities (30 to 20, 10, 5 g L^{-1}) and their effects on the antioxidant defense system, O_2 consumption, CO_2 release and NH3 excretion in *P. monodon* juveniles. The results suggest in low salinity ROS production in hepatopancreas is increased at 5 g L^{-1} of salinity, which might have caused increase in antioxidant enzyme synthesis.

The results in the present study found histological images and parameter hepatopancreas damages, when the organisms were maintained at 1g L^{-1} for A24h^{+}, VAR -SAL group, showing small recovery in the hepatopancreas structure, including normal appearance in the structure, with less dilated tubes and increasing B cells in compared to A24h. Likewise, Díaz et al. (2010b) and Masson et al. (2012) analyzed the histological recovery of shrimp hepatopancreas after re-acclimation of stressed conditions to control the salinity reported. Crustacean hepatopancreas is a key organ for reserve mobilization during peak energetic demand, such as during molting and reproduction (Marcolin et al. 2008). However, storage and/or use of reserves seem to depend on the species and its physiological condition. The same response was obtained in other osmoregulatory investigations, which found that osmoregulatory homeostasis was restored in approximately 24 h (Bursey & Lane 1971, Castille & Lawrence 1981, Li et al. 2017). Firstly, similar durations for recovery have been observed in association with salinity tolerance tests for other penaeid species (Ferraris et al. 1986, Chen & Lin 1994a, Saoud & Davis 2003). Secondly, Rosas et al. (1999b) showed that *P. setiferus* (Linnaeus) postlarvae (PL 10 to PL 14) can tolerate diluted media (up to 5‰ units) and are able to adapt in only 2 h to salinity changes. In contrast, Chen & Lin (1994b) found differences in acclimation salinities that may require longer periods for shrimp to maintain hemolymph osmolality, due to the nature of the physiological mechanisms involved with salinity tolerance tests.

The authors suggest that the VAR-SAL protocol, with an initial salinity reduction from 35 to 5 g/L at an initial rate of 16 g L^{-1} per hour (first hour), 9 g L^{-1} per hour (second hour) and 5 g L^{-1} per hour (third hour), was used to simulate a rapid and intense drop in salinity, consequently osmotic stress condition (Charmantier 1998, Rosas et al. 2001), followed by a gradual reduction rate (0.25

g/L/h from 5 to 2 g/L and 0.13 g/L/h from 2 to 1 g/L), allowing a more controlled transition and more time for adjustment of osmoregulation mechanisms. This combination of abrupt reduction followed by gradual transitions between rapid events of osmotic stress and periods of stabilization. This approach allows us to observe how the organism responds to initial intense stresses and its recovery potential, since periods of stabilization provide time for physiological adaptation (Liu et al. 2024). In shrimp, this type of transition may result in adjustments in osmoregulation mechanisms, including increased enzymatic activity and energy demand to maintain osmotic balance. However, the initial more intense stress may induce significant histological damage in organs such as the gills and hepatopancreas, leading to vacuolization, tubule dilation and cellular disorganization, which are common responses to severe osmotic stress (Rosas et al. 2001). Under favorable conditions, where salinity gradually stabilizes, these organisms may have time to repair the damage and reestablish homeostasis, as suggested by Burgents et al. (2004) and Chen et al. (2019). However, in prolonged exposures or with very abrupt variations, stress can become chronic, making complete recovery difficult and compromising long-term health and growth (Chen et al. 2019, Liu et al. 2024).

In conclusion, our data in the present study suggest that *P. vannamei* postlarval acclimation to reduced salinities of 35 to 1 g L^{-1} in 24 h has revealed important information on the physiological responses based on survival and histological damage. The CON-SAL group, showed survival above 80%, using a constant salinity reduction rate of 1.45 g L^{-1} with small histological damage to gills and hepatopancreas. Additionally, better health status of the hepatopancreas should be considered the most recommended and efficient protocol management during the acclimation process. Improving the survival of PLs directly impacts final yield potentials in a shrimp farming operation besides the ability to acclimate PLs to low salinities in the shortest possible time, resulting in production cost reduction. These findings emphasize the importance of adopting appropriate acclimation rates to minimize osmotic stress in *P. vannamei* postlarvae. The combination of classical references (Van Wyk et al. 1999, McGraw et al. 2002) and recent studies (Cao et al.

2024, Liu et al. 2024) reinforces the need for protocols that balance acclimation time and the intensity of salinity changes to optimize the survival and health of organisms in cultured environments.

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CAPITULO 3

Articulo 2: Physiological and metabolism responses of white-legged shrimp, *Penaeus vannamei* postlarvae exposed to hyporosmotic stress induced by reduced salinity acclimation in a short period of time: Two ranges of salinity (35-5g/L and 5-1g/L)

Manuscrito en revisión por co-autores para someterse Aquaculture

Physiological and metabolism responses of white-legged shrimp, *Penaeus vannamei* postlarvae exposed to hyporosmotic stress induced by reduced salinity acclimation in a short period of time: Two ranges of salinity (35-5g/L and 5-1g/L)

Abstract

Although *Penaeus vannamei* is a euryhaline species that tolerates a wide range of salinities (0.5 to 40 g/L), there is ambiguity in its osmoregulation pattern and variations in its tolerance to low salinity waters. In this study was determining the critical range (35-5 and 5-1g/L) of salinity reduction on the health of *Penaeus vannamei* postlarvae over 24 hours of acclimatation (LW24H): Group 1 (35-5 g/L) included *1^{a}Shock*, *1^{b}Fast* (0.46% per 4 hrs), and *1^{c}Gradual* (1.45 g/L per 22 hrs) treatments. Group 2 (5-1 g/L) included *2^{a}Shock*, *2^{b}Fast* (1.45 g/L per 4 hrs), and *2^{c}Gradual* (0.25-0.13 g/L per 22 hrs) treatments. Control groups (35 g/L and 5 g/L) were maintained without reduction, with rate of change of 40%/h. At the end of LW24H, all treatments were maintained for an additional 24 hours during a non-acclimation period (LW24H+). Survival was determined and PLs were collected to determine the oxygen consumption rate (OCR) and histological analysis of gills and hepatopancreas during trial period. Results showed that there was a decreasing trend in survival rates in both groups, being significantly higher in the CN35 (35-5 g/L) and lowest in *2^{a}Shock* and *2^{b}Fast* treatments (5-1 g/L), with values higher than 80% in LW24H and LW24H+. A trend of increasing oxygen consumption across all treatments, with the highest OCRs (~8.2 mg O2/g/min) in the *2^{a}Shock* and *2^{b}Fast* treatment (5-1 g/L). Histological analysis revealed severe gill and hepatopancreas damage in shock and fast treatments, especially in the 5-1 g/L group, with signs of

vacuolization, necrosis, and disorganized tissue. No recovery was observed in 2ª*Schock* treatments in LW24H**+** period. In conclusion, the most critical range of salinity reduction was 5-1g/L which showed a negative effect on survival, oxygen consumption, and tissue integrity in *Penaeus vannamei* postlarvae and, without any tissue recovery during the non-acclimatization period in *2ªSchock* treatment, suggesting that gradual acclimation of the 35-5 g/L range is most recommended for postlarvae in low-salinity conditions.

Keywords: Structural damage, salinity acclimation, Low salinity aquaculture

1. Introduction

Aquaculture production of euryhaline crustaceans has recently expanded from the coastal environment to inland regions that have a low-salinity groundwater supply (water salinity around 1 to 6 g/L), making use of cleaner water resources and promoting economic development in these areas (Li *et al.*, 2017). Recently, low-salinity water has been increasingly used for aquaculture of euryhaline crustacean species to utilize the available water and maximize economic benefits. In 2020, *Penaeus vannamei* shrimp was classified as the most produced species with 6.8 Mmt (FAO, 2024) and the farming of this organism in low salinity has become a successful practice globally, as it is a euryhaline species known for its strong osmoregulatory capacity and tolerance to salinities ranging from 0.5 to 50 g/L, being the species of choice (McGraw and Scarpa, 2004, Cheng *et al.,* 2006, Walker *et al.,* 2009, Wang *et al*., 2019).

Despite its adaptability, changes in salinity still significantly impact the physiological and metabolic processes of aquatic organisms, which in turn affect their growth and development (Rivera-Ingraham *et al*., 2017, Yuan *et al*., 2017, Velasco *et al*., 2019, Tian *et al*., 2020). Maintaining osmotic balance under fluctuating salinities is energy intensive, causing changes in survival, growth, development, antioxidant defense, and metabolic activities (Sokolova *et al*., 2012, El-Leithy *et al*., 2019). Furthermore, maintaining internal ionic homeostasis, normal cellular function, and other physiological processes in aquatic animals is energy intensive (Setiarto *et al*., 2004, Silvia *et al*., 2004). Importantly, even more energy may be required for osmoregulation at low salinity (Tseng and Hwang, 2008). Some reports have indicated that nearly 20% to 50% of metabolic energy was utilized in

regulating osmotic pressure in response to hypotonic stress in some aquatic animals (Fielder *et al*., 2005, Laiz Carrion *et al*., 2005, Li *et al*., 2017), leading to lower energy availability for animal growth and development when raised at low salinity.

Studies have shown that *Penaeus vannamei* can thrive in low salinity environments (Ednoff, 2001, Samocha *et al*., 1998, 2002), and it is reported that most previous research has focused on the growth of white shrimp at low and medium salinity. Postlarvae (PLs), which are a very important stage for white shrimp, besides being efficient in the osmoregulation process, are few being explored in studies at low salinity to improve the health of PLs that will greatly contribute to the growth performance of shrimp at low salinity (Charmantier *et al*., 2009, Niu *et al*., 2011, Miandare *et al*., 2016). Survival in the initial rearing phase (Postlarvae) depends largely on efficient acclimation protocol to respond properly to salinity changes (Laramore *et al*., 2001, McGraw *et al*., 2002, Roy *et al*., 2009). Postlarvae (PL) are often transported in high salinity; therefore, acclimating them to low salinity is crucial for successful rearing (Samocha *et al*., 2002, McGraw and Scarpa, 2004), so that this organism can respond to the physiological changes caused by these conditions (Roy *et al*., 2009). Rapid changes in salinity disrupt osmotic balance, requiring high energy for osmoregulation (Chong-Robles *et al*., 2014). Gills play a key role in osmoregulation during these transitions (Charmantier, 1998, Péqueux *et al*., 2006, Pham *et al*., 2012) and hepatopancreas has been widely used as a practical method to assess the impact of environmental stressors on shrimp farming (Wu *et al*., 2008, Sun *et al*., 2015). This organ plays essential roles in osmotic regulation during salinity fluctuations, in addition to being crucial for digestion, nutrient absorption and metabolic regulation (Ceccaldi, 1997).

Acclimation to low salinity in *Penaeus vannamei* and other euryhaline crustaceans initiates several physiological adaptations essential for maintaining osmotic balance. Research indicates an increase in oxygen consumption as energy demands rise for osmoregulation to maintain ionic balance (McGraw and Scarpa, 2004). Enhanced activity of Na+/K+-ATPase and carbonic anhydrase in the gills supports ionic regulation (Charmantier, 1998, Henry, 2001), while changes in mitochondrial density within chloride cells aid in energy production for ion transport (Péqueux, 1995). Additionally, decreased membrane permeability helps reduce ionic loss, and increased amino acid oxidation provides alternative energy sources to support osmotic homeostasis under salinity stress. These physiological changes collectively improve survival in low-salinity environments. For organisms such as shrimp, ion exchange across epithelial cell membranes is critical to protect against the hypertonic or hypotonic effects caused by salinity fluctuations while ensuring structural integrity (Li *et al*., 2008).

The growth and development of aquatic animals in low salinity environments largely depend on their ability to adapt to hypotonic stress and regulate osmotic pressure (Péqueux, 1995, Geng *et al*., 2016). The euryhaline white shrimp *Penaeus vannamei* lives in both coastal and oceanic areas and is capable of surviving over a large range of salinities. There is ambiguity on their osmoregulation pattern and variations in their tolerance to low salinity waters. Although several studies address survival and acclimation rates (Aquacop, 1991, Rosas *et al*., 1999, Tsuzuki *et al*., 2000, Davis *et al*., 2004), research on the impacts of reduced salinity (35–5 g/L and 5–1 g/L) on post-larval health, including physiological, metabolism and histological assessments, remains limited. This study aims to identify critical salinity ranges for

post-larvae, improving acclimation strategies to low salinity environments, which is essential for sustainable shrimp farming in inland waters.

2. Material and methods

2.1 Experimental design and shrimp postlarvae acclimation

To determine the effect of the salinity reduction critical range in shrimp postlarvae during in short-term stress. Specific pathogen-free (SPF) shrimp postlarvae were obtained from a commercial hatchery of Mariculture del Pacific, Sinaloa. *Penaeus vannamei* were maintained to laboratory conditions for one weeks prior to the experiment in fiberglass tanks (capacity: 1000 L) with filtered seawater, constant aeration, photoperiod of 12:12 h and the salinity, temperature and pH were 35 g/L, 28.0 °C and 8.0, respectively. PLs were fed with commercial pellets (chemical compositions: 35% crude protein, 8% crude lipid, 3% crude fiber, 14% ash and 10% moisture produced by Hvorash Company, Bushehr, Iran) five times a day (6:00, 9:00, 13:00, 15:00 and 18:00) (Davis and Arnold, 1993, Liu and Lawrence, 1997) during seven day.

After this period, a second acclimation was performed by separating two batches of shrimp in two tanks (T1 and T2) with initial salinity of 35g/L, being that in tank 2, the shrimp have gone through a salinity reduction process from de 35 to 5g/L for period of one week by adding de-chlorinated freshwater and maintained for a further 5 days for adapting of PLs to salinity 5g/L. After properly acclimated, PLs were distributed in experimental units by then, start the trial.

Eighteen-day-old *Penaeus vannamei* postlarvae ($PL_{18,}$ n=1440) with mean initial weight, 0,0028 ± 0.02 g were selected randomly and distributed into Twenty-four

tanks (useful volume of 16 L), and acclimated to low salinity via its decreasing from two ranges during 24 hours period (LW24H): 35 to 5 g/L and 5 to 1 g/L according difference salinity reduction rate (Van Wyk *et al*., 1999, McGraw *et al*. 2002, Esparza-Leal et al., 2016): Group I (of 35 to 5 g/L): *1ªShock* treatment (without acclimation), *1ᵇFast* treatment, constant rate of 0.46% in 4 hrs, *1ᶜGradual* treatment, constant rate of 1.45 g/L in 22 hrs, and, Group II (of 5 to 1g/L): *2ªShock* treatment (without acclimation, direct transfer), *2ᵇFast* treatment, constant rate of 1.45g/L in 4 hrs, *2ᶜGradual* treatment, variables rate, of 0.25 (35 a 5g/L) and 0.13g/L (5 a 1g/L) in 22 hrs. Two groups were reduced salinity by adding de-chlorinated freshwater until achieved the desired salinity and monitored with an optical refractometer. In addition, two control groups (CON35 and CON5) was maintained at a salinity of 35 and 5, respectively with seawater to 40%/h. All experimental groups were evaluated in triplicate and kept for more than 24 hours after the end of the acclimation (LW24H+), without water renewal and end point salinity of 5 and 1g/L, except CON35.

2.2 Physical–chemical parameters

During and at the end of the acclimation period, parameter such as, water temperature (ºC) and dissolved oxygen (mgL-1) were measured using a YSI-55 multiparameter (YSI Inc., Yellow Springs, OH, USA). In LW24H, water samples were collected from each tank to analyze for pH (pH meter YSI 100, Yellow Springs, OH) and total ammonia nitrogen (N– (NH_3^- + NH_4^+)) were analyzed using the method UNESCO (1983). The salinity was measured at each water change to confirm salinity reduction with refractometer equipment.

2.3 Sampling

At the end of acclimation (LW24H) and 24 hours after the end of the non-acclimation period (LW24H+), all postlarvae of trial were counted by experimental unit for evaluated to survival (%) calculated according to the following equations: Survival rate (%) = 100 x (number of shrimp at the end of the experiment) / (number of shrimps at the beginning of the experiment). While 15 whole-body shrimp from each treatment was collected for histological analyzes where were preserved in David solution and 10 live PLs were collected to determine the oxygen consumption rate.

2.4 Oxygen consumption rate as function of low salinity

At the end of the acclimation, oxygen consumption rate (OCR) was measured in a semi-open respirometer system, as described by Díaz *et al.* (2007). The system consisted of 20 chambers of 1 ml each. At the end of 24 hours of exposure in low salinity, and with the desired final salinity, 15 postlarvae from each treatment were transferred to the respirometry system and placed individually in each chamber. All measurements were made between at the end of each acclimation depending on the treatment and group. To minimize the possible influence of body weight on oxygen consumption, we used a narrow weight interval (mean ± SD wet weight, 0.02 ± 1.1 g) of individuals. Initial and final O_2 concentrations were measured with dipping probe oxygen mini-sensors (Loligo Systems, Copenhagen, Denmark) connected to a PC-controlled fiber optic trace oxygen transmitter (PreSens OXY-10 trace transmitter; Precision Sensing GmbH, Regensburg, Germany). The results of the oxygen consumption rate were obtained in mg O_2 h^{-1} kg^{-1} wet weight.

2.5 Histological examination of the gills and hepatopancreas of the postlarvae

To investigate the effects of critical range of salinity reduction in *Penaeus vannamei*, histological examinations were performed to find abnormalities according to routine histological methods using samples of the gill and hepatopancreas of the postlarvae during LW24H and LW24H+ period. In order to avoid autolysis of the tissues, 15 PLs per group were examined following the protocols described by Bell and Lightner (1988), samples were dissected out, preserved in Davidson's solution for 24 hours and, transferred and immersed in the ethanol 70%. Posteriorly, the organisms were dehydrated through a graded series of increasing concentrations of ethanol (100%,90%,80% and 70%), cleared with xylene solutions, and infiltrated with liquid paraffin at 60°C and embedded in paraffin blocks. Histological sections with 5µm thickness were obtained with the aid of a microtome (Leica HistoCore AutoCut) and, mounted on a Poly-L-Lysine solution-coated slides, and stained with Hematoxylin-Eosin (H&E stain). Finally, samples were examined using light microscope (NikonEclipse-E200, Japan) and photographed with digital camera coupled to microscope to detect the structural damages.

2.6 Statistical analyses

Prior to the analyses, survival data on percentage were first arcsine transformed (Zar, 2010). The homoscedasticity of variances and normality were evaluated by Bartlet and Leveneˈs tests, respectively from data of survival, water quality and histological parameters and then a one-way ANOVA within and among treatments was applied. One-way analysis of variances (ANOVA) was followed by Tukey's post-hoc comparison test when significant differences were found. While Kruskal-Wallis

(non-parametric) test was used to analyze the oxygen consumption data. All statistical analyses were executed at a level of significance of $p < 0.05$.

3. Results

3.1 Survival indexes

The effects of reducing salinity from 35 to 5g/L and 5 to 1g/L range on the survival during *P. vannamei* postlarvae acclimation are shown in Figure 1a and 1b. PLs acclimated with reduction of salinity, showed by post hoc Tukey tests a tendency towards reduction in survival and significant difference between the treatments of the 35-5 and 5-1g/L ranges both in LW24H and LW24H+. In LW24H, the survival was significantly lower in the 2^{a}*Shock* (85%) and 2^{b}*Fast* (88.18%) acclimation treatment of 5-1g/L compared to the CON35 (98.33%) treatment of the postlarvae acclimated in 35-5g/L range. Being that the greatest survivals were found in the 35-5g/L range, with 98.33% in the CON35, followed by 1^{c}*Gradual* (97.22%) and 1^{b}*Fast* (95%) treatments (Fig 1). In LW24H+, CON35 treatment of the 35-5g/L range was significantly greater in survival (97.11%) than all treatments of 5-1g/L range with 93.33%, 89.44%, 86.11% and 82.77% of the CON5, 2^{c}*Gradual,* 2^{b}*Fast and* 2^{a}*Shock*, respectively ($p < 0.05$) (Fig 2).

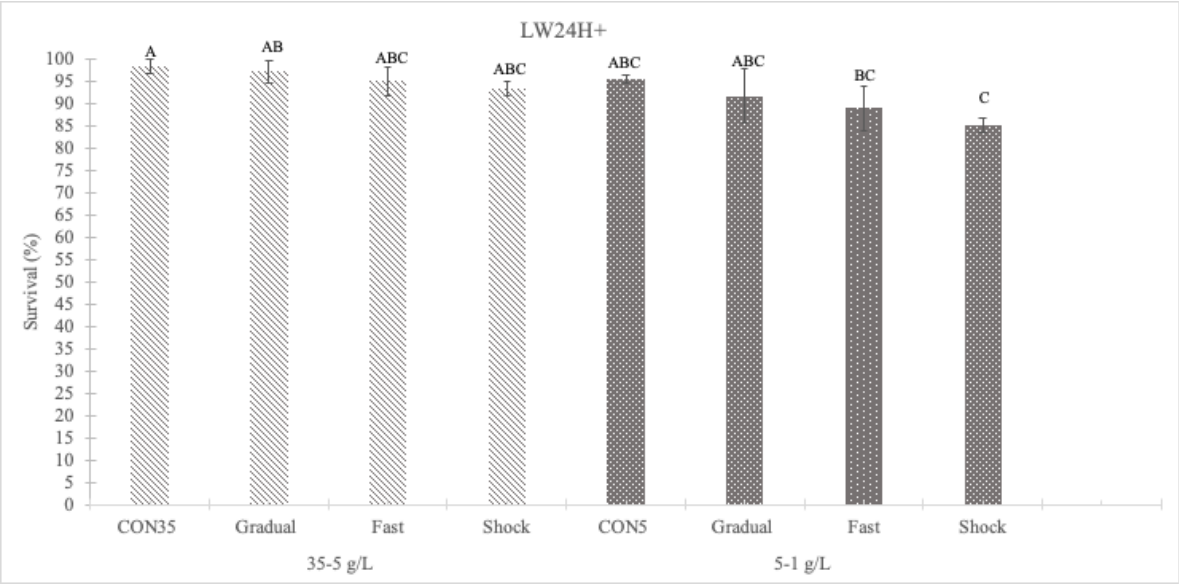

Figure 01: Survival rate of *Penaeus vannamei* postlarvae exposed to two salinity reduction ranges (35–5 g/L and 5–1 g/L) during the 24-hour acclimation period. Different letters indicate statistically significant differences ($p < 0.05$)

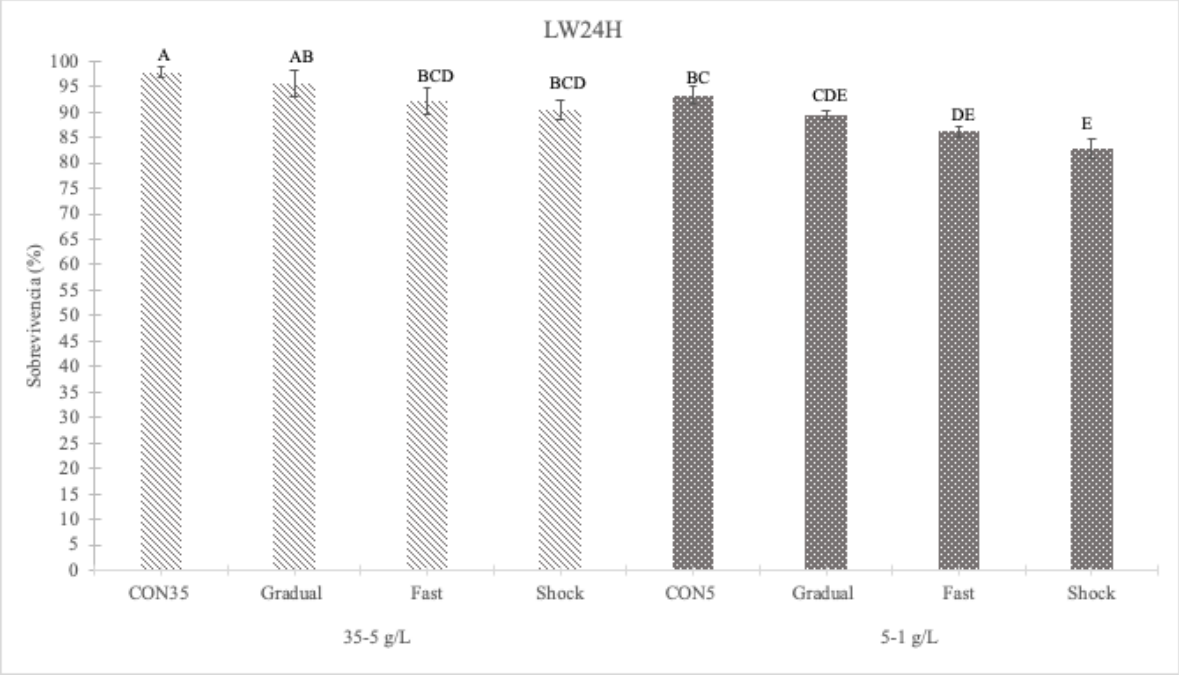

Figure 02: Survival rate of *Penaeus vannamei* postlarvae exposed to two salinity reduction ranges (35–5 g/L and 5–1 g/L) after the non-acclimation period (A24h+).

Different letters indicate statistically significant differences ($p < 0.05$).

3.2 Physical–chemical parameters

For 35-5g/L and 5-1g/L ranges, after 24 hours of acclimation process (LW24H), no significant differences ($p > .05$) were found in the parameters related to water quality, such as temperature (>27ºC), oxygen dissolved (~ 5.30 mg/L) and ammonium (~ 0.2 mg/L) and, remained at levels appropriate for the shrimp *Penaeus vannamei* (Table 01).

Table 1: Means (± SD) values of the physicochemical parameters of water during 24 hours of acclimation of *Penaeus vannamei* postlarvae exposed to the critical range of low salinity ($p < 0.05$).

	Ranges							
	35-5 g/L				5-1 g/L			
Water quality	**CON35**	**1ªGradual**	**1ªFast**	**1ªSchock**	**CON5**	**2ªGradual**	**2ªFast**	**2ªSchock**
Temperature (°C)	27.1 ±0.10	27.27 ±0.06	26.93 ±0.15	27.1 ±0.17	26.93 ±0.06	27.03 ±0.06	27.17 ±0.29	27.03 ±0.06
Dissolved oxygen (mg/L)	5.33 ±0.06	5.25 ±0.06	5.33 ±0.06	5.41 ±0.04	5.46 ±0.06	5.29 ±0.06	5.39 ±0.01	5.29 ±0.04
Ammonium (mg/L)	0.00 ±0.00	0.03 ±0.01	0.00 ±0.00	0.00 ±0.00	0.04 ±0.02	0.03 ±0.01	0.03 ±0.02	0.00 ±0.00
Salinity endpoint (g/L)	35	5	5	5	5	1	1	1

3.3 Oxygen consumption rate as function of low salinity

In LW24H, there was a trend towards an increase in oxygen consumption in both ranges. The highest OCR were recorded at range 5-1g/L salinity reduction, being

PLs exposure in *2ªSchock* and *2ᵇFast* generated a significant increase in the respiratory rate of 8.61mg O_2/g/min and 7.97mg O_2/g/min, respectively. While, *1ªShock* in 35-5g/L range also recorded higher consumption of 8.25mg O_2/g/min. On the land, PLs acclimated in CON35 was significantly lower with 4.85 mg O_2/g/min in 35-5g/L range (Fig. 03).

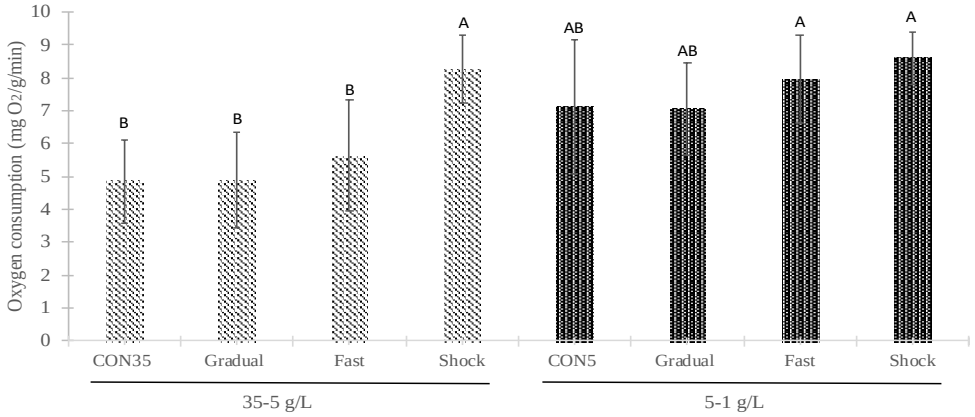

Figure 3*:* Oxygen consumption rate (OCR) of *Penaeus vannamei* postlarvae exposed to two salinity reduction ranges (35–5 g/L and 5–1 g/L) during the acclimation period (LW24H). Different letters indicate statistically significant differences ($p < 0.05$).

3.4 Histological alteration of tissues of *Penaeus vannamei* postarlarvae

Gills and hepatopancreas tissue alterations of *Penaeus vannamei* postlarvae acclimated to 35-5g/L and 5-1g/L range with salinity reduction during LW24H and LW24H+ were identified by histological analysis as shown in Figs. 4, 5, 6 and 7. In group I (35-5 g/L), gill collected during (LW24H) and at the end of the trial (LW24H+) in the PLs acclimated in the control treatment (CON35), the gill filaments of the

shrimp were arranged in an orderly manner, with no lesions observed, displaying a complete branchial structure with normal lamellar structure (Figs. 4a and 4e). In LW24H, PLs exposed to *1ªShock* treatment revealed significant changes in gill anatomy after a sudden reduction in salinity. Severe damage included severe signs of tissue collapse, with most cells distorted and swollen. Gill structure was highly compromised with loss of definition and disorganization of gill filaments. In addition, marked infiltration of hemocytes, suggesting severe inflammation, intense vacuolization and marked necrosis, with areas of cellular destruction visible on the lamellae. (Fig. 4b.

In *1ᵇ Fast* treatment, with rapid reduction of salinity, also caused significant damage, such as signs of thickening of the lamellae, hemocyte infiltration, pronounced vacuolization and with visible swelling of the epithelial cells. The gill filaments were disorganized and distorted, but with areas of preserved structure (Fig. 4c). In PLs exposed to *1ᶜ gradual* treatment, presented less damage in relation to the other treatments, with total preservation of the gills, there was no significant necrosis, vacuolization was absent and hemocyte infiltration was minimal, with the filamentous structure maintained intact. (Fig. 4d).

In LW24H and LW24H+, in the group II (5-1 g/L), the gill filaments of the postlarvae acclimated in CON5 treatment, exhibited gills remained healthy and without signs of alterations, that is, the structure of the filaments was completely preserved, with no signs of disorganization or delay of the lamellae, and the epithelial cells were healthy and functional (Figs. 5i and m). PLs exposed to *2ªShock* treatment, after salinity osmotic shock, exhibited the most severe gill damage of all treatments in the 5-1 g/L group, for example, disorganization of the gill lamellae, the

lamellae lost part of their integrity, signs of hemocyte infiltration, suggesting tissue inflammation, severe necrosis in some areas and vacuolization indicating epithelial edema (Fig. 5j). In *2^{b}Fast* treatment, with rapid reduction of salinity, presented damage to the gills, such as hemocyte infiltration, vacuolization and cell death in some areas (necrosis) (Fig. 5k). In PLs exposed to *2^{c} gradual* treatment presented some damage when acclimated to the gradual reduction in salinity, such as thickening of the lamellae, small areas with necrosis were observed, but most of the lamellae remained healthy, some cells presented vacuoles, as well as hemocyte infiltration, with little visible inflammation, with less intense damage being observed than the other treatments in the 5-1g/L group (Fig. 5l).

After the non-acclimation period (LW24H+), in the group I, PLs acclimated to *1^{a}Shock* treatment (35-5 g/L), evident epithelial edema was observed, with the presence of vacuolization. The inflammatory response was observed with hemocyte infiltration. The lamellae maintained most of their structure, with specific areas showing cellular disorganization and necrosis, and no recovery was observed after the period non-acclimation (Fig. 4f). In the *1^{b} Fast* treatment, the gills showed significant evidence of hemocyte infiltration and vacuolization. An improvement in the tissue is observed, with reduced inflammation and some areas maintaining cellular integrity (Fig. 4g). In contrast, PLs exposed to *1^{c}Gradual* treatment, with almost no necrosis and vacuolization, with only a few cells showing swelling, without significant evidence of hemocyte infiltration and free from signs of inflammation. The gills are well organized and preserved, with defined lamellae and healthy tissue structure, resulting in a virtually preserved structure, with few signs of stress or damage (Fig. 4h).

In LW24H+, in the group II (5-1 g/L), PLs exposed to *2^{a}Shock* treatment, severity of the damage remained high, suggesting that the treatment did not allow adequate recovery of the gills. The structure of the filaments was compromised, with some regions showing loss of organization and distortion of the gill lamellae, with damage such as infiltration of hemocytes between the gill filaments, necrosis and vacuolization in the epithelial cells (Fig. 5n). In *2^{b}Fast* treatment, a slight improvement was observed, but the damage is still noticeable, with some areas showing cells exhibiting significant vacuoles, inflammatory cells and slight cellular disorganization (Fig. 5o). PLs exposed to *2^{c}Gradual* treatment, showed visible improvement, the gills were preserved, the lamellae maintained complete structure and organization, with no visible signs of significant necrosis or vacuolization, there was no visible hemocyte infiltration or inflammation, suggesting a significant structural recovery compared to the histological damage exhibited in the same treatment during LW24H period (Fig. 5p).

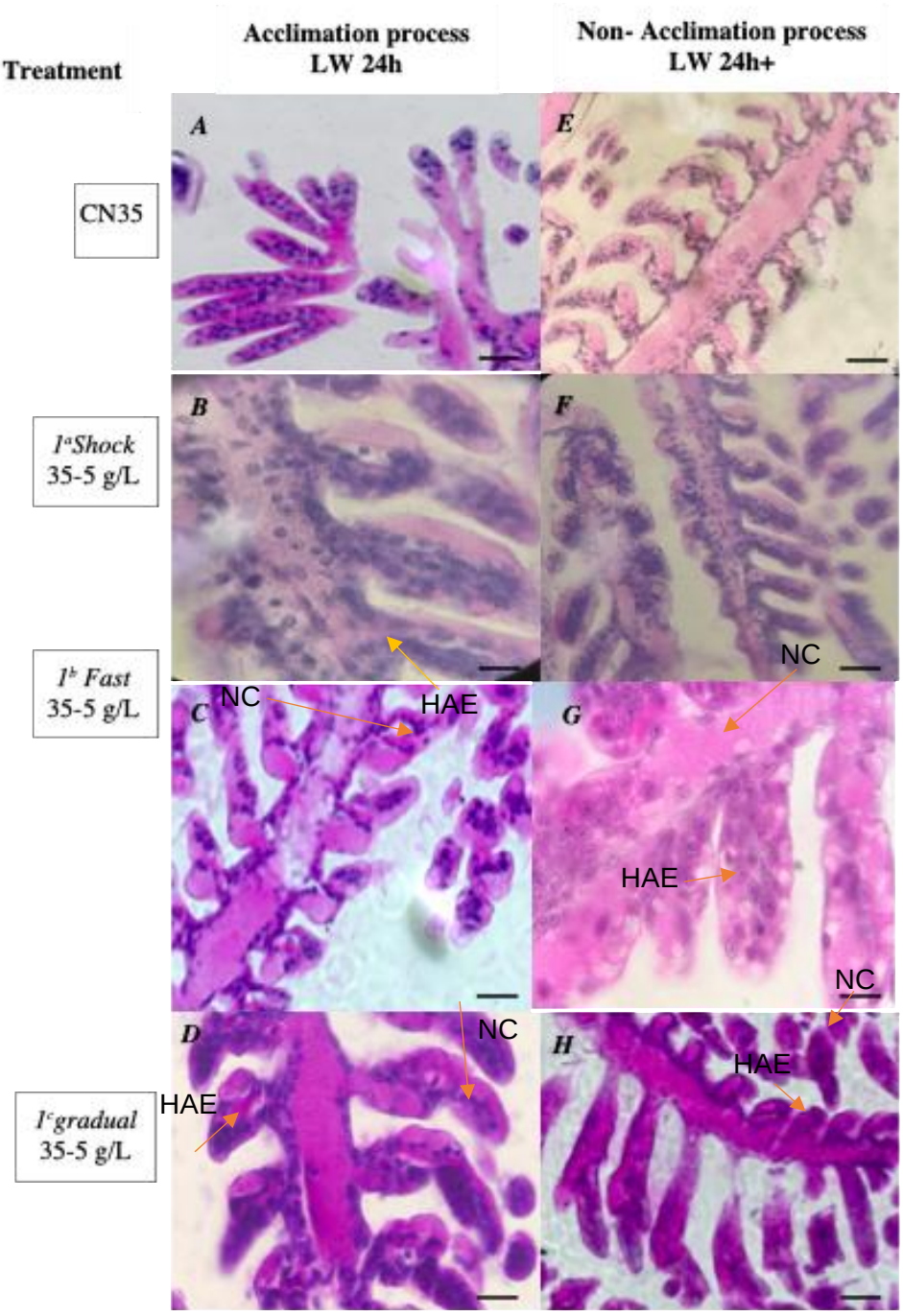

Figure 04: Histological changes in the gills of post-larvae of *Penaeus vannamei* exposed to different salinity reduction treatments in the 35-5 g/L range, during the acclimation (LW24H) and non-acclimation (LW24H+) periods. In LW24H: (A) In the control treatment (CON35), the gills showed organized filaments and complete

structure without lesions or signs of inflammation, (B) In the *1ªShock* treatment (osmotic shock), the gill filaments exhibited severe damage, including tissue collapse, hemocyte infiltration, intense vacuolization, necrosis, and filament disorganization; (C) In the *1ᵇFast* treatment (rapid reduction), significant changes were observed, such as thickening of the lamellae, hemocyte infiltration, and pronounced vacuolization, although some structural areas remained preserved; (D) In the *1ᶜGradual* treatment (gradual reduction), the gills exhibited almost total structural preservation, without significant necrosis, absence of vacuolization, and minimal hemocyte infiltration. In contrast, in LW24H+: (e) In the control treatment (CON35), the gill filaments maintained their intact and organized structure; (f) In the 1aShock treatment, evident epithelial edema, vacuolization and hemocyte infiltration were observed, in addition to areas with necrosis and disorganization, with no signs of tissue recovery; (g) In the 1bFast treatment, there was moderate improvement, with reduced inflammation and greater structural preservation, although vacuolization still persisted in some areas; (h) In the 1cGradual treatment, the gills remained well preserved, with no significant signs of necrosis, vacuolization or inflammation, evidencing tissue recovery and structural organization. Sections of tissue were stained using Hematoxylin and Eosin (H & E). Magnification 40x and 60X, respectively. NC: necrosis, HAE: hemocyte infiltration, Vacuolization,

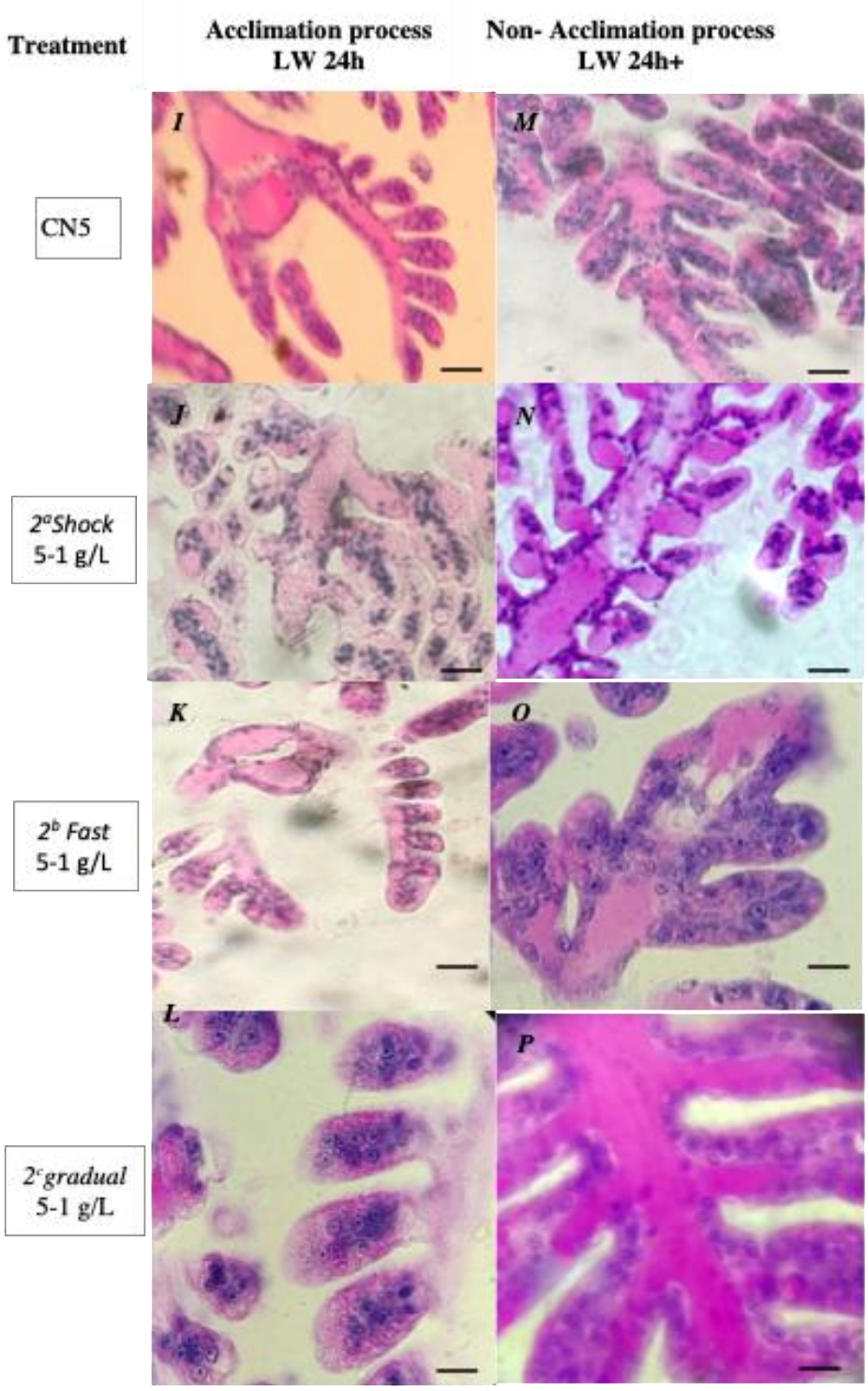

Figure 05: Histological changes in the gills of post-larvae of *Penaeus vannamei* exposed to different salinity reduction treatments in the 5-1 g/L range, during the acclimation (LW24H) and non-acclimation periods. In LW24H: (i) In the control

treatment (CON5), the gills exhibited healthy and preserved filaments, with no signs of disorganization or cellular alterations, with functional epithelium; (j) In the 2aShock treatment (osmotic shock), severe damage was observed, such as disorganization of the lamellae, loss of integrity, hemocyte infiltration, necrosis in some areas and vacuolization indicative of epithelial edema; (k) In the 2bFast treatment (rapid salinity reduction), moderate damage was identified, including hemocyte infiltration, isolated areas with vacuolization; (l) In the 2cGradual treatment (gradual salinity reduction), thickening of the lamellae, small areas with necrosis and discrete hemocyte infiltration were observed, but the majority of the lamellae remained healthy, with less intense damage compared to the other treatments. In the LW24H+: (m) In the control treatment (CON5), the gills remained healthy, with no signs of disorganization or structural changes; (n) In the 2aShock treatment, severe damage persisted, with disorganization of the lamellae, epithelial vacuolization, and no signs of structural recovery; (o) In the 2bFast treatment, there was a slight improvement, but significant vacuolization and infiltration of inflammatory cells were still observed, with cellular disorganization; (p) In the 2cGradual treatment, structural recovery was evident, with preserved lamellae, absence of significant necrosis, minimal infiltration of hemocytes, and absence of signs of inflammation, standing out as the most effective treatment in minimizing histological damage. Sections of tissue were stained using Hematoxylin and Eosin (H & E) Magnification 40x and 60X, respectively. The letters in the figure indicated that: T (hepatopancreas tubule), B (B-cell, Blasenzellen), F (F-cell, Fibrillazellen), R (R- cell, Restzellen), E (E-cell, Embryonalzellen), L (star shape-like lumen), ALU (abnormal lumen), and REC (ruptured epithelial cells).

In Group I (35-5 g/L), histological analysis revealed that postlarvae (PLs) in the control treatment (CON35) showed typical hepatopancreatic architecture, with well-organized tubular structures, a star-shaped lumen, and distinct cell types, including Blassenzelen (B), fibrillenzen (F), and restzellen (R) cells were clearly observed in Figs. 6a and 6e throughout LW24H and LW24H+ periods. However, PLs acclimated to the *1^{a}Shock* treatment during LW24H, involving a sudden reduction in salinity, exhibited lesions in the hepatopancreas, characterized by abnormal lumens, sharp reduction in lumens, significant tubule dilation, with many areas showing signs of collapse or deformation. The epithelial cells showed marked vacuolization and the tubes showed evident rupture, and inflammation (necrosis), all signs that the tissue is under severe osmotic stress (Fig. 6b). In the *1^{b}Fast* treatment, with rapid reduction in salinity, the epithelial cells were less compact, and the tissue structure was affected, with an abnormal lumen, showing dilated tubes, signs of pronounced vacuolization and considerable disorganization of the tubules. The damage was somewhat less severe than in the shock treatment (Fig. 6c). In the *1^{c}Gradual* treatment although some vacuolization was observed, the tissue remained relatively preserved. The structure of the tubules was maintained, and damage was minimal, being showed most preserved cells, the lumen of the tubules remained regular in a star-shape and relatively good preservation of the tubular structure, with minimal presence of dilated tubes (Fig. 6d).

In Group II (5-1 g/L), PLs in the control treatment (CON5) in both study periods (LW24H and LW24H+) (Figs. 7j and 7n). In contrast, in LW24H, PLs exposed to *2^{a}Shock* treatment experienced severe histological disruptions caused by osmotic shock, including lumen deformities, sharp reduction in lumens, tubule

disorganization, dilated tubes, extensive necrosis, and intense vacuolization (Fig. 7j). The *2^{b}Fast* treatment also exhibited signs of cellular damage, such as irregular lumen, dilation of the tubes, atrophied tubes, and evidence of vacuolization, though to a lesser extent than the shock treatment (Fig. 7k). PLs exposed to *2^{c}Gradual* treatment displayed relatively intact tubules with minor vacuolization, most tubules are relatively preserved, with few affected areas, indicating that gradual acclimation alleviated some histological impacts (Fig. 7l).

After the LW24H+ period, in the 35-5 g/L group, PLs exposed to the *1^{a}Shock* treatment continued to show pronounced damage, with abnormal lumens, dilated tubules, disorganization of the tubules and persistent vacuolization, as well as the cells showing signs of necrosis and disintegration, with little change, indicating no recovery (Fig. 6f). In the *1^{b} Fast* treatment, moderate vacuolization, thickening of the epithelium and tubule disorganization were observed (Fig. 6g). Conversely, PLs in the *1^{c}Gradual* treatment maintained good tissue structure with limited stress indicators, suggesting effective recovery (Fig. 6h).

For Group II (5-1g/L), post-LW24H+ analysis showed that the *2^{a}Shock* treatment sustained severe damage, with abnormal lumens, intense vacuolization, dilated tubules, and necrotic cells, indicating no recovery (Fig. 7n). The *2^{b}Fast* treatment exhibited moderate damage, such as abnormal lumen, dilated tubules, the presence of vacuolization and disorganization of the tubes (Fig. 7o), while PLs exposed to *2^{c}Gradual* treatment showed slight recovery, with preserved tubule structures and minimal vacuolization (Fig. 7p). These findings highlight suggest that abrupt acclimation treatments, particularly in the 5-1 g/L range (*2^{a}Shock*), induce severe histological damage to the hepatopancreas, whereas gradual acclimation (*1^{c}Gradual*

and 2^{c}Gradual) effectively reduces stress, allowing better structural preservation and potential recovery.

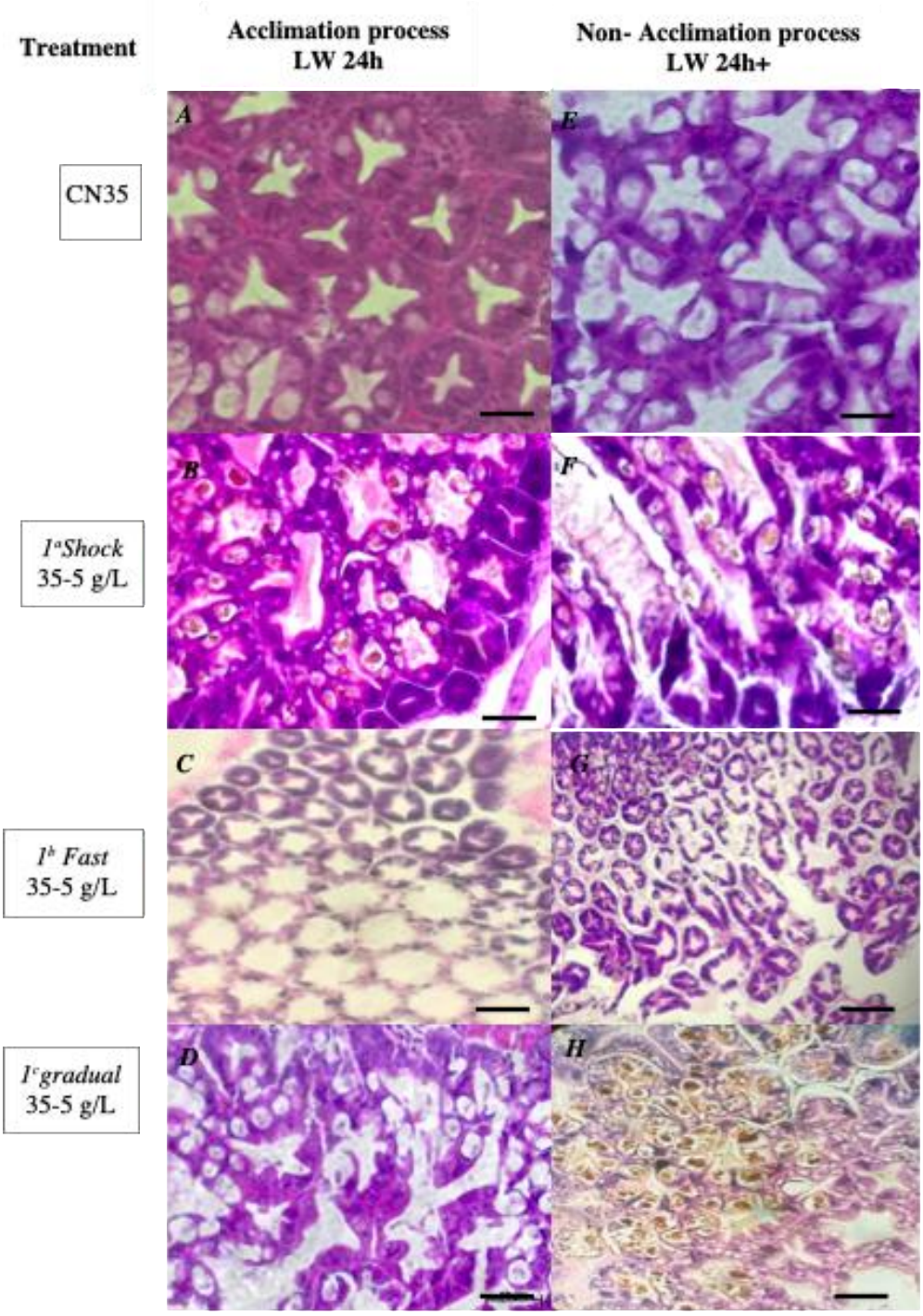

Figure 06: Histological changes in the hepatopancreas of *Penaeus vannamei* postlarvae exposed to different salinity reduction treatments in the range of 35-5 g/L during the acclimation (LW24H) and non-acclimation (LW24H+) periods. In LW24H: (a) In the control treatment (CON35), the typical hepatopancreas architecture was preserved, with well-organized tubules, star-shaped lumen, and distinct cell types, such as Blassenzellen (B), Fibrillenzellen (F), and Restzellen (R); (b) In the 1aShock treatment (abrupt salinity reduction), severe lesions were observed, including significant tubule dilation, lumen deformities, marked vacuolization of epithelial cells, structural collapse, and necrosis; (c) In treatment 1bFast (rapid salinity reduction), the tissue showed moderate damage, with abnormal lumen, dilated tubules, pronounced vacuolization, and tubule disorganization, but with less severity compared to treatment 1aShock; (d) In treatment 1cGradual (gradual salinity reduction), the hepatopancreas remained relatively preserved, with regular lumen, organized tubules, and minimal damage, such as discrete vacuolization. LW24H+: (e) In the control treatment (CON35), the structure of the hepatopancreas remained completely preserved, maintaining its organization and functionality; (f) In treatment 1aShock (abrupt salinity reduction), severe damage persisted, with abnormal lumens, dilated tubules, structural disorganization, marked vacuolization, and necrosis, indicating no recovery; (g) In the 1bFast treatment (rapid salinity reduction), moderate vacuolization and tubule disorganization were observed compared to the 1aShock treatment; (h) In the 1cGradual treatment (gradual salinity reduction), the hepatopancreas maintained a good tissue structure, with limited signs of stress, evidencing effective recovery. Sections of tissue were stained using Hematoxylin and Eosin (H & E) Magnification 40x and 60X, respectively.

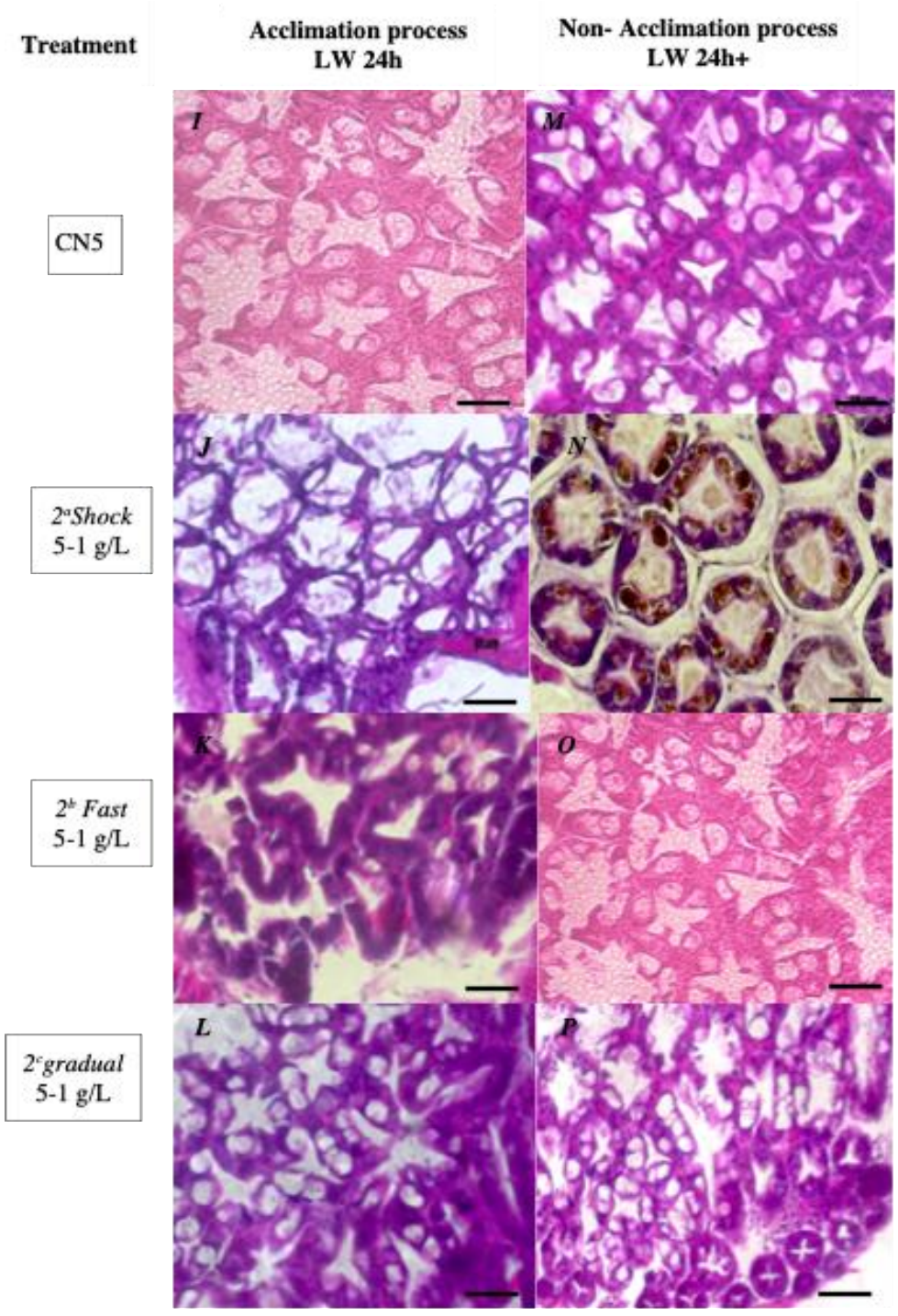

Figure 07: Histological changes in the hepatopancreas of *Penaeus vannamei* postlarvae during acclimation (LW24H) and non-acclimation (LW24H+) periods,

subjected to different salinity reduction treatments in the range of 5-1 g/L. In LW24H: (j) In the control treatment (CON5), the hepatopancreas showed preserved tubules, star-shaped lumen and absence of structural lesions; (k) In the 2aShock treatment (osmotic shock), severe damage was observed, such as tubule disorganization, lumen deformities, dilated tubules, extensive necrosis and intense vacuolization; (l) In the 2bFast treatment (rapid salinity reduction), moderate damage was identified, including irregular lumen, dilation and atrophy of the tubules; (m) In the 2cGradual treatment (gradual reduction of salinity), the tubules remained relatively preserved, with minimal vacuolization and little damage observed, indicating that gradual acclimation reduced histological impacts. LW24H+: (n) In the control treatment (CON5), the hepatopancreas remained healthy, with organized tubular structures and no alterations; (o) In the 2aShock treatment, severe damage persisted, with dilated and vacuolized tubules, abnormal lumen and necrosis, with no signs of recovery; (p) In the 2bFast treatment, moderate improvement was observed, but with the presence of vacuolization, abnormal lumen and tubular disorganization still evident; (q) In the 2cGradual treatment, the tubules showed signs of significant recovery, with structural preservation, without the presence of vacuolization and necrosis.

4 Discussion

The use of *Penaeus vannamei* in aquaculture is primarily due to its adaptability to a broad salinity range, from 0.5 to 45 g/L (Castille and Lawrence, 1981; Lester and Pante, 1992: Roy et al., 2010). This species exhibits hypo-osmoregulation at higher

salinities and hyper-osmoregulation at lower salinities, enabling satisfactory growth and survival near freshwater salinities (Laramore *et al*., 2001; Mcgraw *et al*., 2002; Esparza-Leal *et al*., 2010). Furthermore, *Penaeus vannamei* is a strong osmoregulator, rapidly adjusting hemolymph osmotic concentration during salinity shifts (Hernandez Rodríguez and Diaz Herrera, 1995; Brito *et al*., 2000; Soyel and Kumlu, 2003; Re *et al*., 2004; Díaz *et al*., 2004b). However, acclimation is essential to enhance tolerance, especially when postlarvae are transferred from high-salinity hatcheries to low-salinity grow-out systems, to ensure stability and survival (Charmantier *et al*., 2002; Anger *et al.*, 2008) since the osmoregulation process represents an increase in energy requirement when shrimp is raised at salinities below its isosmotic point (28 g/ L).

Therefore, to gain a better understanding of the physiological and health status of *Penaeus vannamei* species, postlarvae were acclimated under different osmotic stresses by reducing the salinity from 35-5 to 5-1 g/L ranges at different salinity rates during the 24 hours acclimation period (LW24H) and then maintained for another 24 h non-acclimation (24LWh+) at an endpoint salinity of 5 or 1 g/L. Crustaceans' survival is dependent on their ability to maintain the composition of their body fluids when transferred to an environment that differs from their normal environment (Lignot *et al*., 2000; Chong-Robles *et al*. 2014). In our study, the effects of salinity reduction from 35-5 to 5-1 g/L ranges on survival of *P. vannamei* post-larvae showed a tendency towards reduced survival and a significant difference between the treatments of both salinity ranges. In LW24H, survival was significantly lower in the *2^{a}shock* (85%) and *2^{b}Fast* (88.18%) treatments of 5-1g/L group, and significantly higher in the 35-5g/L group, with 98.33% in CON35, followed by the *1^{c}gradual*

(97.22%) and *1^{b}Fast* (95%) treatments. The same decreasing trend was observed in LW24H+, with the CON35 treatment in the 35-5g/L group being significantly higher in survival (97.11%) than all treatments in the 5-1g/L range with 93.33%, 89.44%, 86.11% and 82.77% of CON5, *2^{c}gradual, 2^{b}Fast* and *2^{a}shock*, respectively. The results of survival at LW24H revealed that PLs were able to tolerate salinity reduction from 35 to 5 g/L and 5-1 g/L in 24 hours regardless salinity reduction range and speed of this change, as all values were greater than 80%. These results corroborate that *Penaeus vannamei* is able of hypo-osmotic regulation as has been widely reported in the literature (Hernandez Rodrıguez and Diaz Herrera,1995; Brito *et al*.,2000; Soyel and Kumlu, 2003; Re *et al*., 2004; Dıaz *et al*., 2004b).

Similar results were found by Jayasankar et al. (2009), when investigating the acclimation, survival and growth of post-larvae (PL) and juveniles of *Penaeus vannamei* in low salinity conditions (1 and 5 g/L), demonstrated that after gradual acclimation from a salinity of 30 g/L to 5 g/L, survival rates of the postlarvae (PL15) were significantly higher compared to the 1 g/L treatment. At salinities of 5 g/L, survival of postlarvae was over 85%, while at 1 g/L, survival rates were much lower. When shrimps were transferred directly from 25‰ to 1‰, the survival rate dropped to 20% after 5 h and none survived after 24 h.

The authors explained that survival was significantly higher at 5 g/L than at 1 g/L because, at extremely low salinities (such as 1 g/L), postlarvae face greater difficulty in maintaining osmotic balance, resulting in more intense osmotic stress, which overloads the physiological mechanisms of ionic regulation, impacting survival capabilities, because of the large shift in the ionic profile of the environment and hemolymph, and the animal was unable to cope with such vast changes. At 5 g/L,

osmoregulation conditions are less challenging, allowing postlarvae to maintain better homeostasis, resulting in higher survival rates (Lignot et al., 2000; McGraw & Scarpa, 2004; Huong et al., 2010).

Anand et al. (2023), also demonstrate that the survival of the Indian white shrimp (*Penaeus indicus*) was significantly influenced by salinity conditions and development stage. During the post-larval stage, the optimal salinity of 25 ppt resulted in the highest survival rates (97% at PL12 and 79–81% at PL4), while extreme salinity levels (3 and 48 ppt) reduced survival rates, mainly at PL4, with values of 61–62% and 54–56%, respectively. Rahi et al. (2021), who demonstrated a significant decrease in the survival rate of *Penaeus monodon* exposed to low salinity conditions. These authors observed that survival was reduced from 96% at 25 ppt salinity to 75% at 5 ppt, highlighting the physiological limitations of shrimp in tolerating hyposaline conditions.

Abrori et al*.* (2022), when investigating the responses of *Penaeus vannamei* post-larvae to different levels of osmotic stress, with progressive reductions from seawater salinity (35‰) to low salinity (5‰) also recorded significantly higher survival rates (90%) in post-larvae subjected to gradual acclimation to 5‰ compared to those exposed to more abrupt reductions, such as 10‰ (80%) and 15‰ (58%), being that the greater survival of *Penaeus vannamei* in gradual acclimation to 5‰ were due to the greater time available for osmotic adaptation. Thus, as Jaffer et al., 2020, who sought to reexamine the osmoregulatory capabilities of Pacific white shrimp (*Penaeus vannamei*) under low salinity conditions, recording changes in the ionic composition and osmolality of the hemolymph at different salinity levels (1, 5, 7, 15 and 25 ppt) over a period of 21 days and found high survival in all treatments,

ranging from 91.7% to 95.8%, demonstrating that the species has potential for cultivation in low salinity waters, as long as acclimation is carried out gradually, corroborating the survival rates of *Penaeus vannamei* post-larvae in the present study that were also negatively impacted by abrupt reductions in salinity, especially in treatments that simulated osmotic shocks (*2ªShock* treatment (5-1 g/L)), while the highest values were recorded in the gradual treatment in both ranges of 35-5 and 5-1 g/L ranges.

Although the shrimp *Penaeus vannamei*, a type of crustacean that lives in coastal and oceanic environments, and its larvae develop in the ocean, while post-larvae, juveniles and adults live in estuaries and lagoons (Ogle et al., 1992), commonly studied for their ability to tolerate salinity variations, when exposed under conditions of salinity reduction, studies reveal that the survival of euryhaline crustaceans exposed to different rates of salinity reduction indicate that rapid changes can negatively impact survival and adaptation, while slower rates allow better osmotic adjustment (McGraw and Scarpa 2004; Jayasankar *et al*., 2009; Abrori et al., 2022), corroborating the results of our study that showed that under acclimation conditions, the survival of postlarvae exposed to two salinity ranges (35-5 and 5-1 g/L) at different rates of salinity reduction, all treatments recorded values above 80%, with the Gradual and CON35 treatments resulting in better survival rates than abrupt changes.

These results reinforce the ability of *Penaeus vannamei* to tolerate a wide range of salinities, from 0.5 to 50 g/L, and the importance of the acclimation process, especially in cases of postlarvae transfers from high salinity systems to low salinity systems in culture farms, as demonstrate how shrimp adjust the hemolymph osmotic

concentration to rapidly adapt to changes in salinity. Although survival using a sudden reduction in salinity was lower compared to the other treatments in both ranges, survival rates were considered excellent, with 35–5 g/L being higher compared to a range of 5–1 g/L, validating that shrimp can still osmoregulate more efficiently at moderate salinity levels, while the 5–1 g/L range , even with good survival values, is considered a more extreme change, and salinity levels as low as 1 g/L pose a greater challenge to the osmoregulatory mechanisms of shrimp, which may be unable to maintain adequate osmotic balance.

The authors' hypothesis is that shock and fast treatments in the 5-1 g/L range were considered the most critical and the least suitable as a protocol to achieve greater survival in *Penaeus vannamei* postlarvae, as they provide a much more severe osmotic challenge, and extremely low salinities cause greater stress, making it difficult to maintain homeostasis, resulting in more mortality due to failure of ion transport mechanisms, more drastically affecting the shrimp's physiological ability to cope with changes (Chen *et al*., 2015; Zhang *et al*., 2023), showing that it would be preferable to maintain the endpoint salinity at no less than 5 ppt in order to achieve maximum survival rates (Jayasankar *et al*., 2009) and suggesting that CON35, *1^{c}Gradual, CON5* and *2^{c}Gradual* treatments are considered more suitable for postlarvae of *Penaeus vannamei* during LW24H and LW24H+. In addition, slow acclimation allowed better adjustment of ionic transport mechanisms, reducing osmotic stress, and the increase in the activity of the Na+/K+ ATPase enzyme favored the regulation of ionic balance, contributing to greater survival in low salinity environments. These factors indicate that gradual acclimation provides more

adequate conditions for the physiological adjustment necessary to maintain homeostasis in low salinity environments.

Osmoregulation is an energy-dependent process, and aquatic animals are forced to expend additional energy to modulate and stimulate ion transport mechanisms when challenged with salinity stress (Tseng and Hwang, 2008; Li et al., 2007; Li et al., 2017). Furthermore, oxygen consumption and other indexes related to respiration and metabolism have been used to assess physiological responses under various stressful environments in decapod crustaceans (Nelson et al., 1977; Li et al., 2017; Kumar et al., 2024). Thus, the authors of the present study when investigating the effect of salinity reduction in the 35-5 and 5-1 g/L ranges on oxygen consumption in *Penaeus vannamei* postlarvae during the acclimation process (LW24H), recorded a tendency to increase in both groups, with the highest OCR recorded in the salinity reduction of 5-1 g/L group. PLs exposure in *2^{a}Schock* and *2^{b}Fas*t generated a significant increase in the respiratory rate of 8.61 mg O2/g/min and 7.97 mg O2/g/min, respectively. Meanwhile, "1^{a}Shock" in the 35-5 g/L range also recorded a higher consumption of 8.25 mg O2/g/min. On the other hand, PLs exposed in CON35 treatment were significantly lower with 4.85 mg O2/g/min in the 35-5 g/L group.

Similar our finding, Li *et al*. (2007), found good indicators for assessing energy utilization, oxygen consumption of *Penaeus vannamei* shrimp at 3% were significantly higher than those of shrimp at 17 and 32% salinity**,** suggesting increased metabolic demand for osmoregulation. Comparably, Rosas *et al*. (2001) investigated the effect of salinity acclimation on oxygen consumption of juvenile white shrimp *Penaeus vannamei,* found that oxygen consumption increased as salinity

decreased, with the highest values observed at 5‰ and the lowest at 30‰ (first hours and after 24 hours of acclimation), suggesting that the increased oxygen consumption at lower salinities reflects the greater energy expenditure required for osmoregulation under such conditions. Rosas et al. (1997) reported an increase of 158% in the oxygen consumption in *Litopenaeus setiferus* and *L.schmitti* Burkenroad postlarvae acclimated to salinities lower than 5‰ in comparison to shrimp acclimated at 37‰.Thus, as Abou Anni *et al*. (2016), who reported that higher oxygen consumption was required in juvenile pompanos reared at 3% salinity than for those reared at other experimental salinities (6, 12, and 32%). Huang *et al*. (2019), studying the growth and lipidomic responses of juvenile Pacific white shrimp *Penaeus vannamei* to low salinity also indicates that shrimp reared at 3% salinity require more energy than those at 30%.

These results are consistent with of Pillai *et al*. (2002), when studying the metabolic response of *Metapenaeus monoceros* to osmotic stress caused by abrupt changes in salinity, observed that oxygen consumption was significantly increased at very low salinities (5, 10, 15‰). This increase was interpreted as an elevated metabolic response to deal with osmotic stress, since the shrimp needed to intensely adjust their osmotic and ionic balance to adapt to extreme conditions (Gilles, 1979). While, in the study conducted by Xie *et al*. (2021), the physiological responses of Pacific white shrimp (*Penaeus vannamei*) subjected to different saline acclimation regimes were investigated.

The results demonstrated that the oxygen consumption rate varied significantly according to the acclimation protocol adopted. Specifically, shrimp exposed to abrupt reductions in salinity showed a sharp increase in oxygen consumption,

indicating a greater energy demand for osmoregulation processes. Díaz-Herrera *et al*. (2004) studied the impact of temperature and salinity fluctuations on oxygen consumption, ammonium excretion and osmoregulation of *L. stylirostris*, demonstrated that extreme variations in salinity, such as reductions to very low levels (close to 1 g/L), result in an increase in the rate of oxygen consumption due to high osmotic stress.

The oxygen consumption results reported by Rahi *et al*. (2021) indicated that the energy expenditure of *Penaeus monodon* was directly affected by salinity conditions. The study observed a significant increase in the oxygen consumption rate under low salinity conditions (5 ppt), reaching values of up to 2.5 mg O_2/g/h, compared to the lowest values recorded at 25 ppt (approximately 1.7 mg O_2/g/h). These results highlight that exposure to hyposaline conditions increases the metabolic demands of shrimp due to the higher energy cost associated with osmoregulation. Specifically, the authors observed that the increase in oxygen consumption was particularly pronounced during the first 24 h of exposure, suggesting an intense initial impact on the basal metabolism of the organisms.

This metabolic increase is directly related to the activation of osmoregulatory mechanisms, such as Na^+/K^+-ATPase activity in the gills, and the need to compensate for the loss of essential ions, such as sodium and potassium. This aligns well with the observations on the *2^{a}Shock* and *2^{b}Fast* treatments in the 5-1 g/L range in the present study, being these results consistent with the findings that such acclimation can lead to higher energy demands in shrimp.

According to our study, *Penaeus vannamei* demonstrated a clear metabolic response to the acute decrease in salinity, with significant changes in respiratory

rates and adjustments in metabolic efficiency. The adjustment involved both the modulation of oxygen use and the use of different energy pathways to cope with osmotic stress. Thus, it is possible to suggest that the behavior of shrimp in the present study, which, under reduced salinity registered increase in oxygen consumption, probably due to osmotic stress and the demand for metabolic adjustment.

The response in shrimp may involve processes of metabolic regulation, which include changes in enzyme activity and management of energy consumption to maintain homeostasis, due to the higher energy demands for osmoregulation. This highlights the similar physiological challenges that crustaceans face in low salinity environments**.** Second, Ye *et al*. (2009) and Ma *et al*. (2021), the energy channeled by crustaceans to respond to salinity acclimation leads to a significant reduction in growth, considering that the energy budget for osmotic adjustment is much higher. Therefore, fluctuations in water salinity seem to inhibit energy metabolism, which may cause oxidative damage (Choi *et al*., 2008).

Our findings showed that rapid salinity reduction causes a significant impact on the metabolism of *Penaeus vannamei* postlarvae and that organisms acclimated to reduced salinity in the 5–1 g/L recorded significantly higher oxygen consumption rates compared to the 35–5 g/L range. The increased OCR in treatments such as *2^{a}Shock* and *2^{b}Fast* under lower salinity suggests increased osmotic stress, requiring the shrimp to expend more energy for increased metabolic activity to maintain homeostasis. This adjustment may result in lower overall energetic efficiency and potentially affect long-term survival if the low salinity environment persists.

Oxygen consumption increases as shrimp activate their osmoregulatory mechanisms and adjust ion transport to cope with the abrupt change in salinity concentration. This requires additional energy, resulting in a higher respiratory rate. Furthermore, although to reduced salinity in the 35-5 g/L range represents an osmotic challenge, it is considered in the literature to be more moderate. Although osmotic stress still occurs, the shrimp body is able to adjust more efficiently, resulting in lower oxygen consumption compared to the 5-1 g/L range, where the osmotic imbalance is much more pronounced and difficult to regulate. The authors suggest that crustaceans under chronic or sudden osmotic stress may enter a state of metabolic shutdown to conserve energy, which can directly impact culture success, especially in situations where environmental changes occur abruptly and uncontrollably.

This supports the results found in their study with post-larvae of *Penaeus vannamei*, where oxygen consumption was highest in the range of 5-1 g/L, i.e., the increase in oxygen consumption reflects the physiological response to severe osmotic stress, where ion transport mechanisms are highly demanded. which can impact overall health and productivity, considering the most critical range of 5 to 1g/L, when it comes to oxygen consumption. This is particularly important for aquaculture practices, as understanding the oxygen demands and metabolic responses of shrimp can guide better management strategies during acclimation periods (Li *et al*., 2007).

The growth and development of aquatic animals in a low salinity environment depends largely on their ability to respond to hypotonic stress and regulate osmotic pressure (Péqueux, 1995; Geng *et al*., 2016). Recently, the regulatory mechanisms,

target organs, physiological and metabolic characteristics of osmotic pressure regulation in euryhaline crustaceans have been studied (Rainbow & Black, 2001; Li *et al*., 2017). Studies have shown that low salinity conditions can lead to significant damage to the hepatopancreas and gills in shrimp postlarvae. The hepatopancreas, crucial for digestion and metabolism, can suffer oxidative stress, cellular damage, and altered metabolic activity when exposed to low salinities. Additionally, the gills, being vital for ion transport and osmoregulation, when animals transfer from high to low saline waters (Charmantier, 1998; Péqueux *et al*., 2006; Pham *et al*., 2012; Ponce-Palafox *et al.* 2013; Li *et al*., 2017).

In addition to studying oxygen consumption and survival in post-larvae of *Litopenaeus vananmei.* The authors of this work also investigated the effect of different osmotic stresses using different salinity reduction rates in two salinity ranges (35-5 g/L and 5-1 g/L) on the histological structure of the gills and hepatopancreas, evaluating tissue damage during periods of LW24H and LW24H+. Histological images showed alterations in the gills and hepatopancreas of *Penaeus vannamei* postlarvae. Histological analysis of the gill has been used as a practical means for assessing the environmental stress in crustacean culture, especially in shrimp (Sun *et al*., 2015, Abol-Munafi *et al*., 2020).

In the LW24H period, the results showed a significant variation in histological damage in the gills of *Penaeus vannamei* postlarvae, according to the salinity reduction range and the speed of this change. Sudden and rapid salinity reductions (*1^{a}Shock, 1^{b}Fast, 2^{a}Shock* and *2^{b}Fast*) in both 35-5 g/L and 5-1 g/L ranges, resulted in considerable damage to the gill structures. However, the most severe damage was observed in the 5-1 g/L range, especially in 2^{a}Shock, compared to all other

treatments in the 35-5 g/L range, such as severe loss of structural integrity of the gill filaments, accompanied by intense necrosis, pronounced vacuolization and significant hemocyte infiltration, suggesting severe tissue inflammation.

On the other hand, the results obtained after the non-acclimation period (LW24H+) highlighted marked differences between the salinity reduction treatments in the gill structures of postlarvae (*Penaeus vannamei*), particularly between the groups subjected to the salinity ranges of 35-5 g/L and 5-1 g/L. The severity of the damage was more evident in the abrupt reduction treatments, while the gradual protocols proved to be more effective in tissue preservation and recovery. In group I (35-5 g/L), the organisms exposed to the *1^{a}Shock* treatment resulted in epithelial edema, vacuolization and hemocyte infiltration, reflecting an intense inflammatory response, indicating that the abrupt salinity reduction severely compromised the structural integrity of the gills, without allowing adequate recovery after LW24H+.

The *1^{b}Fast* treatment resulted in a slight improvement, but areas of cellular infiltration and necrosis were still observed. In contrast, postlarvae subjected to the *1^{c}Gradual* treatment had well-organized and preserved gills, with well-defined lamellae and healthy tissue. The absence of significant necrosis and vacuolization in this group highlights the effectiveness of gradual reduction in maintaining histological integrity and minimizing osmotic stress.

In group II (5-1 g/L), the abrupt treatments (*2^{a}Shock*) also showed severe and persistent damage, including hemocyte infiltration, necrosis, and vacuolization. Although the *2^{b}Fast* treatment showed a slight improvement compared to *2^{a}Shock*, damage such as necrosis and cellular disorganization was still evident. In contrast, the *2^{c}Gradual* treatment showed significant recovery, with well-organized gills, no

hemocyte infiltration, and minimal signs of structural damage. These results suggest that gradual reduction protocols allow osmoregulation mechanisms to efficiently adjust to new conditions, promoting tissue recovery and minimizing the impact of salt stress.

The damages found at both 35-5 and 5-1g/L during LW24H and LW24H+ periods are consistent with the literature indicating that large osmotic variations cause cellular collapse and overload of osmoregulatory mechanisms in shrimp, leading to possible failure of ion transporters and consequent cellular necrosis (Díaz-Herrera *et al*., 2010; Ponce-Palafox *et al*., 2013). Second, Zhu *et al*., 2006 and Liu *et al*., 2007, these findings indicate that rapid ion loss due to extreme salinity changes overloads the osmoregulatory system, leading to compromised tissue integrity. This result is in agreement with previous studies that demonstrated that sudden salinity changes cause significant injury to the gills of marine shrimp. Shen et al. (2024), when evaluating the effects of different salinity reduction intervals (30 ppt to 0, using different reduction rates: 30 ppt, 15 ppt, 10 ppt and 5 ppt for 24 h) on physiological processes, including osmoregulation, antioxidant responses and apoptosis mechanisms in *Eriocheir sinensis* megalopae, found that rapid salinity reductions (30 ppt/24 h) caused severe damage to the anterior gills, including epithelial lysis, pillar cell rupture and filament compaction, indicating extreme osmotic stress. In contrast, gradual reductions (5 ppt/24 h) preserved the structural integrity of the gills, with no apparent damage, suggesting that gradual adjustments allow for more efficient physiological adaptation and minimize tissue injury.

Duan *et a*l. (2018) investigated how acute sulfide stress affects the physiology and immunity of white shrimp (*Penaeus vannamei*) gills. Sulfide stress caused

disorganization in the gill structure, with vacuolization and damage to the filaments. This change compromised the respiratory and osmotic regulation functions of the gills. These authors concluded that sulfide directly affects the structure and function of the gills, essentially by overloading the osmoregulation and oxidative stress response processes. Over time, the immune system and physiological mechanisms are insufficient to mitigate the ongoing damage, resulting in long-lasting structural and functional impairment of the gills. Liu *et al*. (2007) through histopathological changes in gills of Pacific White Shrimp (*Penaeus vannamei*) exposed to different salinity levels, with the aim of evaluating damage to the gills and hepatopancreas of shrimp after abrupt changes in salinity. Shrimp subjected to rapid salinity reductions (20 a 5 g/L) presented necrosis in the gill lamellae, disorganization of epithelial cells and vacuolization. These damages were more pronounced in abrupt transitions, as in the case of salinity shocks, similar to what was offered in their *1*[a] and *2*[b]*Shock* treatment (35-5 and 5-1 g/L).

Second, Li *et al*. (2023), while investigating the response of *Macrobrachium nipponense* (freshwater shrimp) to various salinity levels, reported that shrimp exposed to high salinity (up to 22‰) caused significant damage to gill tissues, including vacuolization, disruption of gill filaments, and structural disintegration of epithelial cells. These findings indicated physical stress and cellular adaptation challenges under salinity stress. Thus, as reported by Cheng *et al*. (2013) that investigated the effect of low salinity on the structure and function of the gills of blue crabs (*Callinectes sapidus*) and observed that when exposed to salinity of 5 g/L, there was dissolution of the gill lamellae and degeneration of the epithelial cells, similar to the application of treatments of 5-1 g/L. The cumulative effect of low salinity

causes severe histological damage, compromising respiratory function. Fregoso-López *et al*. (2017), evaluated the impact of stocking density and nitrogen compounds in low salinity water (1.9 g/L) on the gills of *Penaeus vannamei* over a 10-week culture cycle and analyses revealed that higher stocking density (120 shrimp/m²) under low salinity conditions led to significant gill damage, including hypertrophy, lamellar fusion, vacuolization, and increased hemocyte infiltration. The high nitrogen concentrations and low salinity increase osmotic stress and compromise gill osmoregulation. These conditions overload the gills, reducing their adaptive capacity and contributing to the observed damage.

Quintana and Martınez (2005) found oedema in gills when cultured *L. schmitti* at low salinity (8 g/L), and they attributed it to a reduction in osmoregulatory capacity, which affects vascular permeability (Neufeld *et al*.,1980). Chen *et al*. (2022), investigated how stress caused by high salinity levels affects survival, gill histological structure, enzymatic activity and free amino acid content in the mollusk *Sinonovacula constricta* over time. Exposure to different salinities caused contraction of the gill lamellae and tissue dissolution after 48 hours, indicating severe damage. The lamellae experienced thickening and disorganization, with vacuolization and necrosis evident at higher salinities. Nan *et al*. (2024) evaluated how ammonia stress impacts homeostasis in the gills of *Penaeus vannamei* under physiological conditions of saltwater (30‰) and low salinity (3‰). Ammonia stress caused severe contraction of gill filaments, deformation and rupture of vessels in the gills, indicating serious tissue damage under both salinity conditions.

Wang *et al*. (2022) reported that aquatic animals exposure to salinity changes can have a great impact on the physiological activities. It has been found that when the

antioxidant capacity is unbalanced and severely biased toward ROS generation, it can lead to an increase in oxygen ions, free radicals, and peroxides, which can effectively induce oxidative stress (Liu *et al*., 2007; Lushchak, 2011; Paital and Chainy, 2012, Bal *et al*., 2021). Therefore, fluctuations in water salinity seem to inhibit energy metabolism, which may cause oxidative damage (Choi *et al*., 2008). Studies carried out by Liu *et al*., 2007 and Li *et al*., 2017 also report that in low salinity has been shown to induce oxidation status and generate excess ROS to damage cells and tissues in shrimp. Oxidative stress and inflammation are caused by salt stress, but the body protects itself from damage in a short time through the response of the antioxidant system and the anti-stress mechanism (Wang *et al*., 2023). Furthermore, Mu *et al*., 2019 confirm that the gills and hepatopancreas are the main organs for protection of organism from oxidative damage.

The comparison between the two salinity ranges (35-5 g/L and 5-1 g/L) highlights the greater severity of damage observed in the 5-1 g/L range, particularly in the rapid reduction treatments (*2^{a}Shock* and *2^{b}Fast*), such as lost their normal structure, the increase in the number of intercellular hemocytes, presence of vacuolization, necrosis in both in LW24H and LW24H+ period that are attributed to the difficulty of gill cells in maintaining adequate osmotic balance. This finding may be related to the greater difficulty of postlarvae in tolerating extremely low salinities in short periods of time.

When exposed to low concentrations of ions in the environment, gill cells absorb excess water, which results in cellular swelling and collapse of structures such as lamellae. This process leads to a greater metabolic effort to compensate for osmotic losses, overloading cellular mechanisms and increasing energy consumption to

regulate ion transport and membrane permeability. In addition, rapid salinity reduction increases osmotic stress, compromising physiological mechanisms, such as can compromise the transport of essential ions (sodium and potassium), through Na+/K+-ATPase, a critical enzyme for osmoregulation, thus as also associated with the accumulation of reactive oxygen species (ROS), causing oxidative and inflammatory damage in the gills, as observed in other studies on shrimp exposed to salinity variations (Jia *et al*. 2014; Chen *et al*., 2021; Yang *et al*., 2024).

In contrast, the results of histological analyses on gills of postlarvae acclimated to the gradual reduction treatments (*1^{c}Gradual* and *2^{c}Gradual*) demonstrated substantial preservation of gill structures functional integrity of the gills of *Penaeus vannamei*, both in 35-5 g/L and 5-1 g/L range, with minimal hemocyte infiltration and absence of significant necrosis, being considered the most efficient protocol for acclimation of postlarvae of *litopenaues vannamei*. This reinforces the need to apply more gradual salinity reductions in culture systems, since abrupt variations are associated with greater impairment of gill function and, consequently, higher mortality in shrimp post-larvae.

The lower intensity of damage, particularly in the *1^{c}Gradual* treatment (35-5 g/L), suggests that the transition between these salinity levels, although also challenging, allowed a slightly more effective adaptation of osmoregulatory mechanisms (Li *et al*., 2007; Bett and Vinatea-Arana, 2009). Furthermore, the minor damage found in this treatment, shows that gradual adaptation to reduced salinity of 35 to 5 g/L provides sufficient time for the physiological mechanisms of osmotic compensation to be adjusted, minimizing cellular stress (Chen *et al*., 2014) and, have been shown to be the most effective approaches to minimize the impact of saline stress and promote

tissue recovery, which has direct implications for management practices in low-salinity aquaculture systems.

Histological analysis of the hepatopancreas has been used as a practical means for assessing the environmental stress in the shrimp culture (Wu *et al*., 2008; Sun *et al*. 2015). This organ in shrimp is essential for osmoregulation, particularly under salinity fluctuations, and plays a major role in digestion, nutrient absorption, and metabolic regulation (Ceccaldi, 1997). The results found in group I (35-5 g/L), PLs acclimated in control treatment (CON35) showed typical hepatopancreatic structure, with well-organized tubules and well-defined Blassenzelen (B), fibrillenzen (F), and restzellen (R) clearly defined in both periods evaluated (LW24H and LW24H+) (Figs.6a, e). In contrast, the *1^{a}Shock* treatment during LW24H, with abrupt salinity reduction, resulted in significant damage, including irregular lumen, tubule dilation, vacuolization, and necrosis (Fig. 6b). In *1^{b}Fast* treatment with rapid reduction, also presented pronounced vacuolization and tubular disorganization, but to a lesser extent than in the abrupt shock (Fig. 6c). Now the *1^{c}Gradual* treatment, preserved the tubular structure, with minimal damage and regular lumen, indicating greater tolerance to osmotic stress (Fig. 6d).

In Group II (5-1 g/L), PLs exposed to the control treatment (CON5) maintained intact hepatopancreatic structure in both study periods (Figs. 7i, n). However, during LW24H, the *2^{a}Shock* treatment induced severe histological changes, including tubular disorganization and dilation, and extensive necrosis, reflecting high osmotic stress (Fig. 7j). The *2^{b}Fast* treatment caused moderate damage, with irregular lumen and mild necrosis, but with less severe damage than in the abrupt shock (Fig. 7k), while the *2^{c}Gradual* treatment partially preserved the integrity of the tubules, reduced

the histological impact, with minimal damage and limited vacuolization (Fig. 7l).

After the LW24H+ period, in the 35-5 g/L group, PLs in the *1^{a}Shock* treatment continued to exhibit marked damage, with no signs of recovery (Fig. 6f). In *1^{b}Fast*, showed moderate vacuolization, while in *1^{c}Gradual,* showed clear signs of structural recovery (Fig. 6f–h, respectively). In Group II (5-1 g/L), the *2^{a}Shock* treatment maintained severe damage, indicating no recovery (Fig. 7n), while the *2^{b}Fas*t treatment showed moderate damage, with tubular disorganization and vacuolization. The *2^{c}Gradual* treatment demonstrated slight recovery, with partial structural preservation (Fig. 7o–p, respectively). These results indicate that abrupt treatments, especially in the 5–1 g/L range (*2aShock*), cause severe histological damage to the hepatopancreas, while gradual acclimation (*1^{c}Gradual and 2^{c}Gradual*) minimizes stress, promoting greater structural preservation and potential recovery.

The histological damage found in the present study with the salinity reduction of 35-5 and 5-1 g/L ranges were reported in other studies, such as Wang *et al*. (2019), investing the physiological responses of *Penaeus vannamei* during temperature fluctuation a low salinity (5‰), showed that with the decrease of temperature, stellate tubule lumen appeared dilatation, and some vacuoles appeared and ruptured to make the epithelial cell layer thinner were observed (Almohanna and Nott 1989; Wang *et al*., 2016). Pathak *et al*. (2018), investigating the effects of potassium supplementation in saline groundwater to improve the growth and histological structure of the hepatopancreas of the Pacific white shrimp (*Penaeus vannamei*), cultured at salinities of 5, 10 and 15 ppt, found histological changes in the hepatopancreas at salinities of 5 and 10 ppt with low potassium levels, with significant damages, including increased vacuolization, cellular dysfunction, focal

necrosis and tubular disorganization. These changes reflect a response to environmental stress, compromising metabolic functions and energy storage capacity.

Zhang *et al.* (2023), when investigating the effects of chronic exposure to ammonia nitrogen (ammonia-N) on the growth performance, morphological and physiological alterations, and transcriptome changes of the hepatopancreas and gills of white shrimp (*Penaeus vannamei*), the histological results revealed significant differences between the hepatopancreas and gills of *Penaeus vannamei* exposed to chronic ammonia-N. Histologically, the hepatopancreas showed evident signs of cellular damage, including tissue degeneration, with cells exhibiting vacuolization and structural disorganization. Na+/K+-ATPase activity increased significantly, indicating that the organ was overburdened with the effort to maintain osmotic balance. The alterations suggest an inadequate physiological response to cope with oxidative stress induced by ammonia-N.

In the study on alkaline stress (350 mg/L) in *Penaeus vannamei* by Zhang *et al*. (2023), the authors found damage similar to the present work in hepatopancreas, observing that high alkalinity caused marked vacuolization (as an attempt to store and regulate water and ions), necrosis (indicating irreversible damage due to the accumulation of oxidative stress and cellular imbalance) and cellular disorganization in tubular cells, compromising the integrity and function of liver tissue. These histological damages reflect the shrimp's limitation in adapting to high alkalinity environments and demonstrate that osmotic balance and ionic homeostasis are significantly affected in *Penaeus vannamei*.

Similarly, Sun *et al*. (2015), investigated the transcriptomic and histological

responses of the hepatopancreas, muscles and gills of *M. nipponense* under chronic hypoxia. Significant histological changes were observed in the tissues of *Macrobrachium nipponense*. In the hepatopancreas, vacuolization was intense, reflecting accumulation of oxidative stress and difficulties in metabolic function. These changes suggest insufficient cellular adaptation to maintain homeostasis under low oxygen levels, affecting the structural integrity and vital functions of the tissues.

In the study investigated by Wu *et al*. 2008 on the toxic effects of cadmium (Cd) and zinc (Zn) on the hepatopancreas of *Penaeus vannamei*, it was found that shrimp exposed to varying concentrations of Cd caused significant vacuolization, necrosis and cellular degradation in the hepatopancreas. Heavy metals Cd and Zn accumulated in hepatopancreas cells, promoting oxidative stress that leads to the destruction of cellular organelles and liver dysfunction. In contrast to the findings by Li *et al*. 2008, juveniles *Penaeus vannamei* acclimated to the desired salinity by changing 2‰ per day from 22‰ to 3.0, 17.0 and 32.0‰, the hepatopancreas of the euryhaline shrimp *Penaeus vannamei* gradually acclimated at different salinities did not exhibit histological alterations (Diaz *et al*. 2010; Liu *et al*. 2016).

Han *et al*. (2018), when investigating how *Penaeus vannamei* responds to a gradually adjusted low pH environment (pH 6.65-8.20) compared to a normal and high pH environment maintained for 28 days, the hepatopancreas showed lesions at the beginning of exposure to low pH (1 day), including damage such as vacuolization and cellular disorganization. With time and adaptation to low pH, these tissues showed signs of recovery, indicating a physiological adaptation to restore the integrity of the villi and liver cells, promoting nutrient absorption and digestive

function, corroborating what was observed in the gradual treatments (*1^{c}* and *2^{c}Gradual*) of both 35-5 and 5-1 g/L after 24 hours non-acclimation.

The results of the present study suggest that reduction of salinity depending on the differences rates used during and after acclimation process has impact on the physiology in turn on the histological alterations in postlarvae stage. The comparison between the two salinity ranges (35-5 g/L and 5-1 g/L) highlights the greater severity of damage observed in the 5-1 g/L range, as excessive vacuolization observed in shrimp exposed reduction of salinity indicate a limited capacity to store energy reserves. In addition, hemocytic infiltration suggests an exacerbated inflammatory response to the adverse environment, while necrosis and irregular dilation of the lumens highlight severe structural damage, particularly under conditions of sudden and rapid salinity reduction (for example in *2^{a}Schock* treatment), the cells of the hepatopancreas of *Penaeus vannamei* probably faced an intense osmotic challenge. Very low salinity causes an excessive influx of water into the cells, overloading the osmoregulatory mechanisms that attempt to maintain ionic and water balance.

This overload induces damage, such as vacuolization in the cells as an initial adjustment response, a cellular defense mechanism to deal with excess water. If osmotic stress persists, the cells cannot maintain their integrity, resulting in necrosis and loss of tissue function (Pillai *et al*. 2002; Han *et al.* 2018; Zhang *et al*. 2023). According to Charmantier, 1998 and Henry, 2001, when *Penaeus vannamei* and other euryhaline shrimp are exposed to reduced salinities, they experience osmotic stress that requires adjustments to internal ionic balance, placing increased energy demands on the hepatopancreas, which supports homeostasis through active ion transport and regulatory processes. These observations support the

recommendation for gradual salinity reduction in *Penaeus vannamei* aquaculture practices to minimize physiological stress and maintain tissue integrity (Jayasankar *et al*. 2009; Zhang *et al*. 2023).

The results found between the two salinity ranges (35-5 and 5-1 g/L) reinforce the effectiveness of gradual acclimation in minimizing histological damage caused by osmotic stress. In particular, the *1^{c}Gradual* and *2^{c}Gradual* treatments were more efficient in preserving the structural integrity of the hepatopancreas and promoting more robust tissue recovery. In contrast, abrupt reductions, particularly in the range of 5-1 g/L (*2^{a}Shock*), caused severe and irreversible damage. Previous studies indicate that abrupt changes in salinity can destabilize osmoregulatory mechanisms, exacerbate oxidative stress, and increase inflammatory damage, corroborating our findings (Li *et al*. 2017; Zhang *et al*. 2023; Yang *et al*. 2024). The preservation of hepatopancreas structure in gradual treatments demonstrates the crucial role of adequate acclimation in mitigating negative impacts in low-salinity culture systems. These findings provide valuable information for the development of management practices that promote the health and welfare of *Penaeus vannamei* in aquaculture environments.

5 Conclusion

The study concluded that *Penaeus vannamei* can tolerate a wide range of salinities (0.5-40 g/L), but the salinity reduction range and the speed of this change evidence significant impact on survival, oxygen consumption and histological integrity of the gills and hepatopancreas of *Penaeus vannamei* postlarvae. The results demonstrated that under abrupt reduction conditions, they impose substantial

physiological stress and that in the (2aSchock) treatment of 5 to 1 g/L range recorded lower survival, higher oxygen consumption and severe histological damage to the hepatopancreas and gills, including vacuolization, hemocyte infiltration and necrosis and less intense in the rapid reduction treatments (2bFast) and, without evidence of recovery after the non-acclimatization period (LW24H+), the most critical salinity range being considered at 5-1 g/L. In contrast, gradual reduction of salinity in the of 35-5 g/L range minimized these effects, promoting higher survival and better structural preservation of tissues. Furthermore, signs of recovery were observed in the structures of the gills and hepatopancreas after LW24H+ period, suggesting that gradual acclimation allows the physiological mechanisms of osmoregulation and cellular repair to adjust to the new environmental conditions more efficiently. Therefore, the findings reinforce the importance of gradual acclimation protocols, especially in 35-5 g/L range in mitigating osmotic stresses and improve physiological resilience in low salinity farming environments. Adopting practices that minimize abrupt changes in salinity can significantly improve the well-being and survival of *Penaeus vannamei*, promoting more sustainable production in aquaculture systems.

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5.Conclusiones generales

Los hallazgos destacan la importancia de las estrategias de manejo durante el proceso de aclimatación a salinidades bajas, evaluando parámetros críticos como la supervivencia, consumo de oxígeno y alteraciones histológicas en branquias y hepatopáncreas.

En el primer estudio, se demostró que la tasa de reducción de salinidad tiene un impacto significativo en la estructura histológica de los tejidos evaluados durante periodo de aclimatación (A24h). Aunque todos los grupos mantuvieron una supervivencia superior al 80% al final del periodo de aclimatación (A24h), en el periodo de no aclimatación ($A24h^{+}$) se observó una supervivencia significativamente menor en el tratamiento de reducción variable (VAR-SAL), en comparación con el grupo control (CON) y la reducción constante (CON-SAL).

El segundo estudio complementó estos hallazgos al evaluar los efectos de diferentes rangos de reducción de salinidad (35-5 g/L y 5-1 g/L) y sus tasas de aclimatación.

Una tendencia de reducción en la supervivencia fue observada en ambos rangos tanto en el periodo de LW24H cuanto en LW24H+, y todos los tratamientos se mantuvieron arriba del 80%, siendo significativamente mayor en el tratamiento CON35 y *1^{c}Gradual* en 35-5 g/L en comparacion a los tratamientos de rangos 5-1 g/L.

Además, una tendencia de aumento en el análisis de consumo de oxígeno fue registrada y confirmó una mayor demanda metabólica en los tratamientos abruptos, reflejando una sobrecarga energética significativa en estos grupos.

En contrapartida, estos resultados subrayan la importancia de adoptar protocolos de aclimatación gradual y controlados en la acuicultura de baja salinidad, ya que mitigan el estrés osmótico y reducen el daño histológico en postlarvas de *Penaeus vannamei.*

Futuras investigaciones deben enfocarse en los mecanismos moleculares involucrados en la osmorregulación y la recuperación tisular para optimizar aún más las estrategias de manejo en sistemas de cultivo de baja salinidad.

Este estudio proporciona una base científica sólida para el desarrollo de técnicas que maximicen la resiliencia de *Penaeus vannamei* frente a cambios ambientales, fortaleciendo así su producción en diversas condiciones acuícolas.

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ANEXO

---------- Forwarded message ---------
De: **Dr. Fernando Vega-Villasante** <fernandovega.villasante@gmail.com>
Date: lun, 6 ene 2025 a las 13:44
Subject: [LAJAR] Editor Decision
To: Dr. Mario Alberto Galaviz Espinoza <mgalaviz@uabc.edu.mx>

Dr. Mario Alberto Galaviz Espinoza:

We have reached a decision regarding your submission to Latin American Journal of Aquatic Research, "Litopenaeus vannamei post-larval survival and histological changes in gill and hepatopancreas acclimated to low salinity in the short-term: Does dissolved salt content matter in defining their reduction rates?".

Our decision is to: MANUSCRITO ACEPTADO

Estimado Dr. Galaviz, he considerado que su manuscrito responde plenamente a los cuestionamientos y sugerencias de los revisores. Por lo anterior me da gusto informarle que ha sido aceptado. Le solicito envíe una versión final, agregando los nombres de los autores y sus afiliaciones, así como compruebe que el formato cumple cabalmente con lo exigido por LAJAR. En el atado incluyo un artículo ejemplo para que se guíe con este.

Agradecemos su confianza en LAJAR y la paciencia que ha tenido con este proceso editorial.

Saludos afectuosos

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