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Research Article

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 h acclimation period (A24h). Two treatments were based on adapted protocols available in the 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 4 h: 1 h at a 16 g L^{-1}, 2 h at 9 g L^{-1}, and 3 h at 5 g L^{-1} per hour, which was followed by a reduction of 0.25 g L^{-1} per hour from 5 to 2 g L^{-1} over 13 h and, finally, a slower reduction of 0.13 g L^{-1} per hour from 2 to 1 g L^{-1} over the last 8 h. A control group was maintained at 35 ± 1 g L^{-1} with 40% h^{-1} saltwater exchange. At the end of A24 h, all treatments were maintained for 24 h during a non-acclimation period (A24 h^{+}). 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 A24 h^{+}. Histological gill damage was observed in CON-SAL and VAR-SAL at A24 h, but VAR-SAL was the only group showing progressive damage at A24 h^{+}. Both treatments resulted in hepatopancreas changes, but damage was more severe in VAR-SAL at both A24 h and A24 h^{+}. These results support that the reduction rates should be defined as a concentration function, since 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 (A24 h). Few studies have investigated short-term histological changes during PL acclimation to low salinity, emphasizing the significance of these findings.

Keywords: *Penaeus vannamei*; postlarvae; 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, in 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),

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which explains why it has been considered one of the preferred crustacean species for inland aquaculture (McGraw et al. 2002). Although *P. vannamei*'s 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 are transferred and acclimated to commercial farm-water conditions. Therefore, an efficient acclimation protocol is crucial for shrimp responding 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 (Moullac & Damez 1991, Samocha et al. 1998, McGraw et al. 2002, Saoud et al. 2003).

Postlarvae exchange ions across epithelial cell membranes during acclimation to ensure 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 increased oxygen consumption and metabolic activity (Laramore et al. 2001, Decamp et al. 2003, Lin et al. 2012). However, cellular damage is 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) (Moullac & Damez 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 to 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 L^{-1} over a short period (24-48 h), 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 evaluated 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 1,000 L 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 was maintained below 0.5 mg L^{-1} via 100% water exchange daily, and photoperiod was natural (12:12 h, light:dark). Shrimp were fed at 100% of estimated biomass with a commercial feed formulated for PL 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

The following week, 360 PL22 (0.048 ± 0.02 g) were randomly selected and distributed in nine experimental units with a volume of 5 L 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 h (A24h) using the following protocol treatments: a) CON-SAL, a constant rate of salinity reduction at 1.45 g L^{-1} per hour, regardless of salinity level; and b) VAR-SAL, following adaptations from Van Wyk et al. (1999) and McGraw et al. (2002). In the first 4 h, 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, 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 h. Finally, salinity was further reduced from 2 to 1 g L^{-1} at 0.13 g L^{-1} per hour over the last 8 h. 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 h.

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 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 Mousa & Taha (2003) protocols. At the end of the 24 h (A24h) of acclimation, PLs were kept for an additional 24 h (A24 h^{+}) in their corresponding tanks at the final salinity (1 g L^{-1}); likewise, the control group was kept for 24 h more.

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 A24 h^{+}.

At the end of A24 h and A24 h^{+}, 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 A24 h or A24 h^{+}) / (number of alive PLs at the beginning of A24 h or A24 h^{+}).

Histopathological and statistical analyses

Additionally, at the end of both periods (A24 h or A24 h^{+}), 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 poly-L-lysine solution-coated slides, and stained with hematoxylin-eosin. The sections were examined under a light microscope (Nikon Eclipse-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).

Before 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 Bartlett and Levene's tests, respectively, and two-way analysis of variances (ANOVA) to determine statistical differences between treatments. For water parameters, two-way ANOVA was performed to identify differences among experimental groups (control, CON-SAL, and VAR-SAL) and times (A24 h and A24 h^{+}), followed by Tukey's *post-hoc* comparison test when significant differences were found. While the Kruskall-Wallis (non-parametric) test was used to analyze the number of B cells, ruptured epithelial cells, dilated central tubules, and degenerated tubule lumen in the hepatopancreas. All statistical analyses were executed at a significance level 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. Table 1 shows the parameters' mean (standard deviation, minimum, and maximum values). Figure 1 shows salinity variation according to the experimental groups throughout the trial.

At the end of A24 h, survival rate was higher than 80% in all experimental groups, without showing a statistical difference ($P < 0.05$). Significant differences were found at the end of A24 h^{+} ($P < 0.05$) when lower survival was recorded in VAR-SAL (65.47%) in comparison with control (83.33%) and CON-SAL (81.07%) (Table 2).

Table 1. Mean ± standard deviation (SD), minimum (Min), and maximum (Max) values of water quality parameters in the control (35 g L^{-1}) and 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

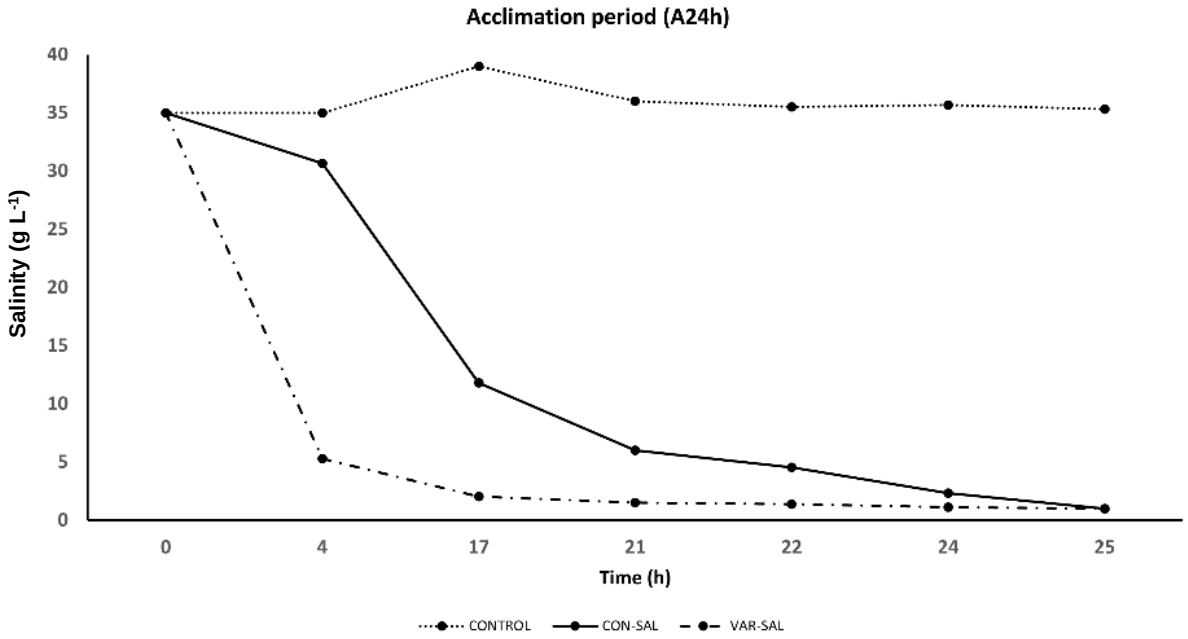

Figure 1. Salinity variation throughout 24-h acclimatation (A24h) of *Penaeus vannamei* postlarvae to 1 g L^{-1} of salinity and the additional 24 h (A24 h^+) in the control (35 g L^{-1} of salinity) and treatments with constant salinity and variable salinity reduction rates (CON-SAL and VAR-SAL, respectively).

Histopathology structure of postlarvae

For both A24 h and A24 h^+, control exhibited normal gill filament structure and no changes in tissue structure (Figs. 2a,c). Shrimp acclimated following a salinity reduction constant rate (CON-SAL) showed histological changes compared with the control, characterized by increased intercellular hemocytes at A24 h. However, no progressive changes were observed 24 h later (A24 h^+) (Figs. 2b,e). In the VAR-SAL treatment at A24 h, 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 A24 h^+, shrimp from VAR-SAL exhibited progressive histological changes characterized by the appearance and increase in high-density vacuoles, indicating filament edema intensification (Fig. 2c,f).

At the end of A24 h, 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

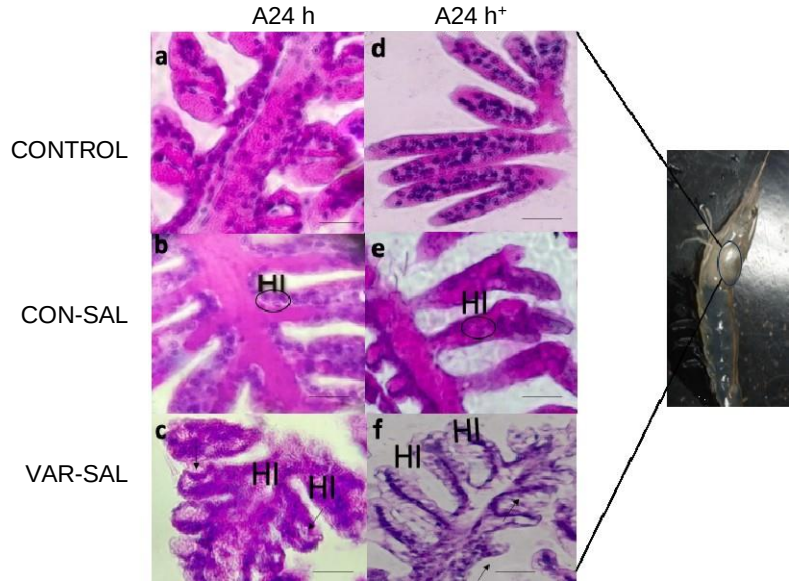

Figure 2. Histological microscopy of *Penaeus vannamei* post-larval gills in the control (35 g L^{-1}) and treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of 24 h of acclimation (A24 h) to 1 g L^{-1} (a, b, and c, respectively) and of the additional 24 h (A24 h^{+}) (d, e, and f, respectively). a) Control at A24 h showed normal structure of gill filaments and no changes in tissue structure. b) CON-SAL at A24 h showed an increase in intercellular hemocytes (HC) compared to control. c) VAR-SAL at A24 h showed several histological changes, such as loss of gill filament structure, an increase in hemocytic infiltration, and appearance of vacuoles resulting in a roughening of the surfaces of the gill filaments (epithelial edema). d) Control at A24 h^{+} still showed no histological damages. e) CON-SAL at A24 h^{+} still showed more intercellular hemocytes than control, but with no increase compared with CON-SAL at A24 h. f) VAR-SAL at A24 h^{+} still showed loss of gill filament structure, but increased inflammation and appearance of high density of vacuoles resulted in the filament edema intensification. Tissue sections were stained using Hematoxylin and Eosin (H&E), 40x. HI: hemocytic infiltration.

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 (A24 h) and the additional 24 h (A24 h^{+}). Different superscript letters within rows indicate significant differences ($P < 0.05$).

	Control	CON-SAL	VAR-SAL
A24 h	88.67 ± 4.42	83.60 ± 4.45	84.07 ± 5.21
A24 h^{+}	83.33 ± 4.16^{a}	81.07 ± 4.58^{a}	65.47 ± 6.27^{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 A24 h^{+}, 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.

For A24 h and A24 h^{+}, VAR-SAL showed a significantly lower number of B cells and higher dilated central tubules, ruptured epithelial cells, and dilated tubule lumen compared with CON and CON-SAL. No statistical differences were observed between CON and CON-SAL, except for B cell number at A24 h^{+}, which was significantly higher in CON-SAL (Table 3).

DISCUSSION

As expected, 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 (A24 h) - 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

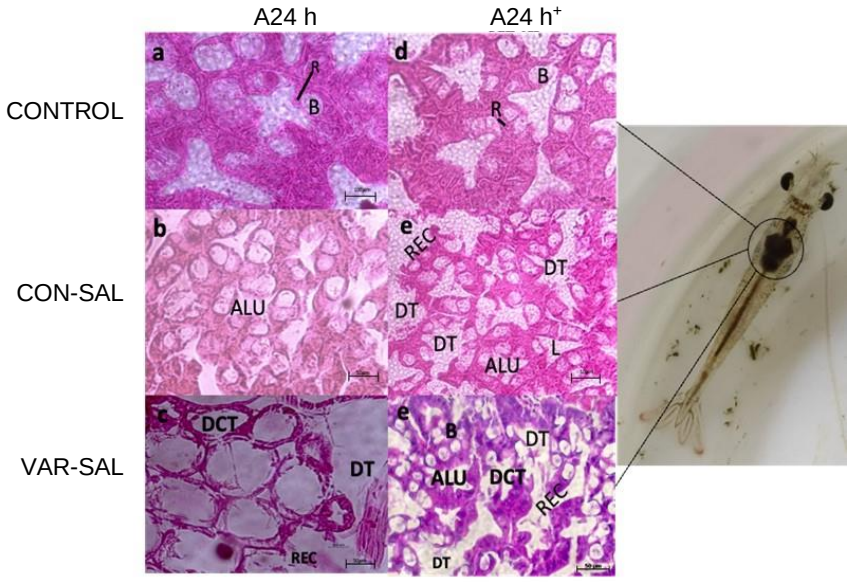

Figure 3. Histological microscopy of *Penaeus vannamei* post-larval hepatopancreas in control (35 g L^{-1}) and treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of 24 h of acclimation (A24 h) to 1 g L^{-1} (a, b, and c, respectively) and the additional 24 h (A24 h^{+}) (d, e, and f, respectively); a,d) Control showed a normal hepatopancreas structure with star shape-like lumen and presence of blasenzellen cells (B) and restzellen cells (R) in A24 h and A24 h^{+}, respectively. In 24 h, 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 the control. c) VAR-SAL exhibited histological changes in the tubular glands and epithelial cells, including cells (B and R cells) that were not observed; the tubule lumen showed dilatation, ruptured epithelial cells (REC), degenerated tubule (DT), storage vacuoles were absent, and epithelial tissue was lost. In A24 h^{+}, e) CON-SAL exhibited vacuolization of the epithelium, abnormal structure of the lumen, REC, and DT. f) VAR-SAL was observed in tubule lumen dilatation, REC, DT, but a recovery in the structure in comparison with the A24 h time, exhibiting a reduction of dilatation in tubule lumen resulting in a normal appearance in some of the hepatopancreatic tubules; additionally, B cells were observed again. Hematoxylin-eosin. Bar 50 and 100 µm. L: star shape-like lumen, ALU: abnormal lumen, DCT: dilated central tube.

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 treatments with constant and variable rates of salinity reduction (CON-SAL and VAR-SAL, respectively) at the end of the 24 h of acclimatation (A24 h) and the additional 24 h (A24 h^{+}). Different superscript letters within rows indicate significant differences ($P < 0.05$). B cells: blassenzelen cells, REC: ruptured epithelial cells.

Parameter	A24 h			A24 h^{+}		
	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}

the VAR-SAL treatment, salinity reductions began with a rapid decrease in the first 4 h, 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, 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 h. Finally, salinity was further reduced from 2 to 1 g L^{-1} at 0.13 g L^{-1} per hour over the last 8 h.

This protocol was strategically implemented to simulate osmotic stress conditions that may occur in

culture environments, where abrupt salinity changes require rapid physiological responses. Such conditions allow for assessing the adaptive response capacity of *P. vannamei* to rapid environmental changes (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 shrimp resilience under rapid and gradual reductions, incorporating strategic change points (5 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 A24 h 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 *P. vannamei*'s ability 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 h 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 to tolerate 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, the 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 24 h, which revealed a significant survival reduction for VAR-SAL compared with the other experimental groups.

The histopathological analysis results in the gill and hepatopancreas revealed changes that most likely explain the results recorded for survival. For A24 h, PL gill filaments acclimated in the CON-SAL exhibited moderate and reparable structural changes and increased intercellular hemocytes compared to the control. Nevertheless, no progressive changes were observed at A24 h^{+}. On the other hand, several histological damages were observed in VAR-SAL, including vacuolization presence, hemocyte infiltration, and loss of its normal structure. Moreover, damages in this treatment were progressively severe at A24 h^{+}, showing an epithelial edema characterized by vacuolation. Fregoso-Lopes 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, resulting in histological alterations in gills such as oedema and inflammation (haemolymph and haemocytic infiltration) during the first five 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}), 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 to 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, increasing 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 et al. (1997) 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 aerobic metabolism rate. Böer et al. (2007) reported that *Clione limacina* organisms obtain energy from the hepatopancreas lipid reserve to manage 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 A24 h and A24 h^+ 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, which were more severe in VAR-SAL, such as vacuolization and cellular disorganization in the hepatopancreas at A24 h. Still, a histological recovery trend was observed in this treatment when data on hepatopancreas damage between treatments over time were compared. In the 24 h^+, a normal appearance in structure was observed with a less dilated structure and more B presence compared with A24 h. This finding is consistent with research demonstrating the ability of *P. vannamei* to exhibit adaptive plasticity, which suggests that tissue damage caused by osmotic stress may be partially reversible if conditions are stabilized, as observed in the VAR-SAL treatment. Despite the first 4 h of rapid salinity reduction, the last 20 h were performed gradually and slowly, indicating stabilized salinity conditions, contributing to some individuals showing signs of recovery after 24 h^+ (Li et al. 2008, Wu et al. 2009).

Similar results were reported by Chen et al. (2015) and Gao et al. (2017), showing that the hepatopancreas of *P. vannamei*, exposed to abrupt changes in salinity, can suffer significant structural damage (vacuolization, tubule dilation, and cell necrosis). Still, 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, which, depending on intensity, may not be completely reversible, leading to permanent tissue sequel (Jayasankar et al. 2009, Cao et al. 2024), especially in organs that require high energy demand for osmoregulation processes. Therefore, although the present study observed some signs of recovery in the A24 h^+ period, possibly 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 its structural integrity, reducing vacuolization and improving tubule organization, which suggests a potential for recovery (Calvo et al. 2011, Zhang et al. 2023). Gradual acclimation protocols were observed that maximize survival and reduce osmotic stress, allowing more effective tissue recovery, which is essential for the sustainable management of *P. 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 A24 h. These 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 was significantly higher in PLs acclimated in VAR-SAL than in CON-SAL and CON. Similarly, greater damage was observed significantly in VAR-SAL regarding tubule degradation; corroborating histological images, B and R cells could not be identified 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 the VARS-SAL group during A24 h^+. A notable reduction was observed in some indicators, such as dilated central tubules, REC, and degeneration tubes, compared to the same treatment for A24 h. A small improvement was observed in hepatopancreas tubes when maintained at L g L^{-1} 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) showed that juvenile *P. vannamei* were gradually acclimated 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, a B-cell increase in *P. vannamei* was observed at low salinities; this indicates 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 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. B cells are the main site for digestive enzyme synthesis, accelerating nutrient mobilization in hepatopancreas tubules when an increased energy demand is required to adapt to environmental stress. The decrease of these cells might be due to the

utilization of their nutrient reserves and increased energy demand for osmoregulation (Chen et al. 2021, 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 (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 that low salinity ROS production in hepatopancreas is increased at 5 g L^{-1} of salinity, which might have caused an increase in antioxidant enzyme synthesis.

The results in the present study found histological images and parameter hepatopancreas damages, when the organisms were maintained at 1 g L^{-1} for A24 h^{+}, 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 A24 h. Likewise, Díaz et al. (2010) 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 molting and reproduction (Marcolin et al. 2008). However, storage or use of reserves seems to depend on the species and its physiological condition. The same response was obtained in other osmoregulatory investigations, which have found that osmoregulatory homeostasis was restored in approximately 24 h (Bursey & Lane 1971, Castille & Lawrence 1981, Li et al. 2017). Firstly, similar recovery durations 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. (1999) showed that *P. setiferus* (Linnaeus) postlarvae (PL10 to PL14) can tolerate diluted media (up to 5‰ units) and can 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^{-1} 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^{-1} h^{-1} from 5 to 2 g L^{-1} and 0.13 g L^{-1} h^{-1} from 2 to 1 g L^{-1}), allowed a more controlled transition and more time for adjustment of osmoregulation mechanisms. Gradual transitions between rapid events of osmotic stress and periods of stabilization followed this combination of abrupt reduction. This approach allows us to observe how the organism responds to initial intense stresses and its recovery potential, since stabilization periods provide time for physiological adaptation (Liu et al. 2024). In shrimp, this 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 Burgents et al. (2004) and Chen et al. (2019) suggested. Nevertheless, in prolonged exposures or 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.

Credit author contribution

V. Magalhães: conceptualization, validation, methodology, formal analysis, writing-original draft; M. Galaviz: funding acquisition, project administration, supervision, review, and editing; A. Braga de Souza & S Sanchez: methodology, validation, supervision, review, and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest regarding this research article.

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