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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of salinity on growth performance and survival of second-stage juvenile *Pontastacus leptodactylus* (Eschscholtz, 1823)

İkinci evre *Pontastacus leptodactylus* (Eschscholtz, 1823) jüvenillerinin büyüme performansı ve yaşama oranı üzerine tuzluluğun etkileri

Hasan Batuhan Emre Özdoğan

Eğirdir Faculty of Fisheries, Isparta University of Applied Sciences, 32100, Isparta, Türkiye

E-mail adress: hasanozdogan@isparta.edu.tr

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Abstract: The narrow-clawed crayfish, *Pontastacus leptodactylus*, is an economically significant species recognized for its high ecological resilience, particularly its ability to thrive in brackish environments. This research investigated the impact of various salinity levels (0, 2.5, 5, and 10 ppt) on the survival and growth metrics of second-stage juveniles during a 25-day experimental trial. A total of 160 individuals (initial mean weight: 0.033±0.001 g) were allocated into four treatment groups, with each group conducted in quadruplicate. The data indicated that growth performance parameters, such as total length, final weight, and specific growth rate (SGR), were not significantly altered by the salinity treatments ($p > 0.05$), demonstrating that somatic growth was sustained across the tested salinity gradient. In contrast, survival was significantly affected by environmental salinity ($p<0.05$). The maximum survival rate was recorded at 5 ppt (52.5±2.50%), followed by the 10 ppt (45.0±6.45%) and 2.5 ppt (30.0±4.08%) groups, whereas the freshwater control group had the lowest survival (12.5±9.46%). These results indicate that moderate salinity (5 ppt) can optimize survival in juvenile crayfish without impairing growth performance. Implementing low-salinity rearing strategies could effectively address the high mortality rates often observed during the sensitive nursery phase of *P. leptodactylus*.

Keywords: *Pontastacus leptodactylus*, salinity, growth performance, survival rate, aquaculture

Öz: Ekonomik açıdan önemli bir tür olan dar kıskaçlı kerevit, *Pontastacus leptodactylus*, özellikle acı su ortamlarında gelişme yeteneği başta olmak üzere yüksek ekolojik dayanıklılığı ile tanınmaktadır. Bu araştırma, 25 günlük bir deneme süresince farklı tuzluluk seviyelerinin (0, 2,5, 5 ve 10 ppt) ikinci evre jüvenillerin hayatta kalma ve büyüme parametreleri üzerindeki etkisini incelemiştir. Toplam 160 birey (başlangıç ortalama ağırlığı: 0.033±0.001 g), her bir grup dört tekerrürlü olacak şekilde dört deneme grubuna ayrılmıştır. Elde edilen veriler; toplam boy, final ağırlığı ve spesifik büyüme oranı (SGR) gibi büyüme performansı ölçütlerinin tuzluluk uygulamalarından önemli ölçüde etkilenmediğini ($p>0,05$) ve somatik büyümenin test edilen tuzluluk gradyanı boyunca sürdürüldüğünü göstermiştir. Buna karşın, yaşama oranı çevresel tuzluluktan önemli ölçüde etkilenmiştir ($p<0,05$). En yüksek hayatta kalma oranı 5 ppt grubunda (%52,5±2,50) kaydedilmiş, bunu 10 ppt (%45,0±6,45) ve 2,5 ppt (%30,0 ±4,08) grupları izlemiş; en düşük yaşama oranı ise tatlı su kontrol grubunda (%12,5 ±9,46) saptanmıştır. Bu sonuçlar, orta seviyeli tuzluluğun (5 ppt), büyüme performansını olumsuz etkilemeden jüvenil yaşama oranını optimize edebileceğini göstermektedir. Düşük tuzluluk içeren yetiştirme stratejilerinin uygulanması, *P. leptodactylus*'un hassas kuluçkahane evresinde sıklıkla gözlenen yüksek mortalite oranlarını etkili bir şekilde azaltabilir.

Anahtar kelimeler: *Pontastacus leptodactylus*, tuzluluk, büyüme performansı, yaşama oranı, su ürünleri yetiştiriciliği

INTRODUCTION

The evolutionary history of freshwater crayfish reflects a strong capacity for biological resilience. Their transition to freshwater likely occurred as early as the Triassic Period (Scholtz, 1999; Audo et al., 2023), and despite this long evolutionary history, these organisms have retained a remarkable degree of physiological plasticity. Features such as direct development and maternal brood care are widely recognized as specialized adaptations to life in hypo-osmotic environments (Yildiz et al., 2004). However, despite this deep-rooted freshwater heritage, many species—particularly those within the Astacidae family—retain the ability to tolerate brackish conditions (Holdich et al., 1997). This capacity highlights the ecological and applied importance of investigating species that can function across a gradient of salinity conditions.

Among these, the narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823), is an economically significant species widely distributed across the Ponto-Caspian region, including Türkiye, the Balkans, Eastern Europe, and Central Asia, with its distribution extending into Iran, Kazakhstan, China, and the Caspian Sea Basin (Holdich, 2002; Khoshkholgh and Nazari, 2015; Li et al., 2025). Its natural distribution and successful introduction into multiple countries highlight its remarkable ecological robustness. The species can survive in environments where salinity ranges from 4 to 14 ppt (Skurdal and Taugbøl, 2002) and dissolved oxygen levels may temporarily decrease to as low as 2 mg L^{-1} (Hesni et al., 2009; Skurdal and Taugbøl, 2002). In Türkiye, *P. leptodactylus* is the only native crayfish species and has been reported from estuarine environments (Yildiz et al., 2004). Köksal (1988) also reported the species from the Black Sea. This ecological flexibility is further supported by reports of the species occurring in the Caspian Sea at salinities of up to 14 ppt, indicating its capacity to exploit and persist in brackish environments (Hesni et al., 2009; Holdich et al., 1997; Yildiz et al., 2004).

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Despite the demonstrated long-term survival of juveniles and adults under elevated salinity conditions in experimental studies, the ability of *P. leptodactylus* to establish stable populations in saline environments appears to be constrained. Evidence suggests that increased salinity may negatively affect critical life stages, particularly egg development and hatching success (Hesni et al., 2009; Yildiz et al., 2004). Consequently, evaluating this species within a brackish-water context, rather than under marine conditions, provides a more realistic ecological and biological perspective.

In aquaculture, growth performance and survival are key indicators of production success. In decapod crustaceans, growth is governed by the complex interaction of multiple environmental factors, including temperature, dissolved oxygen, salinity, photoperiod, and nutrition. High mortality rates have been reported as a characteristic feature during the first growing period of crayfish (Ulikowski and Krzywosz, 2006), highlighting the vulnerability of early life stages. In addition to these early-stage vulnerabilities, culture conditions such as stocking density has been shown to significantly influence growth performance and survival in juvenile crayfish (Savolainen et al., 2004). Although freshwater crayfish maintain internal osmotic balance through physiological regulation, a notable gap remains in our understanding of salinity stress during these sensitive juvenile stages (Hesni et al., 2009).

In addition to environmental factors, dietary composition represents a critical determinant of development. Recent research has established an optimized feeding regime for first feeding, second-stage *P. leptodactylus* juveniles (Özdoğan and Koca, 2023). By adopting these standardized feeding protocols, it is now possible to isolate environmental variables like salinity as the primary experimental factor influencing growth and survival. Despite earlier documentation of salinity tolerance, recent studies have focused on general physiological responses rather than on direct, stage-specific evaluations of growth and survival. This lack of up-to-date data represents a bottleneck for the development of sustainable brackish-water aquaculture management.

Therefore, the present study aims to evaluate the effects of salinity on the growth performance and survival of juvenile *P. leptodactylus* under controlled conditions. By addressing this gap, the study seeks to provide reliable data on the species' adaptive capacity and to assess its potential for use in brackish-water aquaculture systems.

MATERIALS AND METHODS

Supply of crayfish

The female narrow-clawed crayfish (*P. leptodactylus*) samples with eggs a total length of 10-15 cm were collected from Lake Eğirdir, located in Isparta, Türkiye. Healthy individuals were subsequently moved to the aquaculture research facility at the Eğirdir Faculty of Fisheries, Isparta University of Applied Sciences. These females were housed in 800 L fiberglass tanks, which included PVC pipe segments as shelters. During the maintenance period, the females were fed a commercial trout diet. The incubation phase lasted approximately 20 days until hatching, and the resulting offspring were cultured to the second juvenile stage prior to their use in the trial.

Experimental design

Second-stage juvenile crayfish with an average body weight of 0.033 ± 0.001 g and a total length of 10.0 ± 0.01 mm were randomly assigned to experimental units. The experiment followed a completely randomized design consisting of four treatment groups representing different salinity levels: 0 (control), 2.5, 5, and 10 ppt. Each treatment consisted of four replicates, with each replicate containing 10 individuals stocked in polypropylene experimental units (22 × 34 × 15 cm) with a water volume of 10 L. Salinity levels were established by dissolving commercial sea salt in freshwater, and the target concentrations were maintained throughout the study. The 0 ppt group served as the control. Prior to the experiment, crayfish were gradually acclimated to their respective salinity treatments by increasing salinity in a stepwise manner to reduce osmotic stress. This acclimation procedure was based on the approach described by Veselý et al. (2017). Salinity was monitored daily using a refractometer. A daily water exchange of 5–10% was carried out to maintain water quality, and salinity was readjusted to the target level after each water exchange when required. Throughout the 25-day experimental period, crayfish were fed a commercial microparticulate diet (INVE Aquaculture, Dendermonde, Belgium) with a particle size of 300–500 µm twice daily (10:00 and 17:00) on an ad libitum basis. The nutritional composition of the diet is presented in Table 1. Plastic shelters were provided in each experimental unit to reduce encounters among juveniles and limit cannibalism. Uneaten feed and feces were removed daily by siphoning. The units were continuously aerated and filtered, and the water temperature was maintained at 20 ± 1 °C using thermostatically controlled heaters. Dissolved oxygen ranged from 6 to 7 mg L^{-1}, while pH remained between 7.5 and 8.0. A photoperiod of 12L:12D was maintained throughout the experiment.

Table 1. Nutritional composition of the microparticulate feed*

Ingredient	Content (%)
Crude protein	56.0
Crude lipid	13.0
Crude fibre	1.0
Crude ash	10.0

*Particle size: 300–500 µm. Nutritional values are based on the manufacturer's product specifications (Inve Aquaculture Inc.).

Growth parameters

Measurements were taken on days 14 and 25 to assess intermediate and final growth, respectively. At each sampling point, individual body weight (g) was measured using a precision balance, while total length (mm) was determined using a digital caliper. Growth performance was evaluated using the following standard indices (NRC, 2011; Sevgili et al., 2019):

Weight gain (WG, g): $\mathrm{WG} = \mathrm{Wf} - \mathrm{Wi}$

Specific growth rate (SGR, % day^{-1}): $\mathrm{SGR} = \left(\frac{\ln \mathrm{Wf} - \ln \mathrm{Wi}}{\mathrm{t}}\right) \mathrm{x}100$

Survival (SR, %) = $\mathrm{SR} = \left(\frac{\mathrm{Nf}}{\mathrm{Ni}}\right) \mathrm{x}\ 100$

where Wf and Wi represent final and initial crayfish body weights (g), respectively; t is the experimental period (days); Nf and Ni are the numbers of crayfish at the end and beginning of the experimental period, respectively.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics software, version 23 (IBM, Armonk, NY, USA). Prior to analysis, the data were tested for normality with the Shapiro-Wilk test and for homogeneity of variance with Levene's test. As the assumptions of normality and homogeneity of variance were met, growth performance parameters and survival rates were analysed separately at each sampling point using one-way analysis of variance (ANOVA). When ANOVA indicated a significant treatment effect, Tukey's multiple comparison test was used for pairwise comparisons. The significance level for all tests was set at $p < 0.05$. Results are presented as mean ± standard error (SE).

RESULTS

Growth performance at day 14

On day 14, growth performance indicators of juvenile *P. leptodactylus* were comparable among salinity treatments and no statistically significant differences were detected (Table 2). Initial body weight and total length did not differ significantly among experimental groups, confirming the homogeneity of the groups at the beginning of the trial. Mean body weight ranged from 0.044±0.001 g in the 10 ppt treatment to 0.048±0.002 g in the 2.5 ppt group, while total length varied between 11.70±0.28 mm and 12.44±0.32 mm among treatments. Neither body weight nor total length differed significantly among treatments ($p > 0.05$).

Survival rate at day 14

Survival rate on day 14 ranged from 55.0±6.45% in the 2.5 ppt treatment to 75.0±2.89% in the 10 ppt treatment (Table 2). However, no significant differences were detected among salinity treatments ($F = 1.64$, $p = 0.23$).

Growth performance at day 25

At the end of the experimental period (day 25), growth performance parameters showed a similar pattern, with no significant differences among salinity treatments (Table 3). Final body weight ranged from 0.060±0.003 g in the 10 ppt group to 0.069±0.004 g in the 2.5 ppt group, while total length varied between 14.00±0.84 mm in the control group and 14.75±0.30 mm in the 2.5 ppt group. Neither final body weight nor total length differed significantly among treatments ($p > 0.05$). Similarly, weight gain and SGR did not differ significantly among salinity levels. Weight gain ranged from 0.027±0.003 g to 0.036±0.004 g, while SGR values varied between 2.24±0.01% day^{-1} and 3.31±0.01% day^{-1} ($p > 0.05$).

Survival rate at day 25

Survival rate was significantly affected by salinity at the end of the experiment. The highest survival was observed in the 5 ppt treatment (52.5±2.50%), followed by the 10 ppt (45.0±6.45%) and 2.5 ppt (30.0±4.08%) groups. The lowest survival was recorded in the control group (12.5±9.46%). Tukey's multiple range test indicated that survival rates in the 5 ppt and 10 ppt groups were significantly higher than the control group ($p<0.05$). However, the survival rate in the 2.5 ppt treatment did not differ significantly from those in either the control group or the 5 and 10 ppt groups ($p > 0.05$).

Table 2. Growth performance parameters and survival rate of juvenile *P. leptodactylus* exposed to different salinity levels at day 14

	Control	2.5 ppt	5 ppt	10 ppt	df	F	P
Initial weight (g)	0.033±0.001	0.033±0.001	0.033±0.001	0.033±0.001			
Initial length (mm)	10.00±0.01	10.00±0.01	10.00±0.01	10.00±0.01			
Final weight (g)	0.045±0.002	0.048±0.002	0.045±0.002	0.044±0.001	3	0.96	0.42
Final length (mm)	12.20±0.31	12.44±0.32	12.11±0.30	11.70±0.28	3	1.09	0.36
Survival rate (%)	70.00±9.13	55.00±6.45	70.00±7.07	75.00±2.89	3	1.64	0.23

Data are expressed as mean ± standard error (SE) (n=4 replicates).

Table 3. Effects of salinity on the growth performance and survival of juvenile *P. leptodactylus* at the end of the experimental period

	Control	2.5 ppt	5 ppt	10 ppt	df	F	P
Initial weight (g)	0.033±0.001	0.033±0.001	0.033±0.001	0.033±0.001			
Initial length (mm)	10.00±0.01	10.00±0.01	10.00±0.01	10.00±0.01			
Final weight (g)	0.063±0.001	0.069±0.004	0.063±0.003	0.060±0.003	3	1.04	0.38
Final length (mm)	14.00±0.84	14.75±0.30	14.39±0.23	14.48±0.24	3	0.57	0.64
Weight gain (g)	0.030±0.008	0.036±0.004	0.030±0.003	0.027±0.003	3	1.03	0.39
SGR (%)	2.72±0.01	3.31±0.01	2.24±0.01	2.99±0.01	3	1.45	0.29
Survival rate (%)	12.50±9.46^{b}	30.00±4.08^{ab}	52.50±2.50^{a}	45.00±6.45^{a}	3	8.11	0.03

Data are expressed as mean ± standard error (SE) (n=4 replicates). Different superscript letters within the same row indicate significant differences ($p < 0.05$).

DISCUSSION

The results of this study showed different responses of growth and survival to salinity in juvenile *P. leptodactylus*. Growth performance did not differ among the 0–10 ppt treatments, whereas survival was highest at 5 ppt and lowest in the freshwater control. These findings suggest that the tested salinity levels did not restrict somatic growth, but that survival was more responsive to the rearing salinity. However, because physiological variables were not measured, the higher survival at 5 ppt can only be discussed as a possible consequence of reduced osmotic stress.

The stability of growth parameters observed in the present study is consistent with the salinity tolerance previously reported for this species. Holdich et al. (1997) reported that *P. leptodactylus* possesses a strong capacity to hyper-regulate hemolymph osmotic pressure up to approximately 50% seawater. Kozák et al. (2009) recorded an initial change in heart rate following exposure to increased chloride concentrations, followed by a gradual recovery. Similarly, Sladkova et al. (2023) reported that salinity-induced increases in heart rate were temporary and declined as the crayfish acclimated to the new conditions. These responses may help explain why growth was maintained across the salinity treatments used in the present study. In addition, SGR values obtained in this study (2.24–3.31% day^{-1}) fall within the range reported for juvenile crayfish under various dietary and culture conditions. Previous studies have documented SGR values of approximately 2.4–3.8% day^{-1} in juveniles fed combinations of live and formulated diets, including Artemia, Daphnia, and mixed natural feeds (González et al., 2008, 2011; Harlıoğlu and Harlıoğlu, 2009; Sáez-Royuela et al., 2007). Although these studies involved different species and rearing conditions, the comparable SGR values indicate that growth in the present experiment remained within the range commonly reported for juvenile crayfish.

Importantly, the feeding regime used in the present study was based on the optimized diet identified by Özdoğan and Koca (2023) for first-feeding second-stage juveniles of *P. leptodactylus*. Using the same feed and feeding procedure in all treatments reduced dietary variation and allowed the effects of salinity to be examined more directly. The absence of significant growth differences may therefore be related to both the salinity tolerance of the juveniles and the standardized feeding conditions. Furthermore, the initial stocking density may have been sufficiently low to limit competition for space, shelter, and feed, as well as aggressive interactions among individuals. Mortality, particularly in the control group, reduced the number of crayfish in some experimental units. However, this reduction in density did not result in an apparent compensatory growth response among the survivors. The similar growth values recorded among treatments therefore appear to reflect the ability of the juveniles to maintain growth across the tested salinity range. Although growth was unaffected, survival was significantly influenced by salinity, with the highest survival observed at 5 ppt. The second juvenile stage is a sensitive period in crayfish culture and considerable mortality may occur after juveniles begin feeding independently. Previous studies have reported substantial early-stage losses, particularly during the transition to independent feeding, with mortality reaching up to 90% under suboptimal rearing conditions (Taugbøl and Skurdal, 1992; Ulikowski and Krzywosz, 2006; Sáez-Royuela et al., 2007). Juveniles at this stage may therefore be less able to cope with additional environmental challenges, which may partly account for the differences in survival among the salinity treatments.

Susanto and Charmantier (2001) reported hemolymph osmolality values of approximately 290–380 mOsm kg^{-1} in *P. leptodactylus* embryos and newly hatched juveniles during early development. Their findings show that osmoregulatory capacity develops before or shortly after hatching. Under freshwater conditions, juveniles are exposed to a steep osmotic gradient, requiring continuous ion uptake and water balance regulation. In mildly brackish water, the difference between the surrounding water and the hemolymph may be smaller, which could reduce the energy required for hyper-osmoregulation. This may have contributed to the higher survival recorded at 5 ppt. Hemolymph osmolality was not measured in the present experiment, however, and this explanation remains an interpretation based on previous physiological studies rather than a direct result of the current study.

Kuznetsova (2013) also showed that relatively small changes in salinity can alter the physiological and cardiovascular activity of crayfish. Continuous osmoregulatory activity in freshwater could therefore impose an additional physiological cost on juveniles during this sensitive stage. Such a cost may reduce their capacity to respond to other sources of stress under culture conditions. In addition, Susanto and Charmantier (2001) reported that embryonic development and hatching are possible up to approximately 7 ppt, while higher salinities may impair development. The sensitivity of eggs and early developmental stages to elevated salinity has also been noted by Alvanou and Giantsis (2022). In the present study, survival was highest at 5 ppt, which was within the range reported to permit normal embryonic development and hatching. Rather than demonstrating a species-wide optimum, this result indicates that 5 ppt was the most favorable of the salinity levels tested for second-stage juveniles. At this salinity, the difference between the external medium and internal body fluids may have been reduced sufficiently to lower the cost of ion regulation.

The survival response at 2.5 ppt was intermediate between those of the freshwater control and the 5 and 10 ppt treatments. Although exposure to 2.5 ppt may have reduced the osmotic gradient relative to freshwater, the effect was not consistent enough to produce a statistically distinct survival response. This is reflected in the statistical overlap of the 2.5 ppt group with both the control and the higher-salinity groups. The 5 ppt treatment may have provided a greater reduction in osmotic demand and, consequently, more suitable conditions for survival during the second juvenile stage. Nevertheless, direct measurements of hemolymph osmolality and energy

expenditure would be required to confirm this explanation. Cherkashina (1975) reported that *P. leptodactylus* can tolerate salinity levels of up to 14 ppt in natural habitats. Hesni et al. (2009) further demonstrated that the species can survive short-term exposure to seawater conditions of up to 21 ppt, as also summarized by Alvanou and Giantsis (2022). These tolerance limits are considerably higher than the salinity at which the best survival was recorded in the present experiment. This difference suggests that the maximum salinity an animal can tolerate should not be considered equivalent to the salinity most suitable for long-term rearing or for a particular developmental stage. Similar adaptability to brackish environments has also been reported for *Procambarus clarkii*, which survived gradual exposure to salinity levels of up to 35 ppt under laboratory conditions (Bissattini et al., 2015).

Sladkova et al. (2023) found that the normal daily rhythm of cardiac activity persisted during prolonged salinity exposure, indicating that some basic physiological functions remain stable after acclimation. Together with the salinity tolerance reported by Holdich et al. (1997), this finding supports the ability of *P. leptodactylus* to survive in mildly brackish water. The present results extend this information to second-stage juveniles, which maintained similar growth between 0 and 10 ppt. Survival, however, was higher at 5 ppt than in freshwater. Under the conditions of this experiment, mildly brackish water therefore appeared to be more suitable than freshwater for the survival of this early juvenile stage.

CONCLUSION

In conclusion, the present study demonstrates that juvenile *P. leptodactylus* can tolerate salinity levels up to 10 ppt without any measurable reduction in growth performance, indicating that growth can be maintained across the tested salinity range. In contrast, survival was significantly enhanced under moderately brackish conditions, with the highest survival rate recorded at 5 ppt. This pattern suggests that intermediate salinity levels may provide more suitable conditions for early juvenile survival, possibly because of reduced requirements for hyperosmoregulation.

From a practical aquaculture perspective, these findings provide clear evidence that the incorporation of low-level salinity (approximately 5 ppt) into early juvenile rearing protocols can improve survival outcomes and overall production efficiency. Accordingly, controlled brackish-water conditions can be considered a viable strategy for optimizing nursery-phase management of this species.

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AUTHORSHIP CONTRIBUTIONS

Hasan Batuhan Emre Özdoğan: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing - Original Draft, Writing - Review & Editing.

CONFLICT OF INTEREST

The author declares that there are no financial or non-financial conflicts of interest.

ETHICAL APPROVAL

Local Ethics Committee Approval was not obtained because experimental invertebrate animals were used in this study.

DECLARATION OF AI USE

OpenAI ChatGPT (GPT-5.6 Thinking, accessed in July 2026) was used solely to assist with English-language editing of selected passages in the manuscript, particularly in the Discussion sections. The tool was used to improve grammar, sentence structure, clarity, and readability. All suggestions were critically reviewed, verified, and manually revised by the author to preserve the original scientific meaning. The tool was not used in the study design, experimental procedures, data collection, statistical analyses, or generation of research findings. The author takes full responsibility for the content of the final manuscript. The author reviewed the final manuscript and takes full responsibility for its content.

DATA AVAILABILITY

The data that support the findings of this study is available from the corresponding author upon reasonable request.

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