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Impact of Stocking Density on the Survival, Growth and Injury of Narrow-Clawed Crayfish (*Pontastacus leptodactylus*) Reared in a Flowing Brackish Water System

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ABSTRACT

The crayfish population dramatically declined in most lakes and dams in Türkiye; hence, crayfish culture is a possible option to increase the supply. This study aimed to determine the effects of stocking density on the growth performance and survival of newly-hatched third instars of narrow-clawed crayfish (*Pontastacus leptodactylus*) in a flowing brackish water system. The third instars were randomly stocked in nine tanks (area: 1.7 m^2) for 120 days with three densities of 10, 50, and 100 crayfish/m^2. The results indicated that the stocking density significantly affected the growth performance, survival rate, proportion of the cheliped injury, biomass, length distribution, and feed conversion ratio (FCR). The mean final weights and total lengths were 2.27, 1.40, and 1.08 g and 4.83, 3.73 and 3.51 cm according to the densities, respectively. The highest stocking density increased the total biomass yield and proportion of the cheliped injury but reduced the survival, growth, molting frequency and regeneration of the appendage. The survival (86.3%) and specific growth rate (1.16 cm/day) in the 10 individuals/m^2 group were significantly higher than the other groups. The cheliped injury was also found to be the lowest in the 10 individual/m^2 group ($p<0.05$). Our study results showed that significant differences in FCR value were observed at different stocking densities. In summary, higher stocking density at the end of this study increased the frequency and severity of aggressive interactions of crayfish, limiting molting frequency and growth performance. A density of 10-50 crayfish/m^2 is recommended for better growth parameters while 100 crayfish/m^2 is suggested for higher biomass yield.

INTRODUCTION

Pontastacus leptodactylus (named as Turkish crayfish or Turkish narrow-clawed crayfish) is the most commercial Astacid crayfish species in aquaculture with good consumer preference and is widely distributed in Türkiye and Europe (FAO, 2017). It is a highly-priced crayfish and generally harvested from lakes and dams (Gherardi & Souty-Grosset, 2010; Kokko et al., 2018). In the crayfish market, even 25–30 g individuals have good market value (Boštjančić et al., 2021). It is grown in various parts of the world for human consumption and restocking in natural waters (Franke et al., 2013). Crayfish consumption in Türkiye has traditionally very low; thus, this country was a major supplier of *P. leptodactylus* to western Europe from 1970 to 1984 (Baran et al., 1987; Oray, 1990; Holdich, 1993; Harlioglu & Güner, 2006). However, crayfish population was reported to have dropped significantly from 5000 tons to 200 tons per year after in most lakes and dam reservoirs after 1986 (Bolat, 2001). Probable reasons are thought to be the outbreaks of crayfish plague *Aphanomyces astaci* (Svoboda et al., 2012, 2014), climate change and degraded water quality (Kouba et al., 2014). Among the reasons for the decline in the natural stock population of crayfish, factors such as diseases and parasites, predators, cannibalism, unsuitable environmental conditions such as pollution or drought, and directly or indirectly human factors such as overfishing have been the subject of many studies in recent years (Demirol et al., 2017; Kokko et al., 2018). Additional stocking in most lakes and dams in Türkiye increased its production but not to a desired level (Kale & Berber, 2020). It is still under pressure of the crayfish plague in most dams and lakes (Kokko et al., 2018). In 2021, the annual crayfish production was 1011 tons in Türkiye (Turkstat, 2021).

Crayfish are suitable for culture since they are hardy, productive, and adaptable and do not require high-tech cultivation practices (McClain & Romaire, 2004). Unlike the culture of the aquatic species that need hatcheries and formulated feed, crayfish culture is based on self-sustaining populations with a feed-based food system (Eversole & McClain, 2000). For this purpose, studies should be carried out both in controlled laboratory conditions and in natural environments to get more accurate data on survival and growth parameters. Crayfish culture system is categorized by pond type and dominant vegetation but classification according to the main production strategy is perhaps a better alternative. The culture of crayfish is mostly based on semi-extensive and extensive production systems with juveniles stocked in earthen or artificial ponds of various shapes and sizes (Eversole & McClain, 2000).

The production of cultured species is affected by various abiotic and biotic factors that significantly affect crayfish yield and production in earthen ponds (Shoko et al., 2016; Calabrese et al., 2017; Yaun et al., 2018; Mazlum et al., 2019). Many researchers have reported that the stocking density affects crustacean survival, growth, production, molting frequency and chelae injury (Sun et al., 2016; Mazlum et al., 2017; Yu et al., 2020; Zheng et al., 2020). A high stocking density increases the annual production of crayfish (Mazlum et al., 2020), stress level (Nga et al., 2005), competition for resources (McClain, 1995), living place (Mazlum et al., 2017) and cannibalism (Mazlum & Eversole, 2004, 2005; Romano & Zeng, 2017). This case is valid for many crustaceans such as yabby *Cherax destructor* (Verhoef & Austin, 1999), red king crab *Paralithodes camtschaticus* (Daly et al., 2012), red swamp crayfish *Procambarus clarkii* (Mazlum & Eversole, 2004; Yu et al., 2020) and Chinese mitten crab *Eriocheir sinensis* (Yuan et al., 2018). Although higher rearing density is a common way to increase production and maintain economic feasibility, it has a negative impact on growth, survival rate (Rahman & Verdegem, 2010) and cheliped injury or loss (Zheng et al., 2017). For this reason, stocking density in crayfish depends entirely on whether the pond is covered with well-covered plant material or not.

The aggressive behavior of crayfish in aquaculture is affected by density (Golubev et al., 2016). The social factor has a special effect on crustaceans as their growth is associated with their vulnerability to cannibalism as a result of molting (Savolainen et al., 2004). Cannibalism is one of the crucial limiting factors for the productivity and profitability in aquaculture and can significantly change the population dynamics in the wild (Claessen et al., 2004). Both biotic and abiotic factors affect cannibalism as well as stocking densities, molting status, size heterogeneity, photoperiod, light intensity, and shelter and food availability. These factors depend on the species or species-specific stages (Mazlum & Uzun, 2008; Ariyati et al., 2018). A high stocking density may result in cannibalism of the crayfish especially during the molting process. Similar problems were reported for various crayfish species in the early stages of life resulting in a yield reduction (Duffy et al., 2011; Erol et al., 2017; Mazlum et al., 2017, 2019, 2020).

Crayfish have cannibalistic behavior which limits their productivity and profitability in aquaculture (Franke et al., 2013, 2015; Ghanawi & Saoud, 2012; Saoud et al., 2012) especially in their larval stage (Nakata & Goshima, 2004; Naranjo-Paramo et al., 2004). The juveniles of the crayfish exhibit a strong competition for resources and space (Mazlum & Eversole, 2005). The frequency of cheliped injury or loss significantly increases in higher stocking density condition (Savolainen et al., 2004). For this reason, injuries are becoming more frequent as a result of increased fighting activity of crayfish due to crowding. In natural conditions, the injury rate is associated not only with density but also with the higher proportion of large individuals (Skurdal et al., 1993). Previously-injured individuals are more likely to be re-injured (Kouba et al., 2014). In particular, cheliped injuries are known to affect the health and ability of a crayfish since chelae are important for defense, feeding and mating (Mazlum & Eversole, 2005; Kouba et al., 2016; Romano & Zeng, 2017). A higher stocking density of crayfish causes cheliped loss or injury as a result of the competition for space. Hence, the limited space reduces the survival, body size, growth and molting while it increases the intensity of agonistic interactions in crayfish (Mazlum & Eversole, 2004). Moreover, a higher stocking density tends to cause a lower growth (Jussila et al., 1999), survival (Jussila et al., 1999) and production (Adiyana et al., 2014; Supriyono et al., 2017). Therefore, it is essential to determine the crayfish management strategy to ensure sustainable crayfish production and to increase its economic benefits.

The success of larval production of crayfish depends on conditions, availability of food, shelter, rearing practices and stocking density (He et al., 2021). Among these factors, larval stocking density is known to affect larval performance. In order to solve this problem, comprehensive studies are needed to determine the optimum stocking density to minimize cheliped injury. Thus, the present study was to determine whether stocking density has any effect on the growth performance, survival, molting frequency, cheliped injury, and feed conversion ratio of the third instar of crayfish.

MATERIALS AND METHODS

Study Area

The study was carried out at the Research Center of the Faculty of Marine Sciences and Technology of İskenderun Technical University (formerly Mustafa Kemal University) between 14 June and 14 October 2006 in Dörtyol, Türkiye. The study was conducted in 4 months. The water used in the study was obtained from a well with depth of 160 m. The water temperature was almost constant at 18-19°C with a salinity of 5 parts per thousand (ppt). There are interconnected lakes of different sizes around the well that is used to obtain the groundwater. Besides, while these lakes were previously connected to the Mediterranean Sea, these connections have been closed in the present condition. Lakes still have salinity even if with low concentrations. Wide wooden pieces were placed in each tank close to the water inlet to aerate the groundwater.

Experimental Crayfish and Maintenance

A total of 42 adult female crayfish (*P. leptodactylus*) (TL: 95±0.08 mm) with eggs were obtained with fyke-net (Bolat et al., 2010) from the Egirdir Lake located in Isparta, Türkiye. They were transported to the study site in a styrofoam box without water with cooling units and insulation mesh for the protection of crayfish. Upon arrival, females with eggs were held in fiberglass tanks with 1000 L water and acclimated to hatch in the aquaculture research facility to obtain third-instars young-of-year crayfish (YOY). Hatching occurred 21 days after acclimation and the hatching rate was 97%. PVC pipes were used as refuge and commercial carp diets (35% protein and 12% lipid) were used for feeding the crayfish to develop and shed eggs. Physical and chemical water quality parameters during the acclimation were as follow: temperature of 23.4±0.75°C, pH of 7.82±0.55, dissolved oxygen of 6.83±1.14 mg/L and photoperiod of 12 h: 12 h. During the acclimation period, no sick or dead crayfish were observed.

Experimental Design

Hatched *P. leptodactylus* (Eschscholtz, 1823) larvae were grown up to the third instar stage (YOY: young-of-the-year). YOY crayfish were kept in a 20 m^3 tank and acclimated for weekdays until the start of the experiment. A commercial trout feed pellet (55% crude protein, 10% lipid) was used in feeding. After the acclimation, YOY was transferred to the experimental units with the planned densities. The start size of the total length of the third instar YOY crayfish (n=50) was measured to the nearest centimeter (cm). The average body weight and total length of YOY was 22.4±0.5 mg and 1.2±0.04 cm, respectively (Figure 1).

Figure 1. Third-instar stage (young-of-the-year, YOY) freshwater crayfish used in the study

Three stocking densities were utilized in the study: (10: group 1, 50: group 2, and 100: group 3) crayfish/m^2 corresponding to 17, 85, and 170 individuals per tank, respectively. The crayfish were randomly placed into 9 tanks (1.7 m^2). The bottom of each tank was equipped with plastic pipes (8×10×10 cm) (n=30) and 6 pieces of 40 cm^2 nettings to provide shelter to reduce cannibalism and antagonist behavior before the YOY crayfish were stocked. Each tank had its own water inlet and outlet. The water depth was about 40 cm while the water flow rate was 7 L/per minute in the tanks having continuous brackish water flow.

The experiment was conducted in a 3×3 completely randomized design with three replications at three stocking densities. Crayfish were fed twice a day (08:00 morning and 5:00 pm evening) with the same feed as used during the YOY acclimation (commercial trout pellet feed, 55% crude protein, 10% lipid). Feeding schedule was planned for each treatment based on the growth and mortality rates as determined with the sampling period. The crayfish were fed using a feeding rate as a proportion of their biomass (5% of their mass per day) (Mazlum et al., 2011; Sirin & Mazlum, 2017). The data were recorded by collecting feces, feed residues, crust and dead on the bottom of the tank once per week. The experiment lasted 120 days.

Water Quality Parameter Measurement

Water temperature (°C) and dissolved oxygen (mg/L) were quantified by using an oxygen meter (YSI 55, Yellow Springs Instruments Co., Ohio, USA) while the water salinity was measured using a salinometer (YSI 85) and pH were determined using a pH meter (YSI Model 50) in each morning. Other water quality parameters such as ammonia, nitrite, nitrate, calcium and magnesium were analyzed monthly.

Sampling Procedure

The first measurement was taken two months after the crayfish were stocked and the other data were obtained monthly not to expose the crayfish to stress and damage. Growth parameter, survival and feed conversion rate (FCR) were periodically examined on the 60th, 90th, and 120th days of the experiment for each treatment group. The number of surviving crayfish was counted in each tank and the total lengths (TL) were measured from the tip of the rostrum to the telson to the nearest 0.1 mm using a measure board. In order to measure the weight of the crayfish (WW), they were put on filter paper to remove excess water and then weighed to the nearest 0.01 g by using an electronic balance. The number of molt crayfish was also determined daily. The sex of crayfish was determined, the number of injured chelipeds (called as missing chelipeds and regenerated chelipeds) for males and females were recorded at the end of the experiment. Dead crayfish were removed and counted on daily basis.

Regression analyses were performed for the relationship between total length (TL in cm) and wet weight (WW in g), molting frequency (MF) and stocking density (m^2), and total biomass yield and stocking density (m^2) of the crayfish harvested at the end of the experiment is expressed by the following equation (1):

$$WW = aTL^b \quad (1)$$

and log base 10 equation (2):

$$\log WW = \log a + b \log TL \quad (2)$$

where *WW* is the wet weight, *TL* is the total length, and *a* and *b* are constants.

Data Calculation

All crayfish were measured and weighed at the beginning of the feeding trial, on day 60, day 90 and day 120. At the end of the study, survived crayfish, the total number of molt crayfish and the number of cheliped injury crayfish were determined. The measurements were replicated three times in each stocking density group. Crayfish growth performance was estimated through the assessment of weight gain (WG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), cheliped injury rate (CIR) and mean molting frequency (MMF) by using the formulae given below (equations 3-8):

$$WG = FBW - IBW \quad (3)$$

$$SGR\ (\%/day) = \left[\frac{\ln FTL - \ln ITL}{Rearing\ period(T,\ day)}\right] \times 100 \quad (4)$$

$$SR(\%) = \frac{Final\ number\ of\ crayfish}{Initial\ number\ of\ crayfish} \times 100 \quad (5)$$

$$FCR = \frac{FI}{WG} \quad (6)$$

$$CIR(\%) = \frac{N_{CLIR}}{N_{SC}} \times 100 \quad (7)$$

$$MMF(\%) = \frac{Number\ of\ molt}{Total\ number\ of\ crayfish\ survived} \times 100 \quad (8)$$

where *FBW* is the final body weight (g), *IBW* is the initial body weight (g), *FTL* is the final total length (cm), *ITL* is the initial total length (cm), *FI* is the total amount of feed (g) given to crayfish during the rearing period, N_{CLIR} is the number of crayfish with cheliped loss, injury or regenerate appendage.

The coefficient of variations (CV, %) for the final weight and total length were also calculated for each stocking density using the formula given below (equation 9):

$$CV(\%) = \frac{Standard\ deviation}{Mean} \times 100 \quad (9)$$

Statistical Data Analysis

Data on crayfish growth performance such as final total length (FTL), final wet mass (FWM), weight gain (WG), survival rate (SR), specific growth rate (SGR), feed conversion rates (FCR), molting frequency (MF), cheliped injury (CI), cheliped lost (CL), harvest weight (HW), coefficient of variation (CV) for final wet mass (FWM) and final total length (FTL) were subjected to one-way ANOVA (analysis of variance). Normality and homogeneity were tested by using Kolmogorov Smirnov and Levene tests, respectively. All statistical analyses were carried out by using SPSS (2008) 17.0 software according to Duncan's New Multiple Range Test to identify the 5% level of significance of variance among the different stocking density means. Regression analysis was used to determine the length–weight, molting frequency-stocking density, and total biomass yield-stocking density relationships. All experimental data were expressed as mean ± standard deviation (SD).

RESULTS

Water Quality

The water quality parameters were determined and summarized in Table 1. The water temperature inside the tanks ranged between 21.0 and 24.5°C (mean: 22.7°C) while the dissolved oxygen varied from 5.6 to 6.8 mg/L (mean: 6.2) and the pH value was between 7.0 and 7.8 (mean: 7.4). The nitrite and nitrate concentrations in the flowing water aquaculture system were 0.005 mg/L and 0.17 mg/L, respectively. Groundwater which was used during the rearing period had a salinity of 5 parts per thousand (ppt) in all tanks. No significant differences were found in the measured water quality parameters, which were kept within acceptable limits for the crayfish cultured during the study (Table 1).

Table 1. Water quality parameters of the crayfish in the brackish water flow system during the 120-day trial period

Parameters	Mean ± SD
Water temperature (°C)	22.7±0.06
Dissolved oxygen (mg/L)	6.2±0.09
pH	7.4±0.05
Hardness (mg/L)	47.0±1.6
Calcium (mg/L)	66.5±2.1
Magnesium (mg/L)	63.1±1.2
Salinity (ppt)	5.0±0.44
Ammonia (NH_4^{+})	0.05±0.012
Nitrite NO_2 (mg/L)	0.005±0.02
Nitrate NO_3 (mg/L)	0.170±0.003

Note: Data are presented as mean ± standard deviation.

Growth Parameters

It was observed that the stocking density significantly impacted the growth performance of the crayfish (Tables 2 and 3). Total length, body mass, specific growth rate, molting frequency, survival rate and FCR were all negatively influenced by high stocking density; however, the high density increased total biomass and proportion of the cheliped injury for the larval stage (Table 2).

No significant differences in the initial body mass ($p<0.05$) and lengths ($p<0.05$) among crayfish stocked in the three stocking density groups (Table 2). The final mean weights averages were 2.27, 1.40 and 1.08 g while the average final lengths were 4.83, 3.73 and 3.51 cm (Table 2).

A statistical difference was not observed in the groups with a stock density of 50 and 100 crayfish/m². At the end of the trial, the best growth (by weight and length) was observed in the group with the lowest stock density of 10 crayfish/m². Besides, the calculated mass of the male and female crayfish differed significantly at the end of the 120 days (Table 2; Figure 2).

The relationship between total length (TL) and total weight (TW) of the crayfish harvested at the end of the experiment is expressed by the following equation (1) (Figure 3):

$$Total\ weight = (0.8658 \times TL) - 1.9002; (R^2 = 0.9887) \quad (10)$$

Table 2. Various growth and survival parameters of third-instars crayfish reared at three stocking densities in the 120-day experiment

Parameters	Stocking Density (m²)		
	10	***50***	***100***
Initial length (cm)	1.20±0.04	1.20±0.04	1.20±0.04
Initial weight (mg)	22.4±0.50	22.4±0.50	22.4±0.50
Final length (cm)	4.83±0.80[a]	3.73±0.75[b]	3.51±0.0.75[b]
Final weight (g)	2.27±1.41[a]	1.40±0.84[b]	1.08±0.60[b]
Weight gain (WG, g)	2.25±0.91[a]	1.38±0.34[b]	1.06±0.10[b]
Length gain (cm)	3.63±0.76[a]	2.53±0.71[b]	2.31±0.69[b]
Survival rate (SR, %)	86.3±11.31[a]	72.2±11.33[a]	56.3±13.13[b]
Specific growth rate (SGR, %/day)	1.16±0.98[a]	0.95±0.80[b]	0.89±0.76[b]
Feed conversion rate (FCR)	4.52±0.28[a]	3.77±0.20[b]	1.91±0.30[c]
Cheliped injury rate (CIR, %)	6.3±11.26[a]	16.3±6.35[b]	22.1±8.60[c]
Molting frequency (MF, %)	82.2±1.08[a]	68.0±1.03[b]	47.0±0.98[c]
Total biomass yield/harvest (g)	177.2±23.39[a]	378.9±17.91[b]	431.9±8.54[c]

Note: Data are presented as mean ± standard deviation. Means in any row with the different letters are significantly different among the stocking density ($p<0.05$).

Table 3. The total length (TL, cm), cheliped injury (%), and number (N) of male and female crayfish belonging to different stocks at the end of the experiment

Stocking Density	N	Male	N	Female
Total length (TL, cm)				
10	22	4.99±0.64[a]	22	4.45±0.35[a]
50	94	3.77±0.27[a]	90	3.63±0.22[a]
100	138	3.57±0.18[a]	149	3.21±0.12[a]
Cheliped injury (CI, %)				
10	3	13.6±10.46[a]	3	13.6±10.46[a]
50	13	13.8±5.52[a]	29	32.2±12.11[b]
100	30	21.7±14.88[a]	57	38.6±8.87[b]

Note: Data are presented as mean ± standard deviation. Means in rows (male and female) within a stocking density with the same letters are not significantly different (p<0.05).

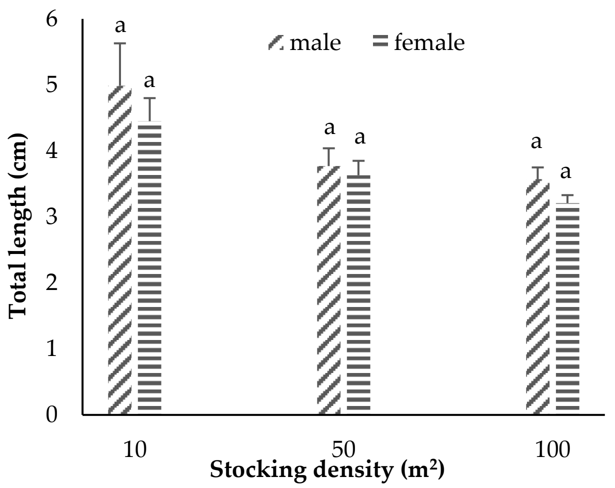

Figure 2. Total lengths according to the stocking density for male and female crayfish

Survival Rate (SR)

Survival rate (SR) of the third instar crayfish after 120 d varied from 56.3% to 86.3% for the stocking densities of 100/m² and 10/m², respectively (Table 2). The survival of crayfish decreased with an increase in the stocking density. The highest SR was found as 86.3% in the group with a stock density of 10 crayfish/m² while the lowest SR was recorded for the density of 100 crayfish/m². There was no significant difference in the SRs between the density groups of 10 and 50 crayfish/m². Besides, the molting frequency (Table 2) and cheliped injury (Figure 4) were significantly different among the stocking density groups (p<0.05) with crayfish in the high-density group. Likewise, the highest SR (86.3%) was observed in the group with 10 crayfish/m² followed by the groups with a density of 50 crayfish/m² and 100 crayfish/m² (72.2% and 56.3%) (Table 2).

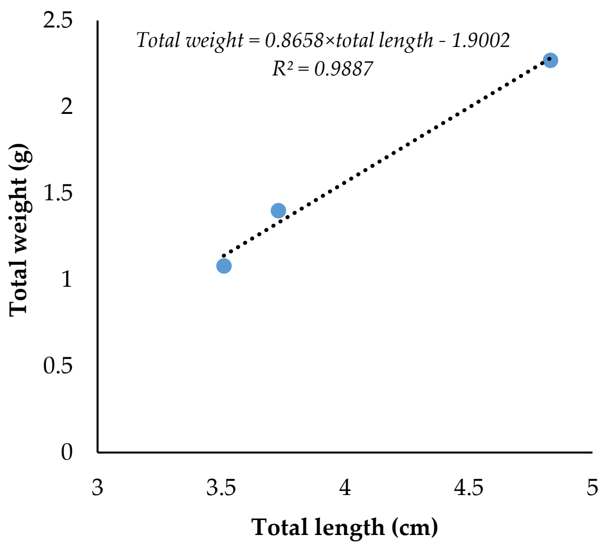

Figure 3. The relationship between the total length (TL) and body weight (TW) of crayfish harvested at the end of the experiment

Specific Growth Rate (SGR)

Stocking density also affected the specific growth rate (SGR), final weight, final length and weight gain (WG) significantly as inversely related to stocking density (Table 2). These values significantly decreased as the stocking density increased. The highest SGR was calculated in the group of 10 crayfish/m² with a value of 1.16 cm and the lowest SGR was in the group of 100 crayfish/m² with 0.89 cm. At the end of the study, the SGR at 10 crayfish/m² was statistically higher than those of the other two groups ($p<0.05$). WG for the density groups of 50 and 100 crayfish/m² was significantly lower than that of the 10 crayfish/m² group. According to the sampling periods, the highest SGR was obtained from the group of 10 crayfish/m² with a value of 1.5 cm on the 60th day.

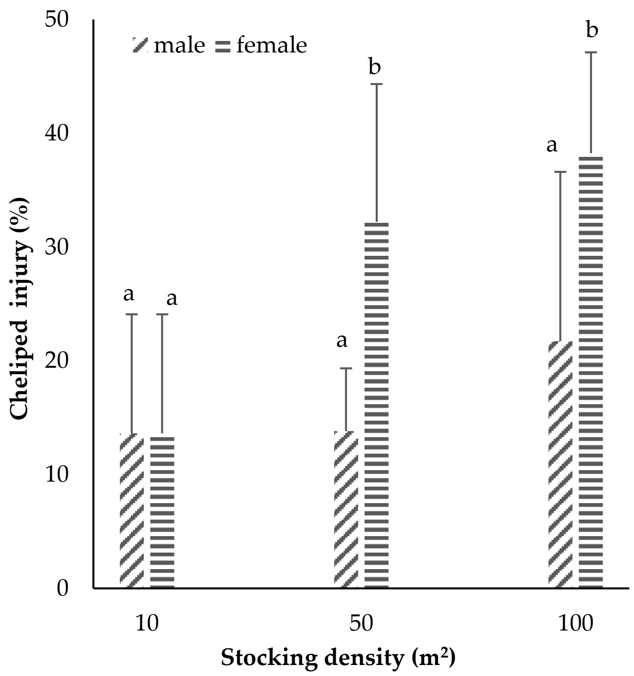

Figure 4. Cheliped injury rate (%) of male and female crayfish third instar for different stocking densities (bars represent the standard deviations of three replicated tanks)

Cheliped Injury Rate (CIR)

Cheliped injury rates (CIR) ranged from 6.3% to 22.1% increasing with stocking density (Table 2). There were significant differences in the CIR values among the three density groups. The CIR was significantly higher at the density of 100 crayfish/m² than those of the other two groups. Besides, the percentage of chelae injury in female crayfish (38.6%) was higher than that of males (21.7%) in the group of the highest stocking density (Table 3). There was no statistical difference in the percent chelae injury rates between the lowest (10 crayfish/m²) and medium (50 crayfish/m²) stocking density for the male crayfish; however, this difference was significant for the female crayfish.

Molting Frequency (MF)

The molting frequency (MF) ranged from 47.0% to 82.2% and was significantly affected by stocking density. The stocking density of 10 crayfish/m² had a significantly higher molting frequency (82.2%) than those of the other groups (Table 3). The molting frequency of the crayfish during the study period was displayed in Figure 5.

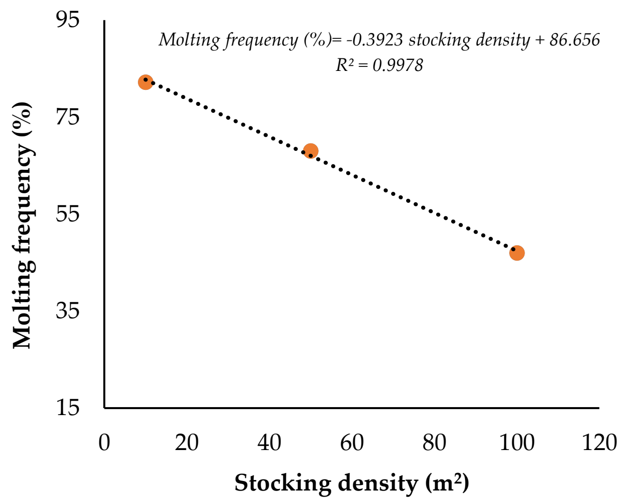

Figure 5. Variation of molting frequency (%) of crayfish according to the three stocking densities over 120-day study period

Feed Conversion Rate (FCR)

Feed conversion rate (FCR) values of the crayfish decreased with the increasing stocking density (Table 2). At the end of the experiment, the best mean FCR value (1.91) was observed in the density group of 10 crayfish/m² and it statistically differed from the other density groups.

Biomass Yield (BY)

It was observed that the total biomass was significantly affected by stocking density (Table 2). The highest crayfish biomass was obtained at 100 crayfish/m² density group (431.9 g), followed by 50 crayfish/m² group (378.9 g) and 10 crayfish/m² group (177.2 g). The differences among the stocking groups were statistically significant ($p<0.05$). Besides, a strong positive relationship was found between the logarithms of the stocking density and the logarithms of the total biomass at the end of the experiment expressed by the following equation (11) (Figure 6):

$$BY\ (g) = (113.3 \times \ln(stocking\ density)) - 79.1; (R^2 = 0.9902) \quad (11)$$

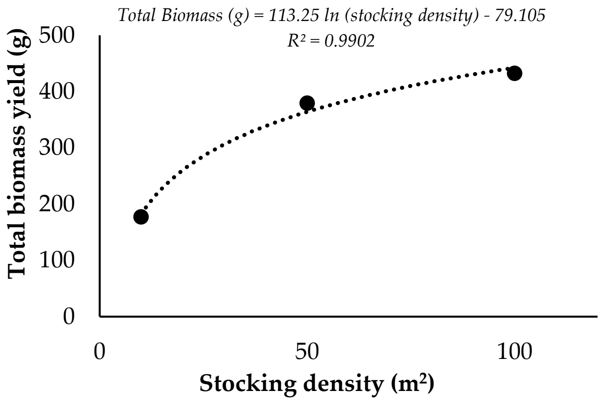

Figure 6. Variation of the total biomass of crayfish according to the three stocking densities over 120-day study period

Body Size (Length Distribution)

Although the male crayfish were larger than the females at the end of the experiment, the difference was not statistically significant (p>0.05) (Table 2). The length distribution varied between 2.0 and 6.2 cm. The distribution in the group with low stock density was more homogeneous and wider than in the other two groups (Table 3). However, there was no significant differences between the total lengths of the male and female crayfish at the end of the experiment (Table 3; p>0.05). It was observed that the crayfish length distribution was not influenced by the stocking density.

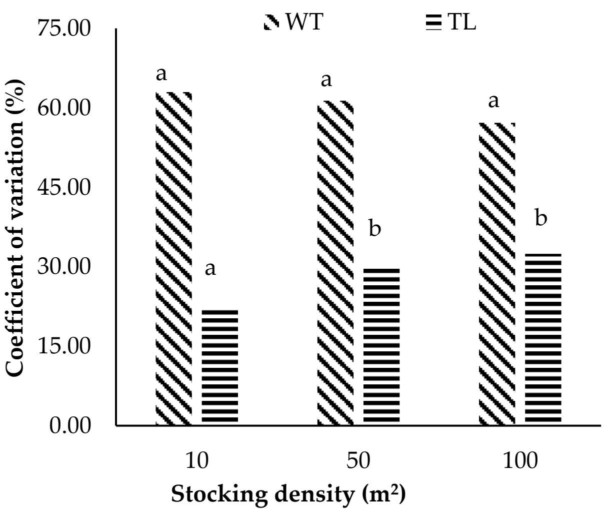

Figure 7. Coefficients of variation (CV) of the total length and body weight of crayfish at three stocking densities over the 120-day study period

The coefficients of variation (CV) values for the body weight did not differ significantly among stocking densities (p<0.05) but not among the total length (Figure 7). The mean total length (size) CV values ranged between 22% (for 10 crayfish/m²) and 33% (for 100 crayfish/m²) while the mean weight CV values ranged from 57% (for 100 crayfish/m²) and 63% (for 10 crayfish/m²) (Figure 7).

DISCUSSION

Successful crayfish culture requires a good survival rate as well as good growth rate (Hammond et al., 2006; Ulikowski et al., 2006; Aksu, et al., 2016). This study was carried out to study the effect of stocking density on the performance of narrow-clawed crayfish production. Stocking density significantly influenced growth performance (both total length and body mass), molting frequency, frequency of cheliped injury, survival and yield of crayfish for the larval stage. Higher stocking density can increase competition among crayfish for space and access to feed and thus reduce the mean size and growth of crayfish (Mazlum & Eversole, 2005; Romano & Zeng, 2017; Mazlum et al., 2020; Yu et al., 2020) as stated in numerous studies on this species (Garcia-Ulloa et al., 2012; Erol et al., 2017; Ponce-Palafox et al., 2018) and on other crayfish species (Geddes & Smallridge, 1993; McClain, 1995; Whisson, 1995; Jones & Ruscoe, 2000). Although the initial lengths and experimental periods of the crayfish are the same, the most important results that make this study different from the other stock studies we have done are; In this study, 5 per thousand salinity was used and the growth results at the end of the study were very different. Mazlum et al. (2020) found the growth at the lowest stock density as 3.2 cm TL in different stock and feed studies, while in this study, they found the growth as 4.83 cm TL at low stock density and 5 per thousand salinity. Ponce-Palafox et al. (2019) stated that *M. americanum* can grow well at low density and they found an inverse relationship between its size and density. The larger animals have advanced growth when reared together with the smaller juveniles at high density (Mazlum & Eversole, 2004, 2005; Mazlum et al., 2007). Hierarchy and cannibalism over smaller animals by the larger ones affect this case (Mazlum & Eversole, 2004;

Mazlum et al., 2007). On the contrary, in the present study, crayfish production (biomass weight) was the highest at the highest stocking density. Depending on the stock density, the final crayfish body mass ranged between 2.27 and 1.08 g while the average length varied from 4.83 to 3.51 cm (Table 2). In a previous study, Qin et al. (2001) reported that marron survival was not improved by size grading. Similar results have also been reported for freshwater prawns (Tidwell et al., 2003, 2004; Peña-Herrejón et al., 2019). Jones & Ruscoe (2000) observed that the stocking size of red claw crayfish had no significant effect on survival. In the present study, the final crayfish biomass weight was significantly higher in the low density group (10 crayfish/m²) as compared to the other two groups ($p<0.05$) (Table 2). An inverse relationship between stocking density and final size has also been observed in studies with cultured *Macrobrachium rosenbergii* (D'Abramo et al., 1995; Tidwell et al., 1998) and *Procambarus clarkii* (Lutz & William, 1989). Moreover, Mazlum (2007) indicated that the length distribution varied among animals in three different density treatment groups.

Survival Rate (SR)

In the present study, the crayfish survival rate (SR) was found to be inversely proportional to the initial stocking density. The SR was found highest (86.3%) at the lowest density (10 crayfish/m²) and lowest (56.3%) at the highest density (100 crayfish/m²) (Table 3). A similar result was reported for *Macrobrachium malcolmsonii* (Mishra, 2017) and some other species (Turan et al., 2012; Xiaolong et al., 2018; Mazlum et al., 2020). Some researchers found mean survivals of less than 50% (D'Abramo et al., 1985; Celada et al., 1993; Sáez-Royuela et al., 1995; Erol et al., 2017). On the other hand, Nystrom (1994) reported high survival rates (75%) for *P. leniusculus* grown at high stocking density similar to the survival results of the current study. Garza de Yta et al. (2012) reported no significant differences in survival (73.3%) of crayfish juveniles fed with mixed meal diets. This result was also confirmed with the findings of Cortes-Jacinto et al. (2004) for *Cherax quadricarinatus* with the SR ranged from 83.0 to 90.0%. Verhoef & Austin (1999) estimated the average survival rate of *Cherax destructor* juveniles at 78%, but found no trend in density even when densities were as high as 150, 300 and 600 individuals/m². Meanwhile, Brown et al. (1995) noted that the survival was significantly lower for *O. virilis* at the two highest densities (27 and 54 individuals/m²). The generally accepted explanation is that high stocking density can increase space and competition for access to forage resources, thereby reducing the average size and growth of crayfish (Mazlum & Eversole, 2005). The stocking density influences the growth, molting and survival of crayfish with an inverse relationship (Ardahan & Jensen, 2016; Mazlum et al., 2017; Sirin & Mazlum, 2017). The relatively high mortality in high stocking density groups is likely to be due to cannibalism as observed in the present study (Table 2). Previous studies have also confirmed that cannibalism during molting could be the main cause of juvenile mortality in prawns (Wu et al., 2001; Romano & Zeng, 2017). The survival rate is also related to nutrition (Cortes-Jacinto et al., 2004). In intensive culture systems, including hatcheries and nurseries, the animals are completely dependent on additional food. Three factors related to feeding should be considered: quality of feed, amount of feed and its availability to the crayfish. D'Abramo et al. (1985) achieved a relatively good 41% survival for newly hatched *P. leniusculus* for 100 days at a density of 200 individuals/m² when appropriate feed was given. In the current study, the growth and survival of crayfish at consistent densities were better than the findings by D'Abramo et al. (1985), Savolainen et al. (2003), and Erol et al. (2017). The better survival combined with the better growth rate results in a higher yield. It is assumed in the current study that most of the deaths were caused by cannibalism or that individuals with cheliped loss were rapidly eaten by the same species. Besides, missing chelae and molting of crayfish were significantly different among the stocking density groups ($p<0.05$) with crayfish in the high-density group having a lower SR than those in the low-density group. Likewise, according to the measurement periods, the highest SR was observed as 92.2% in the group of 10 crayfish/m² at the 60th day followed by 85.0% (50 crayfish/m²) and 73.6% (100 crayfish/m²).

Specific Growth Rate (SGR)

It was found in the present study that the stocking density had a significant effect on the specific growth rate (SGR), final weight, final length and weight gain (WG) (Table 2). These values decreased significantly as the stocking density increased. The highest SGR was calculated as 1.16 cm (10 crayfish/m²) and the lowest was found as 0.89 cm (10 crayfish/m²). The SGR was correlated with stocking density in which higher stocking density resulted in a significantly poor growth. Similar findings were reported by Erol et al. (2017), Gao et al. (2017), and Mazlum et al. (2020). A negative relationship between stocking density and body weight was also reported for *Litopenaeus vannamei* by Krishna et al. (2015). Significant differences in SGR of *F. merguiensis* were also reported for different stocking densities (Araneda et al., 2008). Similarly, Williams et al. (1996), Araneda et al. (2008), and Sookying et al. (2011) reported that higher stocking density had negative effects on the growth of shrimp (Mazlum et al., 2017). According to the sampling periods, the highest SGR was obtained as 1.5 cm on the 60th day from the group of 10 crayfish/m² followed by the groups of 50 and 100 crayfish/m² in the current study.

Molting Frequency (MF)

In the current study, crayfish stocking density significantly and negatively affected the molting frequency (MF). The group with the lowest density (10 crayfish/m²) had a significantly higher MF (82.2%) than the other groups (68.0% and 47.0%) (Table 2, Figure 7). It is known that faster growing and frequently molting crayfish are more vulnerable to cannibalism and predation and exposing them to attacks by conspecifics that are often predators (Lutz, 1983; Mazlum & Eversole, 2005; González et al., 2010; Sirin & Mazlum, 2017). Fatihah et al. (2020) indicated that the use of coral as a substrate showed improvement in the total number of molts in *C. quadricarinatus*. In the present study, a similar trend was observed in a way that the group in the lowest density (10 crayfish/m²) had higher weight gain (2.25 g) and higher MF (82.2%) (Table 2). At the early stage of life, crayfish have a higher risk of mortality than in the later stages and this may explain the higher SR of advantaged juveniles compared to that of early stages. Increase in the amount of shelters is advised to obtain higher SR for crayfish.

Cheliped Injury Rates (CIR)

Crayfish density influenced the mean number of injuries per individual, the proportion of injured individuals and the mean number of chelae injuries per individual in the present study ($p<0.05$) (Tables 2 and 3, Figure 4**)**. Cheliped injury rate (CIR) increased from 6.3% to 22.1% with the increase in stock density (Table 3). The CIR value in female crayfish (38.6%) was higher than in males (21.7%) especially at high stocking density. The differences in the CIR values of the male and female crayfish were not significant at the lowest density (10 crayfish/m²) while they were significant at medium and high densities (50 and 100 crayfish/m²) (Figure 4).

The frequency and severity of injuries in crayfish culture were positively correlated with the density. Our results indicated that the number and severity of injuries increased when the stocking density increased. These findings are consistent with the findings of previous research on crayfish density and aggression. High stocking densities cause increased competition, frequency and severity of aggressive interactions among crayfish (Savolainen et al., 2004; Mazlum & Eversole, 2004, 2005; Kouba et al., 2011). Chela autotomy is considered as an indicator of agonistic encounters between animals (Fiegel & Miller, 1995; Mazlum et al., 2007) and make the crayfish more vulnerable to conspecifics. In the present study, a positive correlation (from 0.88 to 0.81) between the numbers of animals with lost cheliped and the stocking density for female and male. This result is in agreement with the finding of Fiegel & Miller (1995) who found a lower SR in *Procambarus clarkii* crayfish lacking one or two chelae caused by increased contact with conspecifics coupled with trauma associated with injury. The cheliped state (i.e., size, damage, deficiency) has been widely monitored for its effects on fighting ability and survival as most of the injured individuals were eaten by survivors (Skurdal et al., 1988; Figiel & Miller, 1995; Savolainen et al., 2004). Moreover, stress caused by crowding and cannibalism can also induce mortality (Yuan et al., 2018). Since aggressive behavior typically results in cheliped injury and loss, it makes sense that higher density causes more fighting and injuries than low density (Figiel & Miller, 1995; Savolainen et al., 2004). The effect of stocking density on CIR was found to be significant in the present study and this was consistent with previous studies. The CIR in female crayfish (38.6%) was found higher than in males (21.7%) especially at highest density. Mazlum et al. (2017) reported that the missing chelae in *P. leptodactylus* increased mortality due to cannibalism or damage inflicted by conspecifics with unbroken chelae. Our results showed that most of the individuals with injured or lost cheliped were eaten by survivors and the same was observed with the molting process.

Feed Conversion Rate (FCR)

Feed conversion ratio (FCR) has always been a prime concern for crayfish culture. It was observed in the current study that crayfish FCR values varied between 1.91 and 4.52 with significant differences according to the stocking density groups (Table 2). The best mean FCR value (1.91) was observed at the lowest density stock group (10 crayfish/m^2). Similarly, Jones & Ruscoe (2000) also highlighted that the FCR was affected by stocking levels. Increased FCR at higher densities suggests that the supplemental feed had a poor direct nutritional value for crayfish. Similar results were also reported in European freshwater crayfish (*Austropotamobius pallipes*), red swamp crayfish (*Procambarus clarkii*) (Wheatly & Ayers, 1995) and American lobster (*H. americanus*) (Zhuang & Ahearn, 1996; Cortes-Jacinto et al., 2003). Similar to the results of the presents study, FCR values above 1.0 were reported in crayfish (Wheatly & Ayers, 1995; Turan et al., 2012). No evidence was found to support the hypothesis that supplemental feed was of poor value during the nursery stage as differences in culture conditions and study durations of both studies need to be considered.

Biomass Yield

A number of factors affect commercial crayfish biomass yield including water quality, food abundance, management practices, and harvest protocol (Eversole et al., 2006). Total biomass yield was also significantly affected by the stocking density in the current study (Table 2). The highest crayfish biomass (431.9 g) was obtained with the highest density group (100 crayfish/m^2) while the lowest biomass (177.2 g) ($p<0.05$) (Figure 6). Eversole et al. (1999) reported that the total biomass is directly related to stock density while Pinto & Rouse (1996), and Jones & Ruscoe (2000) stated that the weight and yield of red-clawed crayfish is inversely proportional to the stock density. In studies with cultured *Macrobrachium rosenbergii* (D'Abramo et al., 1989; Tidwell et al., 1999; Yu et al., 2020) and *Procambarus clarkii* (Lutz & Wolters, 1986; Villagran, 1993), an inverse relationship was also found between the stock density and final size. Similar results have also been reported showing an inverse relationship between stock density and final size (Chattopadhyay et al., 2013; Paul et al., 2016; Vivek et al., 2017). Ackefors et al. (1989) showed that biomass gain was similar with *A. astacus* juveniles at the lowest (250 individuals/m^2) and highest (1000 individuals/m^2) densities. The biomass yield obtained in the present study was much lower than levels obtained at similar densities with *A. astacus* reported by Keller (1988).

Body Size (Length Distribution)

In the present study, it was expected that the stocking density could affect the size distribution of crayfish and the highest density (100 crayfish/m^2) would reduce the size variation by disrupting the agonistic behavior. Although the males were larger than the females, the difference was not statistically significant ($p>0.05$) (Tables 2 and 3). At the end of the experiment, the length distribution varied from 2.0 to 6.2 cm. The distribution in the group with low stock density was more homogeneous with wider distribution than the other two groups (Table 3). However, it was determined that there were no significant differences between the total lengths of the male and female crayfish ($p>0.05$). Thus, it was concluded that the length distribution of crayfish was not significantly influenced by the stocking density. Similarly, Berber et al. (2012) also highlighted that the length distribution of crayfish in various population lakes was found similar for male and female.

CONCLUSION

This study was carried out to determine the effects of three stocking densities (10, 50 and 100 crayfish/m^2) on the growth performance, survival, molting frequency, cheliped injury and feed conversion ratio of the third instars of narrow-clawed crayfish

(*P. leptodactylus*) reared in a flowing brackish water system.

At the end of the study, stocking density affected the length distribution, the proportion of the cheliped injury, biomass, survival rate, molting frequency, growth performance, and feed conversion ratio (FCR) of crayfish. The highest stocking density resulted in an increased total biomass and proportion of the cheliped injury but reduced the survival, growth performance, molting frequency and retention of the appendage. The crayfish grew and survived satisfactorily in 0–5 ppt water salinity condition, which implied that this crayfish species can be cultured commercially in this salinity condition. Based on the results of the study, stocking density of 10 or 50 crayfish/m^2 is recommended for the culture of third instars. For intensive farming to be economically efficient, stocking density of 100 crayfish/m^2 or more during the first four months of the growing season may be recommended, but this density should be lower for survival, growth performance, and proportion of cheliped injury rate. As a result, stock density is often considered a key factor influencing production and is therefore one of the most important management strategies in crayfish culture cycle.

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Compliance with Ethical Standards

Authors' Contributions

YM: Manuscript design, Field sampling, Laboratory experiments, Data analysis, Drafting, Writing, Editing.

CU: Manuscript design, Field sampling, Laboratory experiments, Drafting, Writing.

Both authors drafted, contributed and approved the final version of the article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Pontastacus leptodactylus is not an endangered or protected species and there was no need for an ethical approval to perform the experiments involving this species in Türkiye.

Data Availability Statement

The data that support the findings of this study are available under reasonable request.

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