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Effect of Different Feed and Stocking Density on Survival and Growth Performance of *Astacus leptodactylus* (Esch., 1823) Juveniles

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ABSTRACT

The aim of this study was to investigate the combined effects of two stocking densities (650 and 1300 juveniles/m^2) and diets (live feed - *Daphnia magna* and commercial trout feed) on survival rate and growth performance of *Astacus leptodactylus* juveniles. Juveniles in the second developmental stage were used in the experiment. Final survival rate and growth paremeters of juveniles were evaluated at the end of experiment (90 days). The results of this study indicated that diets were not found effective on survival rates, though increasing stock densities reduced survival rates. The best final survival rate as 21.99 %, was achieved in the group fed with live feed and kept under the 650 juveniles/m^2 stocking density, whereas the lowest survival rate values as 10.84 % was obtained in the group fed with trout pellet and kept under the 1300 juveniles/m^2 stocking density. At the end of experiment, the highest final body weight was found in group of at 650 juveniles/m^2 stocking density and fed with trout feed. The result of our study also revealed that stocking density had a significant effect on survival rates of crayfish juvenile fed with both feeds.

Keywords: *Astacus leptodactylus*, feeding, stocking density, survival rate, growth

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Farklı Yem ve Stok Yoğunluğunun *Astacus leptodactylus* (Esch., 1823) Juvenillerinin Hayatta Kalma ve Büyüme Performansı Üzerine Etkisi

Öz: Bu çalışmada, iki farklı yem (canlı yem-*Daphnia magna* ve ticari alabalık yemi) ve stok yoğunluğunun (650 ve 1300 juvenil/m^2) *Astacus leptodactylus* yavrularının hayatta kalma ve büyüme performansı üzerine kombine etkileri araştırılmıştır. Denemede II. dönem yavrular kullanılmıştır. Deneme sonunda, yavruların hayatta kalma ve büyüme parametreleri değerlendirilmiştir. Bu çalışmanın sonuçları, diyetlerin hayatta kalma oranları üzerinde etkili olmadığını, ancak artan stok yoğunluğunun yaşama oranlarını azalttığını göstermiştir. En iyi yaşama oranı % 21,99 olarak 650 juvenil/m^2 stok yoğunluğunda canlı yemle beslenen grupta sağlanırken, en düşük yaşama oranı ise % 10,84 olarak 1300 juvenil/m^2 stok yoğunluğunda alabalık yemi ile beslenen grupta elde edilmiştir. Denememe sonunda en yüksek vücut ağırlığı, 650 juvenil/m^2 stok yoğunluğunda alabalık yemi ile beslenen grupta bulunmuştur. Çalışmamız sonucunda ayrıca, yavru kerevit beslenmesinde her iki yem ile beslenen yavrularda hayatta kalma oranları üzerinde stok yoğunluğunun önemli bir etkiye sahip olduğu görülmüştür.

Anahtar kelimeler: *Astacus leptodactylus*, besleme, stok yoğunluğu, yaşama oranı, büyüme

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Introduction

The narrow-clawed crayfish, also known as Turkish crayfish, *Astacus leptodactylus*, is the native freshwater crayfish species of Turkey (Holdich 2002). *A. leptodactylus* has a widespread distribution in lakes and ponds in many parts of the country. Its distribution area was considerably expanded in Turkey after 1985 because of its commercial importance and declined catches from traditional fisheries. Although the domestic demand for crayfish was very low in Turkey, this country has been the main supplier of *A. leptodactylus* to Western Europe from 1970 to until 1986 (Harlıoğlu 2004, 2008). Until 1984, *A. leptodactylus* had an important role as an export product. But after 1985, Crayfish production declined dramatically (from 5000 tonnes to 200 tonnes) (Bolat 2001). The main cause of the decrease in crayfish production was the outbreak and spread of crayfish plague, caused by the funguslike organism, *Aphanomyces astaci*, excessive crayfish harvesting, pollution of water in lakes and overfishing (Bolat 2001; Svoboda et al. 2012, 2014).

Crayfish culture can vary in intensity, from extensive to intensive rearing systems. However, intensive (completely closed) crayfish culture is not practically used in Europe. As a matter of fact, this culture has not proved economically profitable due to high mortality caused by cannibalism amongst cultured crayfish and to high production costs (Kozák et al. 2015). Several environmental factors, such as water temperature, water quality, nutrition, light intensity, photoperiod and density, affect growth and survival of juveniles in intensive crayfish culture (Köksal 1985, 1988; Köksal et al. 1992; Sáez-Royuela et al. 2007; Erol et al. 2010). Among these factors, nutrition is one of the most important factors directly affecting the growth of crayfish (Taugbol and Skurdal 1992; Austin et al. 1997; Savolainen et al. 2004; Mazlum 2007; Sáez-Royuela et al. 2007). Several variables (water quality, the quality and amount of food, food supply frequency, its availability to the crayfish) have played significant roles in crayfish culture since the crayfish are totally dependent on supplemental food in intensive culture systems.

Several studies have been carried out under controlled conditions to evaluate the effect of feed on growth and survival of crayfish juveniles and a wide variety of foods have been tested in juveniles from the onset of exogenous feeding. *Artemia*, *Chlorella*, chironomid larvae, *Daphnia*, earthworms, fresh fish meat, aquatic plants and vegetables have been tried by Köksal (1985), Celada et al. (1989, 1993), Taugbol and Skurdal (1992), Köksal et al. (1992), Blake et al. (1994), Oliveira and Fabiao (1998), Verhoef et al. (1998), Verhoef and Austin (1999), Zaikov et al. (2000), Sáez-Royuela et al. (2001, 2007), Savolainen et al. (2003), González et al. (2008). Besides the exogenous feeding, commercial feeds for crustacean and fish species (Taugbol and Skurdal, 1992; Celada et al. 1993; Sáez-Royuela et al. 1995, 2001; Mazlum 2007) and experimental dry pellets for crayfish and crustaceans (Celada et al. 1989; Savolainen et al. 2004; Ulikowski et al. 2006), have been used in feeding of crayfish juveniles. The results in these studies carried under controlled conditions indicated that the survival and growth of crayfish juveniles have shown a great variability. In addition, it was also stated that the high mortalities were observed in the first period of the independent life of juveniles (Sáez-Royuela et al. 1995; Ulikowski et al. 2006).

In addition to feeding, stocking density is another important factor affecting crayfish growth and survival (Naranjo-Paramo et al. 2004; Ulikowski et al. 2006; Mazlum 2007). Studies under controlled conditions indicated that crayfish growth and survival decreased with increasing stocking density (Naranjo-Paramo et al. 2004; Savolainen et al. 2004; Ulikowski and Krzywosz 2004; Ulikowski et al. 2006; Mazlum 2007; Harlıoğlu 2009; González et al. 2010). In addition, effects of aggressiveness and cannibalism rise with increasing stocking density, resulting in reductions in survival and growth rates, as well as lack of chelae (Savolainen et al. 2004; Mazlum 2007; González et al. 2010).

Several studies have evaluated the effects of different stocking densities on growth and survival of *A. leptodactylus* (Köksal 1985; Ulikowski and Krzywosz 2004; Ulikowski et al. 2006; Mazlum 2007; Harlıoğlu 2009). However, little is known about the combined effect of feeding and density on the growth and survival of *A. leptodactylus* juveniles. The aim of this study was to evaluate the impact of feed type and stocking density on growth and survival of *A. leptodactylus* juveniles.

Materials and Methods

Experimental conditions

This study was carried out in the hatchery of Eğirdir Fisheries Research Institute in 2008. The material for the study consisted narrow-clawed crayfish, *A.leptodactylus* juveniles.

The juveniles were reared from ovigerous females of *A. leptodactylus* kept under the laboratory conditions. Ovigerous females were obtained from Lake Eğirdir in April and May 2008 and were placed in breeding tanks in order to obtain the juvenile crayfish. The eggs hatched in the second week of June. Stage 2 juvenile crayfish were fed with *Artemia* nauplii and *Chlorella* sp. during one week period. The juveniles with mean weight (35±0.005 mg), total length (11.67±0.47 mm) and carapace length (5.87±0.35 mm) were used in the experiment lasting for 90-days period.

Experimental design

The experimental design consisted of two different feed types (live *Daphnia magna* and commercial trout feed) and two different stocking densities (650 juveniles/m^2 and 1300 juveniles/m^2) in triplicates. The stage 2 juveniles were randomly stocked into 12 rearing tanks with a bottom area 1.5 m^2 (975 and 1950 juveniles in each tank at lower and higher stocking density, respectively).

Experimental groups: (D650) crayfish fed with *D. magna* and stocked 650 juveniles/m^2; (D1300) crayfish fed with *D. magna* and stocked 1300 juveniles/m^2; (T650) crayfish fed with trout feed and stocked 650 juveniles/m^2; (T1300) crayfish fed with trout feed and stocked 1300 juveniles/m^2.

The experiment was conducted for 90 days. The measurements of carapax, total length, and weight of the juveniles were performed both at beginning (100 individuals) and at the end of the experiment (all individuals). The number of surviving juveniles in each tank was counted at the end of experiment. The specific growth rate (*SGR*, %/day) and survival rate were calculated as follows:

$$SGR(\%/day) = \frac{100(lnW_t - lnW_0)}{t}$$

W_t and W_0 are the final and initial mean weights of juveniles, respectively, and t is the time in days (t=90 day).

$$Survival\ rate(\%) = \frac{final\ number\ of\ crayfish}{initial\ number\ of\ crayfish} x100$$

Experimental tanks and water quality management

Twelve circular fiberglass tanks with 1.5 m^2 bottom area in flow-through system were used in the experiment. Severel bricks (29x19x13.5cm) with 42 holes were placed in each tank as shelter. Each tank had its own water inlet and outlet. The quality parameters of the incoming water were: pH 7.57, calcium 54.04 mg/L, magnesium 58.66 mg/L, total hardness 338.65 mg/L, O_2 4.99 mg/L. One third of the water in each tank was exchanged every day and aeration was continuously supplied with an airstone in each tank. During the experimental period, water temperature, dissolved oxygen concentration and pH were measured once daily in each tank with a WTW 340i. The temperature ranged from 17 to 19 ^{o}C; dissolved oxygen from 6 to 7 mg/L and pH from 7.5-8.

Feed materials and feeding

Two different feed types as *D. magna* and commercial trout feed (800-1000µ) were used in the experiment. Commercial trout feed was obtained from a feed manufacturing company. *D. magna* were cultured in outdoor ponds. The proximate composition of commercial trout feed and *D. magna* is shown in Table 1. Moisture contents were detected with an automatic moisture analyzer (AND MX-50). The crude protein contents according to Kjeldahl method (Nx6,25) (AOAC 2000a), crude lipid contents by Bligh and Dyer (1959)'s method and crude ash contents according to (Lovell 1981) were done. Crude fibre content was determined according to Standard Association of Official Analytical Chemists (AOAC) methods (AOAC 2000b).

During the experiment, crayfish juveniles were fed daily *ad libitum* with the experimental diets and waste of feed and feces were removed from the tanks by siphoning.

Table 1. Proximate composition (%) of *Daphnia magna* and commercial trout feed used in the experiment

Parameter	*Daphnia manga*	Trout feed
Crude protein (%)	42.05	55
Crude lipid (%)	16.2	13
Crude cellulose (%)	13.5	1.5
Crude ash (%)	14.7	12

Data collection and statistical analysis

The weight and length of crayfish were measured at the beginning and end of the experiment. Total body length and carapace length were measured with digmatic calliper (to the nearest 0.01 mm) and weight measurement were performed by using digital scale with 0.01 g sensitivity. Significant differences among treatment groups were tested by one-way ANOVA and Duncan's multiple range tests at 5% level of significance by applying SPSS (version 11) software.

Results

At the end of 90-days experiment, it was determined that the mean survival rates, final weights, total lengths, carapace lengths and specific growth rates of *A. leptodactylus* juveniles ranged 10.84-21.99 %, 367-653 mg, 23.71-28.68 mm, 12.24-14.70 mm, 2.61-3.22 %, respectively (Table 2). It was seen that the difference between the values of average length and weight obtained from all groups was statistically significant ($P<0.05$). The juveniles fed with trout feed showed a better growth compared to the juveniles fed live feed. The best growth performance was seen on juveniles with low density and with fed trout feed. Both final weights and total lengths in juveniles were negatively affected by increased stocking density.

The highest values (3.22 % and 3.07 %) in specific growth rate were obtained in the groups fed with trout pellet, whereas the lowest values (2.64 % and 2.61 %) were obtained in the groups fed with live feed. It was shown that *SGR* was statistically different between trout feed and live feed groups, but stocking density formed in the relevant groups did not exhibited significant differences on *SGR*.

Survival rates varied from 10.84 % to 21.99 %. Survival of the juveniles decreased with increased stocking density (Table 2). The best survival rate as 21.99 %, was achieved in the group fed with live feed and kept under the low stocking density, whereas the lowest survival rate values as 10.84 % was obtained in the group fed with trout pellet and kept under the high stocking density. The survival rates of reduced with increasing stocking density. The results indicated that stocking density had a significant impact on the survival rate of crayfish juveniles.

Table 2. Final survival and growth indices of juvenile crayfish in the end of experiment ($\overline{X}$ ±SD).

Parameters	Experimental groups			
	D650	D1300	T650	T1300
Total length (mm)	24.04±3.23^{c}	23.71±3.13^{d}	28.68±4.55^{a}	27.71±4.46^{b}
Carapax length (mm)	12.39±1.72^{c}	12.24±1.80^{d}	14.70±2.57^{a}	14.09±2.38^{b}
Weight (mg)	367±1.181^{c}	368±0.168^{d}	653±0.321^{a}	570±0.341^{b}
SGR (%/day)	2.64±0.005^{b}	2.61±0.16^{b}	3.22±0.13^{a}	3.07±0.006^{a}
Survival rate (%)	21.99±2.32^{a}	14.25±1.38^{b}	20.67±3.13^{a}	10.84±2.53^{b}

Mean values in rows with different superscripts are significantly different (P<0.05).

Discussion

Several researchers stated that the juvenile astacids had the poor survival or growth rates when they were fed dry diets as the only food from the onset of exogenous feeding (Taugbol and Skurdal 1992; Ulikowski et al. 2006; Sáez-Royuela et al. 2007; González et al. 2009). For instance, a mortality of 83-90 % in noble crayfish juveniles were recorded with feeding a dry pellets (Taugbol and Skurdal, 1992). They observed that when juveniles were fed with fish and potatoes in addition to dry pellets, mortality was reduced to about 70 %. Similarly, Sáez-Royuela et al. (2007) recorded a mortality of 88.7 % after 100 days when juveniles did not receive live feed. According to the results of these studies, the researchers advised to use the live feed as supplement from the onset of external feeding to guarantee the viability of juvenile crayfish. González et al. (2008), supplementing a dry diet with live *Artemia* nauplii in excess, obtained good results (1283 mg weight, 4459 % weight gain and 3.82 % *SGR*) for juvenile *Pacifastacus leniusculus*. González et al. (2011) indicated that the juvenile crayfish (*P. leniusculus*) receiving decapsulted *Artemia* cysts up to day 50 as supplement to a dry diet showed a faster growth (averaging 13.8 mm carapace length, 610 mg weight 3.05 % day^{-1} *SGR*) at the end of the experiment. In contrast to these results, the commercial trout feed used in the present study resulted in a better growth performance (28.68 mm total length and 653 mg alive weight) than live feed. However, similar survival rates (20.67-21 %) were obtained with both live feed and commercial trout feed. These results suggest that good growth can be achieved by using manufactured diets. This diet or one of similar composition may also constitute a good reference for future dietary studies. Similarly, Verhoef et al. (1998) using a variety of natural and an artificial diet, found similar results for juvenile yabbies *Cherax destructor*. The researchers indicated that the commercial diet used in their study produced growth rates similar to those obtained by feeding live or frozen zooplankton.

Köksal (1985), obtained relatively good survival and growth rates (44.23 % and 430.64-476.16 mg respectively) after 90 days in *A. leptodactylus* juveniles fed trout pellet and flamentous green algae. In our study performed during the same period, the growth of crayfish was better than that reported by Köksal (1985), but the survival rates of juveniles were lower. This may be due to the higher stocking density. In 1985, the same researcher, using different food type in feeding of stage 4 juveniles of *A. leptodactylus* (mean total length 21.1 mm) at the same density, obtained total lengths of 29.2-37.4 mm, weights of 623-1216 mg and survival rates of 50.6-72.4 % after 60 days. In the present experiment, stage 2 juveniles (mean size 11.67 mm) reached 23.71-28.68 mm length and 367-653 mg weight after 90 days. However the survival rates of juveniles were lower the levels reported by Köksal (1985). It is noteworthy that initial sizes used for this study (stage 4 juveniles) were higher than size in our experiment (stage 2 juveniles). The lower survival rates may be due to the initial size of the juveniles used in the experiment. The second-stage that the juveniles become independent from the mother is critical period for crayfish and the highest mortality during the rearing of crayfish occurs in this period but fell considerably during ensuing periods (Ulikowski et al. 2006).

In the present study, crayfish juveniles fed trout feed showed a higher specific growth rate compared to juveniles fed live feed. Sáez-Royuela et al. (2007) indicated that *P. leniusculus* juveniles fed a dry diet (for salmonids) supplemented with live feed (*Artemia* nauplii or *Daphnia*) had a higher *SGR* value (2.83) those fed a dry diet as the sole food (2.24). Ulikowski et al. (2006) used experimental pellets in the feeding of juvenile crayfish and obtained *SGR* ranging from 1.3 (for the third month) to 6.4 % (for the first month) for *A. leptodactylus*. In a similar study of the same species by Harlıoğlu (2009), *SGR* values were found to be 2.41-2.48 % for *A. leptodactylus* when crayfish were provided with natural food. Zaikov et al. (2000), used four different diets for *A. leptodactylus* during up to 1 month of age under 4 diet variants: zooplankton, feed mixture, meat and soyabean meal, and reported the best results of *SGR* in *A. leptodactylus* fed with zooplankton diet (3.56 %) and fed mixture diet (3.15 %). González et al. (2008), supplementing a dry diet with live *Artemia* nauplii in excess, obtained 3.82 % *SGR* for juvenile *P. leniusculus*.

It has been reported that increased stocking density has a certainly negative impact on crayfish growth and survival in previous studies (Ulikowski and Krzywosz 2004; Savolainen et al. 2004; Ulikowski et al. 2006; Mazlum 2007; González et al. 2010). Savolainen et. al. (2004) indicated that final mean weight and mean length in *P. leniusculus* were decreased with increasing stocking densities. The researchers also predicted a reduction of crayfish survival 11 % when stocking density was doubled. The decrease rates of survival when stocking density was doubled in our study for *A. leptodactylus* juveniles were much higher (35.2 % for the live feed groups and 47.5 % for the trout feed groups). The reason of this might be that we had a higher initial stocking densities. In *P. leniusculus*, González et al. (2010) reported 86.33 % and 39.3 % survival in the lowest and highest stocking densities (100 and 1000/m^2) respectively, after 100 days. Ulikowski et al. (2006) reported the high mortality rate of 48-77 % in *P. leniusculus* and *A. leptodactylus* at different stocking densities (600, 1200 juveniles/m^2). Harlıoğlu (2009) reported survival and *SGR* for the same species from 34,3 % to 56 % and 1.10 to 1.40 respectively at different stocking densities, ranging from 234 juveniles/m^2 to 937 juveniles/m^2. Köksal (1985) reported survival rates of 22.5-58 % and at different stocking densities (130-260 juveniles/m^2) in *A. leptodactylus*. The length of weight of juveniles ranged from 20.13 to 20.9 mm and from 201.33-210.5 mg after 45 days, respectively. Ulikowski and Krzywosz (2004) obtained good results in *A. leptodactylus* after four-week rearing period. The survival was 70 % (initial density: 300 juveniles/m^2), 58% (initial density: 600 juveniles/m^2) and 47.8% (initial density: 1200 juveniles/m^2). Harlıoğlu (2009) obtained 57.3-62.6%, 51.3-56.6%, and 42-44.3% survival for *A. leptodactylus* at stocking densities of 234, 468, and 937 juveniles/m^2, respectively. Mazlum (2007) reported good growth rate and survival for newly hatched third instars *A. leptodactylus* at stocking densities of 50, 100 and 200/m^2. In the present study, growth and survival of crayfish juveniles were affected by stocking density; they decreased as initial stocking density increased. This is in accordance with results on *P. leniusculus* (Savolainen et al. 2004; González et al. 2010), *A. leptodactylus* (Ulikowski and Krzywosz 2004; Mazlum 2007). Harlıoğlu (2009) stated that crayfish exhibit density dependent growth even in situations where sufficient food resources are available. The results obtained in our study also support this view. The values of growth obtained in our study for *A. leptodactylus* were similar to those achieved in other studies with similar or even much lower stocking densities; *P. leniusculus* in densities 100-800 individuals/m^2 (Savolainen et al. 2004), *A. leptodactylus* and *P. leniusculus* in densities 600-1200 individuals/m^2 (Ulikowski et al. 2006), for *P. leniusculus* in densities 100-1000 individuals/m^2 (González et al. 2010). Regarding the survival, the values obtained in our experiment was much lower than the levels of the same species reported by other authors (Köksal 1985; Ulikowski et al. 2006; Mazlum 2007; Harlıoğlu 2009). The poor survival rates may be related to the initial stocking density and initial weight and length of the juveniles. In addition, this is due to the food, experimental conditions, and the number of the available shelters.

In conclusion, the crayfish juveniles fed with trout feed exhibited better growth performance than those fed with live feed. This suggest that it might be better to develop an artificial diet for use in the intensive culture of juvenile astacid juvenile crayfish. In addition, the survival of juvenile crayfish were negatively affected by increased stocking density. The best survival rate was obtained at 650 juveniles/m^2 stocking density. This study has clearly demonstrated that stocking density had a significant effect on survival rates of crayfish juveniles.

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