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Effects of Feeding Interval on Growth, Survival and Body Composition of Narrow-Clawed Crayfish, *Astacus leptodactylus* Eschscholtz, 1823 Juveniles

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Abstract

Newly hatched third instars of *Astacus leptodactylus* (mean weight 44.5±3.4 mg and mean total length 10.8±0.4 mm) were randomly stocked in 0.2 m^2 aquariums at rate of 100 crayfish/m^2 fed with trout feed at a quantity 5% of crayfish body weight for 90 day. The amount of diet was adjusted according to the total crayfish biomass calculated for each sampling period (30-days interval). Four feeding intervals were tested: everyday (D), every 2nd (D2), every 3rd (D3), and every 4th day (D4). At the end of feeding period, the feeding interval had significant impact on crayfish survival, growth rate, and yield. The survival of crayfish was the highest in D2 treatment (83.3%) and was lowest in D4 treatment (57.2%). Results indicated that feeding interval in D2 treatment significantly increased survival of crayfish. Growth rates were significantly different ($P<0.05$) between D2 treatment (0.8 mm/day) and D4 treatment (0.5 mm/day) at the end of the 90-day experiment. The best growth rate was observed in D2 treatment group. The yield was found to decrease with increasing length of feeding interval. The highest yield (27.65 g) was observed in D2 treatment, while the lowest yield (12.6 g) was in D4 treatment. The feeding interval had no significant difference on body composition of *A. leptodactylus* juvenile among the treatment groups ($P>0.05$). The study showed that the optimal feeding interval was observed every 2nd day.

Keywords: *Astacus leptodactylus*, feeding interval, growth, survival, body composition.

Yemleme Aralığının Yavru Kerevitlerin (*Astacus leptodactylus* Eschscholtz, 1823) Büyümesi, Hayatta Kalması ve Vücut Kompozisyonları Üzerine Etkileri

Özet

Yeni çıkmış üçüncü aşamadaki *A. leptodactylus* (başlangıç ağırlıkları ve uzunları sırasıyla 44,5±3,4 mg ve 10,8±0,4 mm) kerevitleri canlı ağırlıklarının %5 oranında alabalık yemi ile beslenerek, metrekarede 100 adet kerevit olacak şekilde rastgele 0,2 m^2'lik akvaryumlara 90 gün süreyle stoklanmıştır. Verilen yem miktarı aylık olarak yapılan ölçümler sonucu elde edilen kerevitlerin toplam ağırlıklarına göre ayarlanmıştır. Dört farklı yemleme sıklığı uygulanmıştır. Bunlar sırasıyla: günde bir kez yemleme (D), iki günde bir yemleme (D2), üç günde bir yemleme (D3) ve dört günde bir yemleme (D4) olarak gerçekleşmiştir. Deneme sonunda yemleme aralığının yavru kerevitlerin hayatta kalma, büyüme oranları ve ürün miktarı üzerinde önemli bir etkiye sahip olduğu gözlenmiştir. En yüksek hayatta kalma oranı iki günde bir beslenen gruplardaki kerevitlerde (%83,3) (D2) ve en düşük hayatta kalma oranı ise dört günde bir beslenen gruplardaki kerevitlerde (%57,2) (D4) bulunmuştur. Deneme sonuçları iki günde bir beslenen grupta hayatta kalma oranının yüksek olduğunu göstermiştir. Deneme sonunda büyüme oranları iki günde bir beslenen grupta (0,8 mm/gün) ve dört günde bir beslenen grupta (0,5 mm/gün) istatistiksel olarak farklı olduğu saptanmıştır ($P<0,05$). En iyi büyüme iki günde bir beslenen muamele grubunda bulunmuştur. Ürün miktarı yemleme sıklığının uzunluğuna bağlı olarak azalmıştır. En yüksek ürün miktarı iki günde bir beslenen grupta 27,65 g olarak bulunurken, en düşük ürün ise dört günde bir beslenen grupta (12,6 g) olarak bulunmuştur. Yapılan analizler sonucunda yemleme sıklığının yavru kerevitlerin vücut kompozisyonları üzerine herhangi bir etkisinin olmadığı saptanmıştır ($P>0,05$). Bu çalışma en ideal yemleme aralığının iki günde bir yapılan grupta olduğunu göstermiştir.

Anahtar Kelimeler: *Astacus leptodactylus*, yemleme aralığı, büyüme, hayatta kalma ve vücut kompozisyonu.

Introduction

Astacus leptodactylus (Eschscholtz, 1823) is the only native crayfish species of Turkey (Köksal, 1988). It is considered a valuable fishery resource, as there are no other commercially important species of Crustacean found in fresh waters in Turkey. *A. leptodactylus* lives in cold lakes, ponds, and rivers throughout Turkey. It occurs naturally in the lakes Eğirdir, Beyşehir, Akşehir, Eber, Çivril, Apolyont, and Manyas (Erençin and Köksal, 1977).

While the domestic demand for crayfish was very low in Turkey (Köksal, 1988), Turkey was the main supplier of *A. leptodactylus* to Western Europe from 1970 until 1986 (Baran *et al*., 1987; Oray, 1990; Holdich, 1993; Harlıoğlu and Holdich, 2001). Until 1984, freshwater crayfish played an important role as a high quality live export product, but after 1986, crayfish production declined dramatically in most lakes and dam reservoirs from 5,000 tones to 200 tones, because of the crayfish plague *Aphanomyces astaci* (Schikora, 1903*)*, pollution, overfishing and agricultural irrigation (Diler and Bolat, 2001). Although the plague is still observed in some lakes in Turkey, there has been an increase in the amount of *A. leptodactylus* harvested from the wild (Bolat *et al.*, 2010). Total crayfish harvested in Turkey was about 750 tones in 2007 (Harlıoğlu, 2008). On the other hand, the culture of *A. leptodactylus* in captivity is not carried out in Turkey. Crustacean makes a significant contribution of global aquaculture production of which decapods are the most economically important group. Among freshwater crayfish, *A. leptodactylus* reproduces only once a year has a low fecundity, and a long embryonic development (6-9 months) under natural conditions (Reynolds *et al*., 1992), but females can indeed spawn every year. For this reason *A. leptodactylus* that has emerged as an important culture species. Crustacean, like many other aquatic organisms, are subjected to scarcity of food and prolonged starvation in their natural environment. They are also subjected to periodic fasting during, immediately before and immediately after, molting. As a consequence, they undergo some alterations in their normal physiological and biochemical processes and their nutritional status is altered during these non feeding periods (Hochachka and Somero, 1984). In intensive culture systems, including hatcheries and nurseries, the animals are totally dependent on supplemental food. In addition, crayfish growth is affected by several variables such as water, food quality, food amount, food supply frequency, and its availability to the crayfish (Aiken and Waddy, 1992). For this reason, determining the optimum feeding frequency is one of the key factors for successful crayfish culture. Little information is available on the effects of feeding frequency on crayfish growth. Therefore, *A. leptodactylus* was selected as the valuable fishery resource in fresh water for this investigation in Turkey, being a popular food.

The aim of this study was to investigate the effects of changing food supply interval while the overall feeding rate remained constant. The factors determined to assess the effects were growth rate, survival and body composition in juvenile crayfish.

Materials and Methods

Ovigerous females *Astacus leptodactylus* were collected from experimental ponds at Eğirdir Fisheries Research Institute located in Eğirdir, Isparta, Turkey. Crayfish were immediately packed into insulated live-fish shipping boxes and shipped to Fisheries Research Facility at Mustafa Kemal University where spawning took place.

Prior to experimental use, the crayfish were kept in circular fiber glass tanks provided with shelter (e.g., onion sacks and pieces of polyvinylchloride pipe), and well-aerated water was used in the flow-through system. The animals were fed commercial trout diet to develop and shed eggs. Hatched larvae were reared until the third-instars stage (young-of-the-year, YOY). Total length (TL) of subsamples of the *A. leptodactylus* third instars was measured to the nearest millimeter, to estimate the size of the starting YOY crayfish.

Two hundred and forty juveniles (mean weight 44.5±3.4 mg and mean total length 10.8±0.4 mm) were fed with a commercial trout diet stocked into 60 L aquaria at a density of 20 crayfish per aquarium (100 crayfish/m^2) (Mazlum, 2007). Experiment was conducted in aquaria (n=12) with the dimensions of 80 x 40 x 25 cm (length x width x height; area: 2000 cm^2) in a closed system. A (4 x 3) factorial design with three replications was used in this feeding experiment. A static system was used and 20% of the water in each aquarium was changed with seven days interval. The bottom of each tank was provided with a PVC pipe as a shelter.

All crayfish were acclimatized for a period of one week during which they fed with commercial trout diet of 55% protein, 10% lipid, 1.3% cellulose, 11% ash and 12% moisture (BioAqua Fish Feed, Izmir, Turkey). Crayfish were fed four feeding intervals: these were; 1) everyday (D), 2) every 2nd (D2), 3) every 3rd (D3) and 4) every 4th day (D4) (Table 1). The aquaria system was housed inside a room with natural photoperiod 12 dark: 12 lights and each aquarium were supplied with continuous aeration. The calcium concentration of water was 23 mg/L. Water temperature inside the aquaria ranged from 21 to 29°C (mean of 25°C). Dissolved oxygen ranged from 6.5 to 7.4 mg/L. Water quality was monitored at least once per month, with the exception of temperature and dissolved oxygen, which were measured five times per week using an oxygen meter (Model 55 YSI; Yellow Springs Instruments Company, Ohio, USA), respectively. The pH ranged from 6.8 to 7.6 during the experiments (Accumet pH meter, Model 915, Fisher Scientific, Pennsylvania,

Table 1. Feeding interval for the narrow-clawed crayfish (*A. leptodactylus* Eschscholtz, 1823) juvenile used to assess the effects of feeding interval on growth

Treatment	Feeding Interval	Times (hr)
D	everyday	24.^{00}
D2	every 2nd day	48.^{00}
D3	every 3rd day	72.^{00}
D4	every 4th day	96.^{00}

USA). Ammonia concentration was measured using a multi-parameter ion-specific meter (Model C203 Hanna, Hanna Instruments, Richmond, Virginia, USA). Nitrite nitrogen N-NO_2 (sulphanil method) was determined calorimetrically on spectrophotometer.

The number of surviving juveniles in each aquarium was counted and the total length (TL) of the animals was measured to the nearest 0.1 mm from the tip of the rostrum to telson 30-day intervals over the 90 days experimental period. Juveniles were put on filter paper to removed excess water and weighed to the nearest 0.01 g (wet weight, WWt). In addition, the number of molting animals was counted for each treatment during the study. At the end of the growth trial, crayfish were counted and individually weighed during the 90-day trials. Feed was provided twice a day at a quantity of 5% of their body weight, and uneaten food was removed from each experimental unit daily before next feeding. The amount of feed was adjusted based on the crayfish weight calculated for each sampling period.

Growth, survival, feed conversion ratio (FCR) and body composition were determined for the various sampling periods (30, 60, and 90-days) from 15 June 2008 to 15 September 2008. Survival was calculated as the percentage of crayfish surviving each sampling period. Growth rates (GR) were estimated from the mean TL sampled from treatment to the end of the experiment. The expression used was

$$GR = (TL_f - TL_i)/t$$

where: TL_i is the total length at treatment, TL_f is the total length at the end of the experiment and t is the time in days (Reynolds, 2002; Mazlum and Eversole, 2005). Productivity was determined as biomass increase within the treatment period using the equation:

$$\text{Productivity} = ((W_{tf} - W_{ti}) / W_{ti}) \times 100,$$

where W_{tf} = final tank weight and Wti = initial tank weight. The feed conversion ratio (FCR) was calculated as feed given per wet weight gain.

At the end of the experiment, all crayfish in each aquarium were analyzed to the determination of body proximate composition and stored at -20°C. The proximate compositions of the crayfish were analyzed according to the Association of Analytical Communities (AOAC, 1990) procedures as follows: moisture was determined by oven drying at 105°C for 24 h, crude protein (N x 6.25) by the Kjeldahl method, and crude ash by combustion in a muffle furnace at 550°C for 16 h. The total lipid concentration was determined by extracting with the chloroform–methanol method, described by Bligh and Dyer (1959).

Statistical Analysis

Data were analyzed with the Micro-SAS Statistical Software System (Version 8, SAS, 1999, SAS Institute, Cary, North Carolina). Analysis of variance (ANOVA) was used to determine the effects of feeding frequencies on growth, survival and body composition of juvenile crayfish. The mean score test was used to determine survival rates among the treatments. The least significant difference (LSD) test was used to determine the differences in average weight, TL, FCR, and productivity. Statistical analyses were considered significant at the $P<0.05$ level.

Results

All aquariums were continuously aerated throughout the duration of the study. No statistical differences were observed in the average water temperature and dissolved oxygen (DO) levels among treatments. The lowest DO was 4.2 mg/L, a DO value above the recommended level (Köksal, 1988). Over the duration of the study, water quality parameters averaged were: temperature, 25°C (21–29°C); dissolved oxygen, 6.95 mg/L (6.5–7.4 mg/L); total ammonia nitrogen, 0.048 mg/L; nitrite, 0.015 mg/l; nitrate, 0.017 mg/L; pH, 6.8 to 7.6. The calcium concentration during the experimental period averaged 23 mg/L, which is adequate for the survival of crayfish during the moult (e.g., Mazlum, 2007). These parameters were within acceptable limits for the growth and development of *A. leptodactylus* (Table 2) (Köksal, 1988). No disease problem occurred during the experiment.

The current study indicated that length of feeding frequency over the 90-days had an important impact on growth, survival and yield in juvenile of *A. leptodactylus* are shown in (Table 3). Survival rate was significantly affected with the feeding frequency

Table 2. Mean±SD and range in parenthesis of water quality for third instars of *Astacus leptodactylus* Eschscholtz

Parameters	Mean (± SD)	Ranges
Temperature (°C)	25.0±1.8	21-29
Dissolved Oxygen (DO mg/L)	6.9±0.4	4.2-8.4
pH	7.2±0.3	6.8-7.9
Nitrite (mg/L)	0.015±0.002	0.012-0.019
Nitrate (mg/L)	0.017±0.001	0.013-0.021
Ammonia (mg/L)	0.048±0.04	0.032-0.062
Calcium (mg/L)	23±2.1	19-31

Table 3. Mean and standard deviation (± SD) of final length, weight, percent survival, FCR, molting frequency, yield, GR and yield of *Astacus leptodactylus,* given 55% protein pelleted feed at different feeding frequencies (D-D4) under laboratory conditions, after 90 days

Parameters	Feeding interval			
	D	D2	D3	D4
Final length (mm)	23.44±0.22[a]*	28.4±0.9 [a]	21.93±0.16[b]	21.22±0.16[b]
Final weight (g)	0.86±0.12 [a]	1.02±0.18[a]	0.62±0.09 [b]	0.5±0.04 [b]
Survival (%)	71.67±8.9 [a]	83.33±11.6[a]	67.23±6.8 [a]	57.20±5.9 [b]
FCR	4.31±1.2 [a]	3.6±0.10 [a]	3.84±0.09 [a]	4.72±1.4 [a]
GR (%/d)	0.73±0.03 [a]	0.80±0.06 [a]	0.64±0.04 [b]	0.52±0.02 [b]
Yield (g)	23.31±4.8 [a]	27.65±3.9 [a]	16.74±2.9 [b]	12.6±2.6 [b]

*Mean values (mean ± sd) in rows with different superscript are significantly different (P<0.05). The initially estimated TL and WW_t (n=40) of third instars *Astacus leptodactylus* Eschscholtz were 10.8 ±0.4 mm and 44.5 ±3.4 mg, respectively.

(P<0.05). Survival rate decreased with an increased length of feeding interval. The highest survival rate was in D2 treatment (83.3%), and was the lowest in D4 treatment (57.2%). At the end of the study, feeding interval in D2 treatment significantly increased survival of crayfish (Table 3).

The best growth rate was observed in D2 treatment group. Initial lengths and weights were similar among the four feeding interval regime. Final weights varied from 0.27 to 1.65 g and total length from 15 to 50 mm in the various tanks. The average weight over the 90-day period was 1.02 g for D2 treatment compared with only 0.5 g for D4 treatment. Weight gain of *A. leptodactylus* juvenile in this study was significantly affected by the feeding interval (P<0.05). Weight gain decreased with the increasing rate of feeding frequency. The total length increment in crayfish fed in D2 treatment (28.4 mm) and D treatment (24.4 mm) were significantly higher (P<0.05) than those of crayfish fed at D3 (21.9 mm), and D4 treatments (21.2 mm) (Table 3). The average growth rates (GR) at D2 treatment (0.8 mm/day) and D4 treatment (0.5 mm/day) were found significantly higher than (P<0.05) those of the other treatments. However, there was no significant difference (P<0.05) between D3 and D4 treatments.

The highest yield was 27.65 g, with crayfish fed two days interval, while the lowest yield (12.6 g) was observed in D4 treatment (Table 3). Differences among the yields of the crayfish fed at different feeding frequency were significant. Molting frequency of *A. leptodactylus* at different feeding frequency is summarized in (Figure 1). Molting frequencies in D, D2, D3 and D4 feeding groups were detected as 62.7, 70.4, 52.2, and 35%, respectively. These were significantly different among the treatments (P<0.05). Feed conversion ratio (FCR) for D2 group tended to be better than that of the other groups (Table 3). But, no significant differences were found among the treatment groups.

Proximate composition (which includes percent moisture, protein, lipid, fiber, and ash) of juvenile A. leptodactylus at different feeding frequency are presented in (Table 4**)**. There were no significant differences in percentage moisture, protein, lipid, and ash (wet-weight basis contents) of the tail meat of *A. leptodactylus* juvenile among treatments, which averaged 82.3%, 16.2%, 0.38% and 1.2%, respectively.

Discussion

The current study indicated that length of feeding frequency over the 90-days had a significant impact on growth, survival and yield in juvenile of *A. leptodactylus*. Gu *et al*. (1996) demonstrated that length of feeding frequency caused a marked decreased in mean final length of juvenile of *Cherax quadricarinatus*. But as soon as starved crayfish juveniles were provided with food they began to increase in weight but at a slower rate. Meade and Watts (1997) demonstrated that short term nutrient deprivation did not have long term consequences in terms of weight gain in juvenile *Cherax quadricarinatus*. Crayfish which exhibited minimum weight gain when cultured initially using poor quality feeds exhibited improved weight gain when fed with high quality feed. Thomas *et al*. (2003) found that for *Jasus edwardsii* culture, high ration levels and multiple daily feedings reduced diet competition and agonistic behavior. However, this may have been the result of different-sized individuals establishing dominance hierarchies. Low diet ration levels, particularly rations where energy intake becomes limited, can significantly affect growth rate (Chittleborough, 1975).

In the present study, survival was influenced by frequency periods as increase in length of frequency had negative effect on percentage survival of *A. leptodactylus*. Saez-Royuela *et al*. (2001) in an 80-day rearing period obtained higher survival rates with juvenile crayfish that were fed twice daily than with those that fed once daily. This frequency did not have a significant impact on the growth of juvenile *A. pallipes*. However, food supply frequency was found to have an impact on the growth of *C. quadricarinatus* (Cortes *et al*., 2003) the yabby, *C. destructor* (Mills and McCloud, 1983). The current study indicated that feeding frequency impacts crayfish growth mainly by regulating molting frequency. Molting frequencies in D, D2, D3 and D4 treatments were detected as 62.7%, 70.4%, 52.2% and

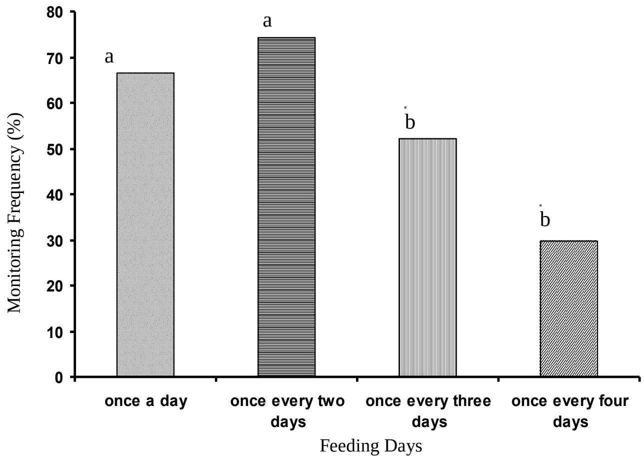

Figure 1. Comparison of molting of *Astacus leptodactylus* Eschscholtz, 1823 juveniles at four different feeding regimes. Molting frequency in bar with different superscript (a, b) are significantly different (P <0.05).

Table 4. Mean (±SD) moisture, protein, lipid and ash percent of tail meat of crayfish harvested from aquarium and fed everyday (D), every 2nd day (D2), every 3rd day (D3) and every 4th day (D4) under laboratory conditions, after 90 days

Parameters	Feeding interval			
	D	D2	D3	D4
Moisture (%)	82.3±0.7	81.9±1.3	82.5±0.3	82.5±1.3
Protein (%)	16.1±0.8	16.5±0.8	16±0.8	16.1±0.8
Lipid (%)	0.3±0.001	0.4±0.00	0.4±0.00	0.4±0.00
Ash (%)	1.2±0.04	1.3±0.10	1.07±0.10	1.2±0.10

*Mean values (Mean±SD) in rows with different superscript are significantly different among treatments (P<0.05).

35%; respectively. As the food supply interval was increased, the frequency of their molt increased (Barki *et al.*, 1997; Saez-Royuela *et al.*, 2001). The probability for cannibalism increases with the increasing rates of molting, since cannibalism usually occurs when an individual is soft after molting (Huner, 2002). As a result, crayfish cannibalism was the main factor that contributed to the lower survival among the stocks crayfish fed once a daily than in those fed once every four days. Mikami and Takashima (1993) demonstrated that feeding frequency restricted the lengthening of the first molting interval to newly hatched phyllosoma larvae of the spiny lobster, *Palinurus japonicus*. Longer periods of time to first feeding lengthened the intermolt period of the first instars of *P. japonicus* and *Thenus* sp. larvae without affecting the duration of later intermolt periods or growth rates (Mikami *et al.*, 1995).

Many studies showed that feeding frequency influenced the growth and survival of crayfish (Wang *et al*., 2006). The effects of feeding frequency on crayfish growth depend on several factors including crayfish species, developmental stages, environmental conditions and nutritional value and energy levels in feeds. Optimum to maximum weight gain was observed in juvenile crayfish when fed twice per day (D'Abramo and Conklin, 1985), but newly hatched crayfish, eight feedings per day at 10% biomass were reported by Tucker *et al*. (2006).

Environmental factors may also influence food consumption rates of crayfish. For instance, temperature regulates processes such as activity, feed consumption, and growth in poikilotherms (Winberg, 1956). Soderback *et al*. (1987) observed a linear increase in food consumption with increased water temperatures in noble crayfish *A. astacus.* D'Abramo and Conklin (1995) confirmed that feeding one ration of artemia salina once a day produced better consumption rates in the European clawed lobster compared with animals fed smaller rations twice daily. The results of this study showed favors once every two days feeding for optimal growth and food conversion. We found that feeding once every four days gave worse food conversion.

As expected, moisture content did not increase or change during the length of feeding frequency. Whyte *et al*. (1986) used the ratio of wet weight of hepatopancreas of whole body as an index of physiological condition in the prawn *Pandalus platyceros* and demonstrated that the ratio decreased on starvation and increased on re-feeding. Armitage and Wall (1982) suggested that changes in moisture content in the body tissue reflect physiological changes during starvation. The finding that starvation caused an increase in moisture content aggresses with previous studies in other crayfish species (Claybrook, 1983). Armitage *et al*. (1973) suggested that in the freshwater crayfish, *Orconectes nailis*, and fluctuations in moisture content occurred during the molt and reproductive cycle. In the present study, *A. leptodactylus* analyses for condition indices were not considered for analysis as described by Schafer (1968) in other crustaceans. This study has demonstrated that juvenile *A. leptodactylus* can tolerate relatively long periods of starvation or low food availability. Cortes *et al*. (2003) reported that when feeding frequency was restricted crayfish required higher energy diet. This may also indicated that excess dietary energy is not utilized by crayfish juvenile.

Our study indicated that optimum feeding interval was reported as every 2nd day for larval crayfish. An increasing the length of feeding interval of *A. leptodactylus* had a positive impact on growth, survival and yield, but not on body composition.

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