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Effects of Feeding Frequency on Growth Performance and Molting Cycle of Two Different Size Classes of Red Swamp Crayfish (*Procambarus clarkii*)

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ABSTRACT

Red swamp crayfish (*Procambarus clarkii*) is an important crayfish species for both aquaculture and ornamental purposes. In this study, growth performance, survival rate, and molting cycle were evaluated with three feeding frequencies, including every other day (2F1), two (F2) and four times (F4) daily in two different age classes (Size I, 15 days old and Size II, 45 days old). The study was carried out in 50 L glass aquariums with three replicates for 12 weeks. The highest final mean weight (FMW) of Size I group was found in F4 (P<0.05). F2 and F4 groups attained higher final mean weight, final mean total length (FMTL) and final mean carapace length (FMCL) for Size II (P<0.05). Although there were no statistical differences in survival rates (SR) of Size I (P>0.05), the highest SR was recorded in F2 for both size groups. Molting cycle of F2 was the highest for Size I and there was a moderate increase from F2 to F4 in Size II group. In conclusion, F4 for Size I and F2 for Size II groups are recommended. Further feeding studies such as starvation and refeeding cycles, feeding rate, feeding time, etc. are necessary for this important crayfish species.

Keywords: Ornamental crayfish, freshwater decapods, meal frequency, feeding regime

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İki Farklı Boy Sınıfındaki Kırmızı Bataklık Kerevitlerinde (*Procambarus clarkii*) Yemleme Sıklığının Büyüme Performansı ve Kabuk Değiştirme Üzerine Etkileri

Öz: Kırmızı bataklık kereviti (*Procambarus clarkii*) hem su ürünleri yetiştiriciliği hem de süs amacı için önemli bir kerevit türüdür. Bu çalışmada, iki farklı boy grubundaki (Boy I, 15 günlük ve Boy II, 45 günlük) kırmızı bataklık kerevitlerinin iki günde bir (2F1), günde iki (F2) ve günde dört (F4) kez olmak üzere 3 farklı yemleme sıklığında büyüme performansları, yaşama oranları ve kabuk değişim periyotları araştırılmıştır. Çalışma 12 hafta boyunca üç tekrarlı olarak 50 L cam akvaryumlarda gerçekleştirilmiştir. Boy I grubu için en yüksek son ortalama ağırlık F4 grubunda bulunmuştur (P<0,05). Boy II grubunda F2 ve F4 sıklıklarının son ortalama ağırlık, son ortalama total boy ve son ortalama karapas boyları daha yüksektir (P<0,05). Boy I grubunun yaşama oranlarında istatistiksel olarak anlamlı bir fark bulunmamasına rağmen (P>0,05), her iki boy grubu için F2 sıklığında en yüksek yaşama oranı kaydedilmiştir. Boy I grubunda F2 sıklığının kabuk değişim sayısı en yüksektir ve Boy II grubunda ise F2'den F4'e doğru bir yükseliş vardır. Sonuç olarak, Boy I grubu için günde dört kez, Boy II için ise günde iki kez besleme önerilmektedir. Bu önemli kerevit türü için açlık döngüsü, yemleme oranı, yemleme zamanı, vb. gibi daha fazla beslenme çalışması yapılması gereklidir.

Anahtar kelimeler: Süs kereviti, tatlısu dekapodları, besin sıklığı, yemleme rejimi

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Introduction

Red swamp crayfish is the most cultured crayfish species in all over the world in 2016 (FAO 2018). They are naturally distributed in the state of Louisiana in South America and in the Northeastern region of Mexico. Natural habitats of this species are stagnant rivers, lakes, marsh areas and wetlands. They are tolerant of low oxygen levels, temperature changes, moderate salinity, drought and pollution (Cruz and Rebelo 2007). These properties play an important role in the preference of *Procambarus clarkii* in aquaculture. In addition, *P. clarkii* has the ability to adjust itself to extreme natural conditions and changes in water levels (Barbaresi and Gherardi

2000). However, Gherardi (2006) reported that this species was invasive in several European countries, which should be seriously taken into consideration.

Apart from aquaculture, crayfish are also one of the most interesting creatures in the aquarium industry and the demand for them increases day by day. For example, *P. clarkii* began to take place in the aquarium sector in Germany in 1985, while *Procambarus zonangulus* in the early 1990s (Holdich 2002). There is no a professional red swamp crayfish farm in Turkey, but they are traded as one of the most common and popular species among nine crayfish species in the aquarium sector in Turkey (Türkmen and Karadal 2012).

As in other aquatic decapod species, crayfish have to molt during certain periods to grow. During this phase, they are vulnerable and are at a constant risk (Bliss 1983). In practical terms, it would be a very useful move to remove these molting individuals from the area in order to prevent attacks from other individuals. There are many factors that affect molting and its cycle, such as species, size, genetic structure, nutrition, feeding behavior, water parameters, physical factors (temperature, salinity, light intensity), environment, presence of shelter, substrate type, pollutants, etc. (Chen and Chen 2003; Chang and Mykles 2011).

Feeding is one of the important factors during the life cycle and different feeding regimes may affect some parameters, such as growth performance, survival rate, and molting cycle in crayfish species. The optimal feeding frequency improves growth efficiency and survival rate and decreases feeding costs and feed related wastes (Mizanur and Bai 2014). Although, there are few studies conducted on the effects of different feeding characteristics of red swamp crayfish on reproduction (Xu et al. 2010), condition indexes (Dörr et al. 2013), body composition (Xu et al. 2013), nutrient digestibility (Wan et al. 2017), immune response (Zhang et al. 2016) and histology (Xiao et al. 2014; Chen et al. 2017) besides growth performance, no study has been conducted on the feeding frequency. Therefore, in this present study, the effects of three different feeding frequencies on growth performance, survival and molting rate of two different sized *Procambarus clarkii* were investigated.

Materials and Methods

Red swamp crayfish (*Procambarus clarkii*) were obtained from a commercial facility in İzmir, Turkey and transported to the Faculty of Fisheries, Ege University, Turkey. Two size groups of this species namely Size I (SI, mean total length 9.36±0.73 mm, 15 days) and Size II (SII, mean total length 12.17±0.88 mm, 45 days old) were randomly distributed to 18 glass aquariums (47x31x34 cm) filled with 10 L of dechlorinated and continuously aerated tap water. Crayfish were stocked at a density of 10 individuals per aquarium with three replicates for each dietary treatment. Both sizes of crayfish were fed with three different feeding frequencies, including every other day (2F1), twice daily (F2) and four times daily (F4). Crayfish were hand-fed to apparent satiety as mentioned in Table 1 for 12 weeks experimental period with a commercial shrimp/crayfish diet (JBL NovoPrawn, Table 2). The feed intake (FI) was recorded biweekly. Uneaten food was siphoned out from the aquariums 1 h after feeding. PVC pipe pieces with a diameter of 2 cm for the SI group and 4 cm for SII group were placed into all aquariums as shelters. Water temperature was maintained at 27.2±1.23 ºC, dissolved oxygen at 9.05±0.48 mg/L (WTW-Oxi 315), pH at 7.59±0.14 (Sartorius PT-10) and ammonium at 0.18±0.02 mg/L (HANNA C205). The water was exchanged with 30%, twice a week. The system was housed in a climate-controlled laboratory with a controlled photoperiod (12 h light: 12 h dark).

Table 1. Feeding frequencies and hours during the experiment

Feeding Frequency	Group	Hours
Every other day	2F1	09:00
Two times a day	F2	09:00, 17:00
Four times a day	F4	09:00, 11:30, 14:30, 17:00

Table 2. The chemical composition of the commercial feed used in the experiments (%, dry matter)

	Pellet Feed
Moisture (%)	8
Crude Protein (%)	37
Crude Fat (%)	5
Crude Fibre (%)	10
Crude Ash (%)	12
Nitrogen Free Extract	28
Gross Energy (MJ/kg)	15.56

*Nitrogen-free extracts (NFE) were calculated as NFE = 100 - (% protein + % fat + % fibre + % ash) Gross energy was calculated using the conversion factors of 23.7 kJ/g for protein, 39.5 kJ/g for fat, and 17.2 kJ/g for carbohydrates

Growth performance was monitored biweekly. Crayfish were individually weighed with an electronic balance (Sartorius BL610, precision of ±0. 01 g). Total length measurements were performed at from the rostrum tip to the telson end whereas the carapace lengths were measured from the tip of the rostrum to the posterior edge of the carapace using a vernier caliper. Growth parameters, including feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR) were calculated as follows: FCR = feed intake / weight gain, SGR = 100 x ([Ln final crayfish weight] - [Ln initial crayfish weight]) / experimental days, SR = 100 x (total crayfish count - dead crayfish count) / total crayfish count.

Growth and molting data were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to rank groups using a statistical software (Statgraphics Centurion XVI, Statpoint Technologies Inc., The Plains, VA) (Zar 1999). All data are presented as "mean±standard error" and in all tests, a significance level of P<0.05 was used.

Results

Growth performances of two different sized red swamp crayfish were given in Table 3 and 4. Final mean weights (FMW) and feed intake (FI) of SI were increased with feeding frequencies (P<0.05). Final mean total lengths (FMTL) and specific growth rates (SGR) of 2F1 group was significantly lower than F4 group (P<0.05). The highest final mean carapace length (FMCL) was recorded in F4 group. There were no significant differences in feed conversion ratios (FCR) among feeding frequencies (P>0.05). Survival rates (SR) ranged from 83.3% to 93.3% without a significant effect by feeding levels (P>0.05). In Size II crayfish, FMW, FMTL, and FMCL of 2F1 group were significantly lower than other groups (P<0.05). FI of all groups was significantly different (P<0.05). SGR of F4 was higher than 2F1 group and the highest FCR was observed in 2F1 (P<0.05). SR of F2 group was 96.7% being statistically different from 2F1 group with 76.7% (P<0.05).

Table 3. Growth parameters of Size I red swamp crayfish fed with different feeding frequencies

	2F1	F2	F4
Initial mean weight (g)	0.07±0.00	0.08±0.00	0.08±0.00
Final mean weight (g)	1.73±0.02^{a}	2.03±0.04^{b}	2.27±0.04^{c}
Initial mean total length (cm)	0.95±0.11	0.92±0.04	0.93±0.07
Final mean total length (cm)	1.36±0.16^{a}	1.51±0.25^{ab}	1.68±0.09^{b}
Initial mean carapace length (cm)	0.51±0.02	0.50±0.01	0.50±0.02
Final mean carapace length (cm)	0.72±0.08^{a}	0.76±0.05^{a}	0.85±0.07^{b}
Feed intake (g)	3.16±0.07^{a}	3.74±0.05^{b}	4.24±0.04^{c}
Specific growth rate (%/day)	3.78±0.04^{a}	3.92±0.07^{ab}	4.04±0.04^{b}
Feed conversion ratio	1.16±0.03	1.16±0.01	1.16±0.01
Survival rate (%)	86.67±8.82	93.33±6.67	83.33±3.33

*In the same line, different letters indicate statistically significant differences (P<0.05) among the treatments

Table 4. Growth parameters of Size II red swamp crayfish fed with different feeding frequencies

	2F1	F2	F4
Initial mean weight (g)	0.28±0.01	0.29±0.01	0.29±0.01
Final mean weight (g)	2.66±0.09^{a}	3.13±0.07^{b}	3.37±0.08^{b}
Initial mean total length (cm)	1.22±0.06	1.20±0.10	1.24±0.09
Final mean total length (cm)	1.98±0.17^{a}	2.44±0.09^{b}	2.50±0.14^{b}
Initial mean carapace length (cm)	0.63±0.05	0.62±0.01	0.64±0.02
Final mean carapace length (cm)	1.01±0.10^{a}	1.25±0.21^{b}	1.26±0.18^{b}
Feed intake (g)	4.73±0.10^{a}	5.24±0.12^{b}	5.74±0.07^{c}
Specific growth rate (%/day)	2.65±0.09^{a}	2.83±0.03^{ab}	2.92±0.02^{b}
Feed conversion ratio	1.20±0.02^{b}	1.12±0.01^{a}	1.12±0.02^{a}
Survival rate (%)	76.67±6.67^{a}	96.67±3.33^{b}	86.67±3.33^{ab}

*In the same line, different letters indicate statistically significant differences (P<0.05) among the treatment

The number of molting of individuals, mean molting frequency (MMF) and molting rate (MR) of red swamp crayfish were given in Table 5 for Size I and Table 6 for Size II. MMF of Size I crayfish ranged from 4.23 to 5.14 with the lowest value in 2F1. The highest molting frequency during the experimental period was found as five times with 28 crayfish, followed by six times with 23 crayfish and four times with 18 individuals. The highest MMF was observed in F2 and F4 groups for Size II (P<0.05). Higher molting frequencies were recorded as five, four and three times with 27, 17 and 14 individuals, respectively.

Table 5. Molting parameters of Size I red swamp crayfish fed with different feeding frequencies

		2F1	F2	F4
n		26	28	25
Number of molting	1	1	0	0
	2	1	0	1
	3	3	2	2
	4	9	5	4
	5	10	8	10
	6	2	13	8
Mean molting frequency		4.23±0.22[a]	5.14±0.18[b]	4.88±0.22[b]

*In the same line, different letters indicate statistical significant differences (P<0.05) among the treatments

Table 6. Molting parameters of Size II red swamp crayfish fed with different feeding frequencies

		2F1	F2	F4
n		23	29	26
Number of molting	1	3	1	0
	2	5	3	3
	3	7	3	4
	4	2	9	6
	5	6	11	10
	6	0	2	3
Mean molting frequency		3.13±0.29[a]	4.10±0.23[b]	4.23±0.24[b]

*In the same line, different letters indicate statistical significant differences (P<0.05) among the treatment

Discussion

The best growth performance was observed in F4 in both size groups in the present experiment. This treatment resulted in a higher feed intake. This is an expected result and consistent within the findings of previous studies carried out in various crayfish species by Sáez-Royuela et al. (2001), Cortés-Jacinto et al. (2003), Ulikowski and Krzywosz (2006) and González et al. (2009). Molting frequencies ranged between 4.9 and 5.1 for Size I and 4.1 and 4.2 for Size II group with the highest value in 2F for Size I crayfish and in 4F for Size II group. However, 2F and 4F were comparable in both sizes.

The mean weights of crayfish used in previous feeding frequency studies varied between 0.4 and 20 g. Crayfish (9 days old and 0.39 g) were fed twice daily by Xiao et al. (2014) and Chen et al. (2017) while those weighing between 1-10 g (2.10, 2.52, 5.39 and 8.13 g) and above 10 g (10.40, 11.35 and 16.00 g) were fed ranging from 1-3 times a day (Jover et al. 1999; Yue et al. 2009; Xu et al. 2010; Dörr et al. 2013; Xu et al. 2013; Hua et al. 2015; Zhang et al. 2016; Wan et al. 2017). Out of six of these cited 10 studies, feeding twice daily was used as the main feeding frequency. This is the reason for the choice of three feeding frequencies below and above twice daily in the present study.

In some previous studies carried out with different crayfish species with average mean weights of 0.03 g, including white-clawed crayfish (*Austropotamobius pallipes*) and signal crayfish (*Pacifastacus leniusculus*), the growth performance increased with feeding frequencies (Sáez-Royuela et al. 2001; González et al. 2009). These authors observed the best FMW, FMCL, and SR at twice daily and once daily feeding frequencies, respectively. In this study, FMW, FMTL, and SGR of Size I group significantly enhanced with increasing feeding frequency. Although a similar trend was observed in Size II group in terms of FMW, FMTL, and FMCL (0.29 g), higher meal frequencies (2F and 4F) were comparable. Cortés-Jacinto et al. (2003) carried out a feeding frequency study in 0.89 g Australian red claw crayfish (*Cherax quadricarinatus*) and fed the crayfish with six, four, two times and once daily. The authors found similar results to our Size II group and FMW and SGR of *C. quadricarinatus* were similar in six and four times daily feeding groups. In contrast, 0.03 g Turkish crayfish (*Astacus leptodactylus*) did not grow well at higher feeding frequencies and the highest FMW and SGR were found at once daily and every other day feeding (Ulikowski and Krzywosz 2006; Mazlum et al. 2011). This could be explained by that optimum feeding frequency may change depending on species, size, dietary protein levels and feeding period (Karadal et al. 2017).

Ulikowski and Krzywosz (2006) found very low survival rates at Turkish crayfish with 22-40%, but Mazlum et al. (2011) recorded higher rates with 57-83% for the same species. In addition, González et al. (2009) determined the similar survival rates (57-82%) for signal crayfish. Unlike these studies, the survival rates of red swamp crayfish in this study were fairly above from this level with the ratios between 77-97% for both size groups. However, Cortés-Jacinto et al. (2003) reported much higher values for Australian red claw crayfish with 87-98%. Holdich (2002) stated that the cannibalism is one of the major factor increasing mortality of crayfish when judged from low survival rates on lower feeding frequencies.

The molting is a significant process in the normal life cycle of all aquatic decapod species. Molting frequency may change with many factors. The stress due to an incorrect and inadequate nutrition affects the period of molting (Chen et al. 2017). Turkish crayfish had a successful molting with 66-74% ratio at once and every other daily feeding, while every three and every four-day feeding frequencies negatively influenced with a ratio of 29-53% (Mazlum et al. 2011). Malnourished or hungry crayfish still enter the premolt phase, but this phase is delayed compared to well-fed and healthy individuals. In a previous research carried out by Renai et al. (2007) on 0.42 g of noble crayfish (*Astacus astacus*), the number of molting was found to be moderately higher in 3% BW feeding group than 1% BW feeding group. Feeding amount can also affect the growth performance of crayfish generally due to changing the frequency of molting (Aiken and Waddy 1992). Furthermore, Whyte et al. (1986) reported that unfed prawn (*Pandalus platiceros*) did not enter the molting phase. In the current research carried out with red swamp crayfish, molting frequency was increased with feeding frequency, which linearly reflected growth performance. Increasing the food amount up to an optimum limit elevates the ratio of energy consumed for growth in crayfish (Renai et al. 2007).

Consequently, optimum feeding frequencies were determined as four times and twice daily for Size I and Size II groups, respectively in this study when growth performance and molting frequency are considered. Further studies related to feeding strategies, including starvation-refeeding periods, feeding rate, feeding time, etc. are necessary for this species.

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