



Growth Performance, Molting Frequency and Carapace Coloration of Two Different Size Classes of Red Swamp Crayfish (*Procambarus clarkii*) Fed with Different Diets

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ABSTRACT

In this study, the effects of three different commercial aquarium feeds were tested on the growth performance, molting frequency and carapace coloration of red swamp crayfish (*Procambarus clarkii*) in two different size classes (4 and 5 cm). For this purpose, six experimental groups were formed according to crayfish size (S1: Size 1 and S2: Size 2) and feed type (BF: bottom fish food, CF: cichlid food and CG: crustacean granules) as S1BF, S1CF, S1CG, S2BF, S2CF and S2CG, and the crayfish were fed with these diets for 12 weeks. The final mean weight (FMW) of the S1CG was higher than the S1BF ($P<0.05$). The final mean total length (FMTL) and final mean carapace length (FMCL) of S2BF were the lowest ($P<0.05$). No significant differences were recorded in the feed conversion ratios (FCR), specific growth rates (SGR) and survival rates (SR) of red swamp crayfish in two different size classes ($P>0.05$). The lowest cheliped injury and cannibalism rates were found in the crayfish fed with crustacean feed in both size classes. The mean molting frequencies (MMF) of the S1CG and S2CG were statistically higher than the S1CF and S2BF, respectively ($P<0.05$). S2CG has the highest final lightness (L^*) value ($P<0.05$). The final redness (a^*) and final yellowness (b^*) values of the S1BF and S2BF groups were the lowest ($P<0.05$). The results showed the positive effects of crustacean feed on all the tested parameters in both size classes. Further studies are needed to investigate the efficient use of species-specific rations in the crayfish species.

Keywords: Cambaridae, crayfish feeding, aquarium feed, growth performance, pigmentation

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Farklı Yemlerle Beslenen İki Farklı Boy Sınıfındaki Kırmızı Bataklık Kerevitlerinin (*Procambarus clarkii*) Büyüme Performansı, Kabuk Değişirme Frekansı ve Karapaks Renklenmesi

Öz: Bu çalışmada, iki farklı boy sınıfındaki (4 ve 5 cm) kırmızı bataklık kerevitlerinin (*Procambarus clarkii*) büyüme performansı, kabuk değişirme frekansı ve karapaks renklenmesi üzerine üç farklı ticari akvaryum yeminin etkileri test edilmiştir. Bu amaçla kerevit boyutuna (S1: Boy 1 ve S2: Boy 2) ve yem tipine (BF: dip balığı yemi, CF: çiklit yemi ve CG: krustase yemi) göre S1BF, S1CF, S1CG, S2BF, S2CF ve S2CG olmak üzere altı deney grubu oluşturulmuştur ve kerevitler bu yemlerle 12 hafta boyunca beslenmiştir. S1CG'nin son ortalama ağırlığı, S1BF'den daha yüksektir ($P<0,05$). S2BF'nin son ortalama total boy uzunluğu ve son ortalama karapaks uzunluğu en düşüktür ($P<0,05$). Kırmızı bataklık kerevitlerinin yem dönüşüm oranlarında, spesifik büyüme oranlarında ve hayatta kalma oranlarında iki farklı boy sınıfında önemli farklılıklar kaydedilmemiştir ($P>0,05$). Her iki boy sınıfında da krustase yemiyle beslenen kerevitlerde en düşük kısıkaç kaybı ve kanibalizm oranları bulunmuştur. S1CG ve S2CG'nin ortalama kabuk değişirme frekansı, sırasıyla S1CF ve S2BF'den istatistiksel olarak daha yüksektir ($P<0,05$). S2CG, en yüksek son parlaklık (L^*) değerine sahiptir ($P<0,05$). S1BF ve S2BF gruplarının son kırmızılık (a^*) ve son sarılık (b^*) değerleri en düşüktür ($P<0,05$). Sonuçlar, krustase yeminin her iki boyut sınıfında test edilen tüm parametreler üzerindeki olumlu etkilerini göstermiştir. Kerevitlerde türe özel yemlerin verimli kullanımı ile ilgili daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Cambaridae, kerevit besleme, akvaryum yemi, büyüme performansı, pigmentasyon

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Introduction

Red swamp crayfish (*Procambarus clarkii*) originated in north-eastern Mexico and Louisiana, USA. During the last decade, cultivation of this species has been widespread in both ornamental and commercial aquacultures because of their resistant and tolerant characteristics (Cruz and Rebelo 2007), attractive behaviors, and appealing coloration and the species is the most cultivated crayfish in the world (FAO 2019). But unfortunately, the red swamp crayfish was reported as an invasive animal from inland waters of many countries of the world (GISD 2021). In Türkiye, this species became more popular in the aquarium sector in the last quarter of the 2000s. Although, there is no common culture of red swamp crayfish in Türkiye, the species is one of the most available crayfish in the aquarium sector.

With the increase in the number of species demanded in the aquarium sector, research on ornamental aquaculture has gained importance. Basics in ornamental aquaculture have primarily originated from conventional aquaculture (Sicuro 2018). For example, the biggest variety of raw materials and dietary additives widely used in ornamental aquaculture is similar to conventional. However, fast development in ornamental aquaculture has caused an ever-increasing demand for enhanced and profitable aquafeeds for the cultivation of finfish and invertebrate species (Boonyaratpalin et al. 2001). Today, ornamental aquafeeds are marketed in a wide spectrum according to the specific characteristics of the species, i.e., living in freshwater, saltwater, brackish water, etc., feeding as carnivorous, omnivorous, herbivorous, etc., being a vertebrate or an invertebrate, or surface and bottom feeding (Sicuro 2018).

Nutrition is extremely important, stating second only to water quality, in the culture of aquatic species (Asoka and Hettiarachchi 2004). The feeding behavior of an animal is critical both in terms of determining its nutritional status and growth and reproductive activities (Hughes 1993), but it also gives information about the place of the animal in the food chain (Poon et al. 2010). Benthic animals have important functions in the food chain and therefore in the ecosystem. For instance, crayfish species, extensively identified as ecosystem engineers, have been known for detritivorous and omnivorous feeding characteristics (Rodríguez-Serna et al. 2010). Plant and detrital resources contain dietary pigments, trace elements and energy (Brown 1995). Therefore, the including these resources in formulated feeds is very important in terms of vital parameters. The number of aquafeeds formulated by commercial companies with raw materials, such as fish and fish derivatives, cereals, vegetable protein extracts, various vegetables, yeasts and algae for ornamental

crustaceans is increasing, currently. However, there is no clear information that reveals the adequacy level of commercial feeds.

Color is one of the significant parameters for the price in ornamental aquaculture (Sicuro 2018). Coloration is the primary determining criterion not only for fish but also for both freshwater and marine invertebrates. For that matter, Kaldre et al. (2015) mentioned the importance of carapace color not only for ornamental aquaculture, but also for crayfish cultivated for consumption. Aquarium enthusiasts and seafood consumers are decided by brightness and vibrant pigmentation to the freshness, quality and health of the animal (Boonyaratpalin et al. 2001). Color differentiation in crayfish depends on several factors, including morphological characteristics, genetics, environmental conditions, and diet (Wade 2010; Kaldre et al. 2015). In ornamental aquaculture, dietary additives, including fatty acids, ascorbic acid, alpha-tocopherol and carotenoids are widely used in aquarium feeds for the enhancement of coloration (Güroy et al. 2012).

The efficiency of commercial feeds on several crayfish species, including Mexican crayfish, *Procambarus llamasii* (Rodríguez-Serna et al. 2010), marbled crayfish, *Procambarus fallax* f. *virginalis* (Kaldre et al. 2015) and Australian red claw crayfish, *Cherax quadricarinatus* (Karadal and Türkmen 2012) are studied previously. However, no research was found on the red swamp crayfish with commercially available aquafeeds. Therefore, the aim of the present study was to evaluate the effects of different commercial ornamental fish and crustacean feeds on the growth performance, molting frequency and carapace coloration of red swamp crayfish (*P. clarkii*) in two different size classes (4 and 5 cm). Because the most popular sizes of these crayfish in the aquarium trade are usually around 4-5 cm, the effects of study parameters were tested in both size groups.

Materials and Methods

Ethical Approval

The present study was carried out in accordance with animal welfare and the ethics of experiment. This study complied with the Guidelines of the EU Directive 2010/63/EU for animal experiments.

Rearing Systems and Crayfish

The study was carried out in the Tropical Aquaculture Laboratory, Faculty of Fisheries, İzmir Katip Çelebi University, İzmir, Türkiye. Two different sizes (Size 1 of 4.00±0.06 cm and Size 2 of 5.13±0.03 cm in total lengths) of red swamp crayfish (*P. clarkii*), reared in the Tropical Aquaculture Laboratory, were stocked in 10 L plastic containers

connected to two recirculating sump systems (300 L each) and adapted for 14 days. A submersible pump (Aquawing AQ6000) placed in glass sump aquarium (140 × 50 × 22 cm) circulated the freshwater and fresh air was supplied to all containers by an electromagnetic air pump (Resun ACO-001), continuously. PVC pipes with 5 cm diameter were placed to containers as much as the number of individuals. The 20% water was removed from the sump aquarium twice a week and chlorine-free tap water was added to the system. The photoperiod was maintained as 12:12 (light:dark). Crayfish were weighed individually at the initial and the end of the trial.

Experimental Design and Diets

Three different commercial ornamental finfish and crustacean feeds were used in the study (Table 1). Crayfish were fed once a day near satiation and uneaten food was removed from the containers after 1.5 h from feeding by siphoning and weighed. Six experimental groups were formed with two sizes of crayfish (S1 and S2) and three different feeds, including Art Akua Bottom Fish Food (BF), AHM Natural Cichlid Granulat (CF) and Tetra Crusta Granules (CG) as S1BF, S1CF, S1CG, S2BF, S2CF and S2CG. The experiment was carried out in 24 plastic containers in four replications for 12 weeks and 8 crayfish were stocked in each container.

Table 1. Proximate compositions of commercial diets used in the experiment

Composition (%)	Art Akua Bottom Fish Food	AHM Natural Cichlid Granulat	Tetra Crusta Menu
Crude protein	50.00	40.34	44.00
Crude lipid	10.00	5.08	11.00
Crude fiber	3.00	1.11	2.00
Crude ash	7.00	6.92	-
Moisture	7.00	6.29	8.00

Water Parameters

An external heater (Hydor ETH 300) is connected to the circulation pump outputs of the systems. Thus, the temperature of a system was kept constant throughout the trial. The water parameters, including temperature, pH and dissolved oxygen of the freshwater in plastic containers were checked daily with AZ 84051 Combo Water Quality Meter during the study. Mean values were recorded as 24.58±0.17 °C for temperature, 7.42±0.14 for pH and 8.97±0.42 ppm for dissolved oxygen.

Evaluation of Growth Performance

Growth performance data were obtained by biweekly weight, total and carapace length measurements. Before weighing, each crayfish was collected from containers and dried on filter paper to remove water from branchiostegites and appendages.

FI (g) = average of the total feed given to each experimental group during the study,

FCR = Feed intake / Weight gain,

SGR (%/day) = 100 x ([Ln Final crayfish weight] - [Ln Initial crayfish weight]) / Experimental days.

Survival and Cannibalism Rate

The number of crayfish that died, injured or lost their claws, and died from cannibalism during the study were recorded according to their tank numbers

SR (%) = 100 x (Number of total crayfish - Number of dead crayfish) / Number of total crayfish,

CIR (%) = 100 x Number of crayfish cheliped injured / Number of total crayfish,

CR (%) = 100 x Number of crayfish died due to cannibalism / Number of total crayfish.

Monitoring of Molting Frequency

At the start of the experiment, each crayfish carapace in the plastic containers was individually

weighed in bulk with an electronic balance (KERN PCB 2500-2, precision of ±0.01 g). Total length measurements were performed 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 digital caliper. At the beginning of the experiment, the same weight of commercial feeds was weighed for each plastic container and were stocked to small boxes, and the crayfish were fed with these feeds during the trial. The feed intake of the crayfish was recorded by weighing the feeds in these boxes during the biweekly measurements. Growth parameters, including feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR) were calculated according to following formulae:

and trial groups. The survival rate (SR), cheliped injury rate (CIR) and cannibalism rate (CR) were determined with following calculations at the end of the trial:

marked with nail polish in different colors from the right side (Ramalho et al. 2010). The crayfish were checked daily and shells (if any) in the containers

were recorded according to their color. For determining intermolt period, newly molted crayfish was marked again from the same area with the same color of nail polish. New marks on individuals were checked until the shell hardened. Numbers of molting

in the experimental groups were recorded and after two days from each molt, weight and total length were measured for collecting molt increment data. The mean molting frequency was calculated with the following formula (Chen and Chen 2003):

$$MMF = [(n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + \dots + (n_k \times k)] / \text{total number of crayfish.}$$

Molting parameters, including mean molting number (MMN), intermolt period (IMP), weight increment at molt

(WIM) and length increment at molt (LIM) were calculated according to the following formulae:

$$MMN = \text{Total number of molting} / \text{Crayfish number,}$$

$$IMP \text{ (days)} = \text{Time of } n+1 \text{ molt} - \text{Time of } n \text{ molt,}$$

$$WIM \text{ (\%)} = 100 \times (\text{Weight after molting} - \text{Weight before molting}) / \text{Weight before molting,}$$

$$LIM \text{ (\%)} = 100 \times (\text{Length after molting} - \text{Length before molting}) / \text{Length before molting.}$$

Carapace Coloration Measurement

Color measurements were taken from all crayfish at the beginning and end of the experiment in order to obtain coloration data. Measurements were taken from the carapace region of the crayfish on a flat surface with a colorimeter (Color Muse, Variable Inc., Tennessee, USA) (Dang et al. 2021). The measurements were performed on top surface (4 mm) of carapace of each crayfish. The colorimeter was set to take absolute measurements in the L^* , a^* , b^* measuring mode (CIE 1976). L^* is the lightness variable (where white: 100 L^* and black: 0 L^*), a^* is the redness where $+a^*$ stands for red, and $-a^*$ stands for green, and b^* is the yellowness where $+b^*$ stands for yellow, and $-b^*$ stands for blue.

Statistical Analysis

The Shapiro-Wilk W and Levene tests were subjected to verify normality and homogeneity of variance before further analysis was undertaken, respectively. One-way analysis of variance (ANOVA) was performed for the analysis of the data of growth performance, molting frequency and carapace coloration. Differences between the experimental groups were ranked Tukey's multiple range test. All means were presented with standard errors (\pm SE). For statistical assessment of the study

data, a statistical software (Statgraphics Centurion XVI, Statpoint Technologies Inc., The Plains, VA) was used (Zar 1999). Differences were considered significant at the 95% confidence interval.

Results

Growth performance parameters of red swamp crayfish fed with different commercial feeds in two different size groups are given in Tables 2 and 3. The final mean weight (FMW) of the S1CG was higher than the S1BF ($P < 0.05$). The final mean total length (FMTL) of the S1CG was the highest among the S1 experimental groups ($P < 0.05$). There were no significant differences in final mean carapace length (FMCL), feed conversion ratio (FCR) and specific growth rate (SGR) of S1 groups ($P > 0.05$). Although there was no statistical difference in the FMWs of S2 groups, the S2CG was higher than the other groups. The FMTL and FMCL of S2BF was the lowest ($P < 0.05$). There was no significant difference in FCR and SGR of S2 groups ($P > 0.05$). Feed intakes (FI) of the experimental groups were statistically increased as BF, CF and CG for two different sizes, respectively ($P < 0.05$). No significant differences were recorded in the survival rates (SR) of red swamp crayfish in two different size classes ($P > 0.05$).

Table 2. Growth performance of Size 1 (S1) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S1BF	S1CF	S1CG
Initial mean weight (g)	1.52 \pm 0.02	1.53 \pm 0.01	1.52 \pm 0.03
Final mean weight (g)	3.69 \pm 0.08 ^a	3.77 \pm 0.12 ^{ab}	4.16 \pm 0.10 ^b
Initial mean total length (cm)	3.98 \pm 0.10	4.03 \pm 0.11	4.00 \pm 0.10
Final mean total length (cm)	5.62 \pm 0.10 ^a	5.59 \pm 0.10 ^a	5.87 \pm 0.11 ^b
Initial mean carapace length (cm)	2.02 \pm 0.03	1.99 \pm 0.04	2.03 \pm 0.01
Final mean carapace length (cm)	2.76 \pm 0.05	2.81 \pm 0.04	2.90 \pm 0.08
Feed intake (g)	2.63 \pm 0.02 ^a	2.83 \pm 0.02 ^b	3.15 \pm 0.05 ^c
Specific growth rate (%/day)	1.06 \pm 0.04	1.07 \pm 0.04	1.20 \pm 0.03
Feed conversion ratio	1.21 \pm 0.04	1.20 \pm 0.07	1.14 \pm 0.06

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the groups

Table 3. Growth performance of Size 2 (S2) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S2BF	S2CF	S2CG
Initial mean weight (g)	3.53±0.02	3.51±0.01	3.50±0.02
Final mean weight (g)	8.77±0.14	8.98±0.22	9.11±0.05
Initial mean total length (cm)	5.10±0.05	5.15±0.05	5.12±0.04
Final mean total length (cm)	6.29±0.06 ^a	6.51±0.07 ^b	6.63±0.12 ^b
Initial mean carapace length (cm)	2.49±0.07	2.54±0.03	2.51±0.02
Final mean carapace length (cm)	3.06±0.04 ^a	3.21±0.05 ^b	3.29±0.09 ^b
Feed intake (g)	5.68±0.06 ^a	6.28±0.07 ^b	7.18±0.05 ^c
Specific growth rate (%/day)	1.08±0.02	1.12±0.03	1.14±0.01
Feed conversion ratio	0.96±0.03	1.05±0.02	1.03±0.04

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the groups

Survival, cheliped injury and cannibalism rates of two different sizes of red swamp crayfish fed with commercial diets are shown in Figures 1, 2 and 3, respectively. The SRs of the S1 groups were 59.38, 75 and 62.63% for the BF, CF and CG, respectively and 84.38% for the BF and CF and 93.75% for the CG in the S2 groups ($P > 0.05$ among their size

classes). The CIRs of the S1BF, S1CF, S1CG, S2BF, S2CF and S2CG were found as 46.88, 40.63, 34.38, 28.13, 25 and 18%, respectively ($P > 0.05$ among their size classes). The CR of S1BF was 21.88%, S1CF and S2CF were 15.63%, S1CG and S2BF were 12.5%, and S2CG was 6.25% ($P > 0.05$ among their size classes).

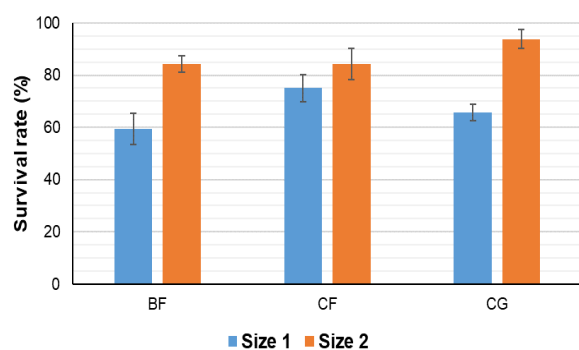


Figure 1. Survival rates at the end of the trial of two different size classes of red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds. Error bars (\pm SE) of each experimental group were shown in the column chart.

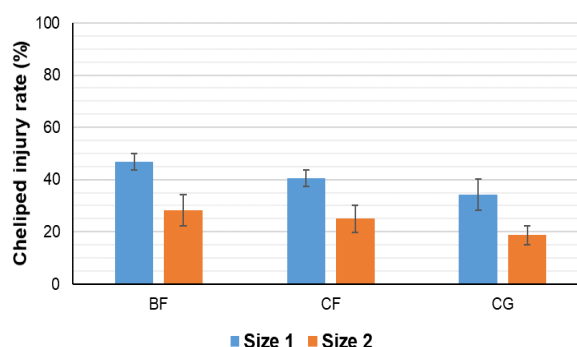


Figure 2. Cheliped injury rates at the end of the trial of two different size classes of red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds. Error bars (\pm SE) of each experimental group were shown in the column chart.

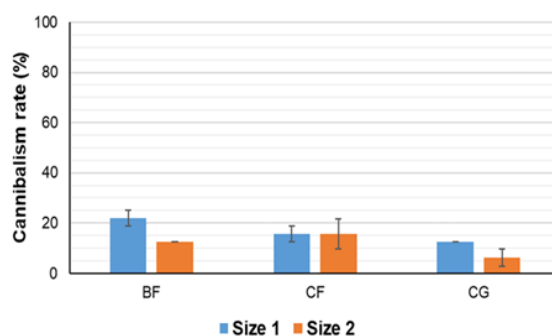


Figure 3. Cannibalism rates at the end of the trial of two different size classes of red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds. Error bars (\pm SE) of each experimental group were shown in the column chart.

Molting numbers and parameters of red swamp crayfish fed with different ornamental fish and crustacean feeds in two different size classes are detailed in Tables 4 and 5. The highest molting numbers were recorded at two and three times in S1 (20 specimens for each) and one time in S2 (60 specimens), irrespective of experimental groups. The mean molting frequency (MMF) and mean number of

molting (MNM) of the S1CG were statistically higher than the S1CF ($P<0.05$). The MMF and MNM of the S2CG were higher than the S2BF ($P<0.05$). The IM of the S1CF and S2BF were the highest among their size classes ($P<0.05$). While the WIM and LIM increased in the order of BF, CF, CG in both groups, a statistical difference was found in the WIM of only the S1 groups ($P<0.05$).

Table 4. Molting parameters of Size 1 (S1) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

		S1BF	S1CF	S1CG
	<i>n</i>	19	24	21
Number of molting	1	1	5	0
	2	6	11	3
	3	6	6	8
	4	4	2	6
	5	2	0	4
Mean molting frequency		3.00±0.26 ^{ab}	2.23±0.13 ^a	3.52±0.29 ^b
Mean number of molting		14.25±2.02 ^{ab}	13.25±0.48 ^a	18.50±1.85 ^b
Intermolt period (days)		28.69±2.54 ^a	38.06±2.40 ^b	24.40±2.10 ^a
Weight increment at molt (%)		48.56±1.43 ^a	51.40±1.11 ^{ab}	54.69±1.46 ^b
Length increment at molt (%)		22.02±1.63	23.47±1.48	24.69±1.42

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups

Table 5. Molting parameters of Size 2 (S2) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

		S1BF	S1CF	S1CG
	<i>n</i>	27	27	30
Number of molting	1	23	19	18
	2	3	5	6
	3	1	3	5
	4	0	0	1
Mean molting frequency		1.18±0.09 ^a	1.41±0.07 ^{ab}	1.65±0.19 ^b
Mean number of molting		8.00±0.71 ^a	9.50±0.65 ^{ab}	12.25±1.11 ^b
Intermolt period (days)		72.08±5.17 ^b	59.91±3.16 ^a	53.40±7.37 ^a
Weight increment at molt (%)		82.85±1.41	84.80±1.57	86.12±1.06 ^b
Length increment at molt (%)		32.70±1.32	34.96±1.71	36.95±2.35

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups

The initial and final carapace coloration parameters of the red swamp crayfish used in the experiment are listed in Tables 6 and 7. There was no statistical difference in the lightness (L^*) of S1 groups ($P>0.05$). The final redness (a^*) and final yellowness (b^*) values of the S1BF were the lowest ($P<0.05$). S2CG has the highest lightness (L^*) value ($P<0.05$). The final redness (a^*) and final yellowness (b^*) values of the S2BF were the lowest ($P<0.05$).

However, significant increases were noted through the initial and final values of the lightness (L^*) of the S1BF, S1CF, S1CG, S2CF and S2CG, the redness (a^*) and yellowness (b^*) of the S1CF, S1CG, S2CF and S2CG groups ($P<0.05$). Also, statistical decreases were recorded in the initial and final values of the lightness (L^*) and yellowness (b^*) of the S1BF and the redness (a^*) of the S1BF and S2BF groups ($P<0.05$).

Table 6. Carapace coloration of Size 1 (S1) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S1BF	S1CF	S1CG
Initial lightness (L^*)	39.62±0.39 ^A	39.75±0.49 ^A	39.02±0.39 ^A
Final lightness (L^*)	41.75±0.43 ^B	41.35±0.52 ^B	41.74±0.72 ^B
Initial redness (a^*)	25.02±0.83 ^B	24.55±0.97 ^A	24.87±0.87 ^A
Final redness (a^*)	17.72±0.81 ^{a, A}	25.72±0.53 ^{b, B}	26.06±0.51 ^{b, B}
Initial yellowness (b^*)	23.63±0.63 ^B	23.83±0.54 ^A	23.49±0.56 ^A
Final yellowness (b^*)	20.46±0.92 ^{a, A}	26.02±0.61 ^{b, B}	25.51±0.47 ^{b, B}

Different small letters in the same line and capital letters in the same column indicate statistically significant differences ($P < 0.05$) among the groups and between the initial and final parameters in each, respectively

Table 7. Carapace coloration of Size 2 (S2) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S2BF	S2CF	S2CG
Initial lightness (L^*)	40.12±0.36	40.11±0.48A	40.17±0.26A
Final lightness (L^*)	40.58±0.33a	43.70±0.66b, B	45.01±0.56c, B
Initial redness (a^*)	26.79±0.75B	27.74±1.09A	28.76±1.05A
Final redness (a^*)	20.69±0.81a, A	30.87±1.70b, B	33.64±1.23b, B
Initial yellowness (b^*)	22.51±0.30	23.29±0.50A	22.21±0.38A
Final yellowness (b^*)	21.97±0.27a	24.77±0.49b, B	25.07±0.64b, B

Different small letters in the same line and capital letters in the same column indicate statistically significant differences ($P < 0.05$) among the groups and between the initial and final parameters in each, respective

Discussion

The effects of different commercial aquarium feeds (bottom fish food, cichlid food and crustacean granules) on growth performance, molting frequency and carapace coloration of 4 and 5 cm red swamp crayfish (*P. clarkii*) were investigated in the present study. The growth performance regarding final mean weight (FMW) and final mean total length (FMTL) of crayfish have been enhanced with crustacean granules, while bottom food was the lowest. Although the protein content of bottom food was higher (50%) than cichlid (40.34%) and crustacean (44%) foods in our study, low protein feeds (cichlid and crustacea) attained higher growth rates. Similar results were reported in the previous study conducted on Mexican crayfish (*P. llamasii*) by Rodríguez-Serna et al. (2010). The authors formed six experimental feeding groups with different kinds of farm animal feeds (rabbit, turkey, pig, tilapia, shrimp and trout) and they stated that high protein contents were in the trout and shrimp feeds with 43.2 and 38%, respectively. The highest FMW, weight gain (WG) and specific growth rate (SGR) were recorded in the group fed with shrimp feed in this previous study and the authors explained that this situation was possible due to lower stability in the water. Obviously, there are main aspects to take into when formulating the

species-specific feed for ornamental crayfish in order to balance between dietary requirements and stability. Also, Goldblatt et al. (1980) identified that this is a nutrient loss problem caused by leaching in crustaceans according to their slow-eating ability. Furthermore, growth of crayfish is also significantly affected by the culture conditions, type of food and dietary ingredients (Jones et al. 2000). However, Kaldre et al. (2015) declared that the weight and length gain of marbled crayfish (*P. fallax* f. *virginalis*) was higher among crayfish fed with astaxanthin-rich discus feed, whilst Harpaz et al. (1998) stated that the growth performance of the Australian red claw crayfish (*C. quadricarinatus*) was not influenced by dietary carotenoids. These results clearly show that there may be important ingredients in the structure of the formulated feed, apart from the protein ratio. For instance, Erol et al. (2017) fed the 2.5 cm narrow-clawed crayfish (*Astacus leptodactylus*) with a 55% protein ratio of trout feed and the authors recorded the highest total and carapace lengths in this group. But we found the highest FMTL and FMCL in the red swamp crayfish fed with crustacean feed among the S1 groups. This, of course, is related to the effectiveness of the species-specific feed. In addition, the shape and structure of feed are the significant criteria for accessibility in the bottom of the water in

crustaceans. Karadal and Türkmen (2012) reported that Australian red claw crayfish (*C. quadricarinatus*) are easier to reach because granule feed sinks faster than flake and stick forms, and this situation directly affects their growth performance. Consequently, all this demonstrates the importance of considering all the needs of the animal when formulating specific feeds.

Survival is vital in crayfish cultivation because of the cannibalism problem (Taugbøl and Skurdal 1992). Rodríguez-Serna et al. (2010) reported 100% survival rate in 1.16 g Mexican crayfish (*P. llamasii*) fed with six different farm animal feeds. Also, Kaldre et al. (2015) stated 89% and 78% survival for 1.15 and 2.45 g marbled crayfish (*P. fallax* f. *virginialis*) fed with carp and discus feeds, respectively. In our study, the Size 1 group, similar to previous studies, has a lower survival rate (ranging from 59.38-75%) than these mentioned results. The reason for this is thought to be related to species behavior. The survival rate in cultured animals has a complex mechanism that is affected by many variables. Previous studies have revealed that growth, survival and cannibalism of crayfish are impressed by various factors, such as water parameters (temperature, dissolved oxygen, etc.), photoperiod, feeding regime, shelter presence, density, competition and species and size differentiations (Mazlum and Eversole 2005; Farhadi et al. 2014; Yu et al. 2020). For instance, Blank and Figler (1996) demonstrated the interspecific dominance of red swamp crayfish (*P. clarkii*) over white river crayfish (*Procambarus zonangulus*) in shelter competition. It can be said that this situation, which is based on competition between species belonging to the same genus, will directly affect the survival rate of white river crayfish (*P. zonangulus*). Based on this example, it is very important to evaluate factors such as food competition and feeding regime in terms of differences between species. However, in many crustaceans, limited shelter, low availability of food, unstable feeding, high stocking density, species' behaviors not only cause aggressiveness but also increase cheliped injury or loss, and even cannibalism in culture conditions (Elgar and Crespi 1992; Taugbøl and Skurdal 1992; Figiel and Miller 1995; Savolainen et al. 2004; Marshall et al. 2005; Yu et al. 2020). In this study, although there was no statistical difference in the cheliped injury and cannibalism rates, the lowest rates were recorded in the groups fed with crustacean feed in both size classes. As mentioned by Farhadi et al. (2014), food presence and proper feeding are significant for maintaining cannibalism in crayfish species. Furthermore, cannibalism in crayfish is closely related to molting, which is their vulnerable period (Taugbøl and Skurdal 1992; Farhadi et al. 2014) and

it also has been detected during inter- and postmolt stages (Elgar and Crespi 1992). Therefore, as the molting period increases, the rate of cannibalism is expected to decrease, as in the results between two different size classes in this study.

In crustaceans, molting is a part of the growth and development (Ghanawi and Saoud 2012) and is an energy-intensive process (Raviv et al. 2008). Therefore, similar results with growth performance were recorded on the molting frequency of Size 2 red swamp crayfish fed with different commercial feeds used in the study. However, molting frequency of Size 1 crayfish fed with bottom food was found to be higher, although statistically similar to those fed with cichlid feed. This can be explained as an increase in the molting frequency due to the fact that it is more frequent in the early periods (Barki et al. 1997). Fockedeý (2005) declared the intermolt period (IM) may increase or decrease by the quality of food. In our study, lower IMs were noted in CG groups in both size classes. However, Paglianti and Gherardi (2004) reported no significant differences in weight increment at molt (WIM) and length increment at molt (LIM) in red swamp crayfish (*P. clarkii*) fed with different plant and animal derived natural food sources. Although our findings are similar to the results of the previous study, except for the WIM in the S1 groups, it is much higher than the mentioned study. Previous findings provided evidence for the positive effects of species-specific formulated feeds on molting parameters, as in this study.

Coloration is a key feature in the selectivity of an aquarium species in the industry and indirectly displays the value. However, diet is the one of major factors that directly affected to the coloration in finfish and shellfish. In crustaceans, carapace coloration primarily depends on two main characteristics: proper carotenoid type (predominantly astaxanthin) for the species and carotenoid level in the formulated diet (Négre-Sadargues et al. 2000; Kaldre et al. 2015; Wade et al. 2017). In addition, Tanaka (1978) has recommended that decapods should be fed carotenoid-included diets for obstructing the color fading. Commercial company of the crustacean feed used in this study stated that the granules contained nutritious carotenoids offer a varied and balanced feeding. Although the commercial company has not presented the exact ration of the feed, it is clearly seen that it is quite effective on the carapace coloration according to the results obtained in the study. These results point those two sizes of red swamp crayfish could successfully utilize the dietary carotenoids from crustacean feed to enhance the lightness and redness of carapace. Lightness (L^*), redness (a^*) and yellowness (b^*) identified as CIELAB color space are significant parameters to assess the coloration in

crustaceans (Long et al. 2017). In this study, these parameters indicate the same statistical levels between cichlid and crustacean feeds, but higher values in crustacean feed. As it is known that there are various carotenoids in cichlid feed, this can be explained as the fact that the crustacean feed has a more balanced ration. However, these parameters also showed that the bottom food led to the carapace color even paler than the initial color rather than improving it. Although the bottom food contains natural carotenoid sources, including krill, gammarus and squid meals, and *Schizochytrium* sp., *Chlorella* sp. and *Spirulina* sp. algae, this situation is thought to be due to nutrient leaching and that the unsuitable feed stability for crustaceans.

In conclusion, red swamp crayfish showed a great enhancement during the feeding with commercially formulated crustacean feed and positive results were revealed when regarding to meet the demand of both aquaculture and aquarium industries. But it should be noted that it is the most expensive feed used in the study. Since feed is the biggest expense in ornamental fish sector, it is very important to maintain profitability in high-capacity productions. Therefore, the use of the crustacean feed in the final product stage is recommended in terms of accelerating growth performance, improving body coloration and balancing profitability.

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