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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Growth performance, molting frequency and carapace coloration of marbled crab (*Pachygrapsus marmoratus*) in different salinity levels

Mermer yengecin (*Pachygrapsus marmoratus*) farklı tuzluluk seviyelerinde büyüme performansı, kabuk değiştirme frekansı ve karapas renklenmesi

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Abstract: This study was conducted to evaluate the parameters on growth performance, molting frequency and carapace coloration of marbled crab (*Pachygrapsus marmoratus* Fabricius, 1787). Crabs were collected from Urla, İzmir. The experiment was performed in 10 L plastic containers filled with 6 L of seawater at four different salinity levels (5‰, 15‰, 25‰ and 35‰). Ten crabs with an initial mean weight of 0.78±0.03 g were placed in each container with three replicates. Crabs were fed once a day with a commercial diet (46% protein and 18% lipid) for 12 weeks. At the end of the study, the final mean weight (FMW) of the 25‰ group was significantly higher than the 5‰ and 15‰ groups (P<0.05). Specific growth rates (SGR) of the 15‰ and the 35‰ groups and feed conversion ratio (FCR) of the 25‰ group were significantly higher than the 5‰ group (P<0.05). The mean molting frequency (MMF) of the 25‰ group was significantly higher than the 5‰ group (P<0.05). Final lightness (*L**) of the 5‰ and 15‰ were significantly lower than their initials (P<0.05). Final redness (*a**) of the 25‰ group was the highest among the experimental groups (P<0.05). Final yellowness (*b**) of the 25‰ group was significantly higher than the 5‰ and 15‰ groups (P<0.05). According to the results, it is recommended to keep the salinity at 25‰ under marble crab rearing conditions. Further studies are needed to reveal the potential properties of this species in marine aquariums.

Keywords: Crab feeding, survival, growth, pigmentation, container

Öz: Bu çalışma, mermer yengeçlerin (*Pachygrapsus marmoratus* Fabricius, 1787) büyüme performansı, kabuk değiştirme frekansı ve karapas rengine ilişkin bilgileri değerlendirmek için yürütülmüştür. Yengeçler İzmir, Urla'dan toplanmıştır. Deney, dört farklı tuzluluk seviyesinde (‰5, ‰15, ‰25 ve ‰35) 6 L deniz suyu ile doldurulmuş 10 L'lik plastik kaplarda gerçekleştirilmiştir. On yengeç (başlangıç ortalama ağırlıkları 0,78±0,03 g) her bir kaba üç tekrarlı olarak rastgele yerleştirilmiştir. Yengeçler, 12 hafta boyunca ticari bir yemle (%46 protein ve %18 yağ) günde bir kez doymaya yakın beslenmişlerdir. Deneme sonunda ‰25 grubunun son ortalama ağırlığı, ‰5 ve ‰15 gruplarından anlamlı derecede yüksektir (P<0,05). ‰15 ve ‰35 gruplarının spesifik büyüme oranları ve ‰25 grubunun yem dönüşüm oranı ‰5 grubundan önemli ölçüde yüksektir (P<0,05). ‰25 grubunun ortalama kabuk değiştirme frekansı, ‰5 grubundan önemli ölçüde yüksek bulunmuştur (P<0,05). ‰5 ve ‰15 gruplarının son parlaklığı (*L**) ilk değerlerine göre önemli ölçüde düşmüştür (P<0,05). ‰25 grubunun son kırmızılığı (*a**) deney grupları arasında en yüksektir ve bu grubun son sarılığının (*b**), ‰5 ve ‰15 gruplarından belirgin şekilde daha yüksek olduğu ortaya konmuştur (P<0,05). Bulgulara göre, mermer yengecinin büyütme koşullarında tuzluluğun ‰25'te tutulması tavsiye edilmektedir. Bu türün deniz akvaryumlarındaki potansiyel özelliklerini ortaya koyabilmek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Yengeç besleme, yaşama oranı, büyüme, pigmentasyon, konteyner

INTRODUCTION

Aquarium enthusiasts have more interested in marine aquariums with the enhancements in aquarium technology. Approximately 1500 fish species have been considered pets in the marine aquarium sector and during the last decade, about 20-24 million fish have been sold annually (Türkmen et al., 2011). Moreover, not only the popularity of fish but also decapod species such as shrimps, lobsters and crabs have become more attention in this sector (Karadal, 2018). Among them, the crabs are preferred in marine aquariums due to their strange appearances. Brachyuran and Anomuran crabs were amongst the most commonly traded decapod species in the saltwater aquarium sector (Penha-Lopes et al., 2005).

The feeding behavior of an organism is very important in terms of determining its living conditions, nutritional status and reproductive activities (Hughes, 1993). Crabs are generally classified as omnivores or detritivores based on their diet. For this reason, they have a key role in the food chain of many ecosystems in nature (Penha-Lopes et al., 2009). Brachyuran crabs' diet mainly consists of limpets, barnacles, mussels and algae (Cannicci et al., 2002). However, artificial feeds can also be used to keep these animals in artificial environments such as aquariums. Hence, it is very important to examine the interactions with the basic water parameters (temperature, salinity, pH, etc.) in the experimental environment where the crabs will be fed with formulated feeds (Cadman and

Weinstein, 1988). Pedapoli and Ramadu (2014) stated the water quality parameters directly impact feed utilization, growth performance, health and survival of crab species. Since the variability of these abiotic parameters can adversely affect aquaculture activities, the importance of studies conducted under controlled conditions has increased. For instance, salinity is one of the most important abiotic parameters affecting larval development, growth, survival, distribution and food stress of crab species (Nurdiani and Zeng, 2007; Bianchini et al., 2008). The juvenile and adult crabs have a wide tolerance of salinity requirements, while their larvae can be vulnerable to varying salinity conditions (Diele and Simith, 2006). While the influences of salinity on various crab species in rearing conditions have been documented by previous studies (i.e., Anger et al., 1990; Anger, 1991; Bas and Spivak, 2000; Jantrarotai et al., 2002; Nurdiani and Zeng, 2007; Ikhwanuddin et al., 2012; Long et al., 2019), only the survival rate and restricted growth of the marbled crab (*P. marmoratus*) at five different salinities have been investigated (Karadal, 2018). In the previous study (Karadal, 2018), the crabs were starved for 140 days in the dry area and five salinities (0‰, 5‰, 15‰, 25‰ and 35‰). No crabs survived for the first 2 weeks in the dry area and with 0‰ salinity. Hence, dry and 0‰ salinity groups were eliminated in this study and according to these results, it was considered to conduct the study at four different salinity levels.

Since the culture of the species traded in the marine aquarium sector is not common, approximately 95% of them are collected from nature (Monticini, 2010; Domínguez and Botella, 2014). Therefore, it is important to consider potential alternatives to these in order to reduce the destruction of the natural populations of popular species. Marbled crab (*Pachygrapsus marmoratus*) is a brachyuran decapod found in the intertidal rocky regions of the Black Sea and Mediterranean (Flores and Paula, 2002; Aydın et al., 2014; Deli et al., 2015). Karadal and Öndes (2018) suggested that there are 35 crab species at the cost of Turkey that can be considered suitable decapods in marine aquariums. Although there are some studies on the natural diets and feeding habits of marbled crab (Cannicci et al., 2002; Cannicci et al., 2007; Silva et al., 2009), no comprehensive feeding study has been found in laboratory conditions. In this study, growth performance, molting frequency and carapace coloration of marbled crab at four different salinity levels were investigated. With this study, the responses of marble crab in terms of growth, molting and coloration parameters at different salinities under laboratory conditions will be recorded and it will be evaluated whether they are suitable for keeping in marine aquariums.

MATERIAL AND METHODS

Collection of crabs

Marbled crabs (*Pachygrapsus marmoratus*) were caught from the rocky shores by hand in Urla, İzmir (38° 22' 17'' N, 26° 45' 44'' E) in June 2018. Crabs, which had same molt stage (hard carapace) and did not have limb loss, were selected for the experiment. A total of 120 crabs were transferred to plastic containers in the Education & Research Unit of Faculty of Fisheries, İzmir Kâtip Çelebi University, İzmir, Turkey.

Rearing systems

The experiment was performed in 10 L plastic containers filled with 6 L of marine water with different salinity levels. Ten crabs (initial mean weights of 0.78±0.03 g) were randomly placed in each container with three replicates (Figure 1). Each container contained PVC pipes with a diameter of 4 cm as shelter and a stone placed in the container. The water temperature was maintained at 18.4±0.3ºC. Salinity, temperature, dissolved oxygen and pH (Hach HQ40D Portable Multi Meter) were checked in each container daily. Dissolved oxygen was 9.27±0.04 ppm, pH was 7.65±0.06 and photoperiod was held at 12:12 (light/dark). The water in all containers was changed at a ratio of 20% once a week.

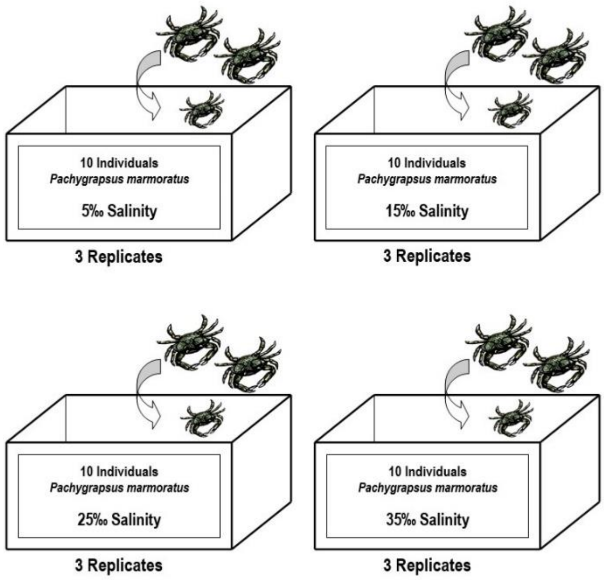

Figure 1. Design of the experimental study

Experimental design and feeding

Four different salinity levels (5‰, 15‰, 25‰ and 35‰) were used to determine the growth performance, molting frequency and carapace coloration of marbled crab. Animals were kept and fed (with the same feed used in the trial) in the rearing system for two weeks for acclimation. In the trial, crabs were fed once a day with 1 mm commercial gilthead seabream diet (46% protein and 18% lipid) and uneaten food was removed after 1.5 h from feeding by siphoning and weighed. At the beginning of the experiment, the same weight of commercial feeds was weighed for each plastic container and was stocked in small boxes and the crabs were fed with these feeds during the trial. The feed intake (FI) of the crabs was recorded by weighing the feeds in these boxes during the biweekly measurements. The experiment was carried out in 12 plastic containers in three replications for 12 weeks.

Evaluation of growth performance

Weight (g) and length (cm) measurements were carried out biweekly during the experiment. All animals were dried with a paper towel to remove the water before the measurement process. Crab weights were individually recorded with an electronic compact scale (SF-400D, precision of ±0.01 g). Carapace length (CL; length of the carapace along the midline) and carapace width (CW; width of the frontal region of the carapace) were measured with the digital caliper. Dead animals were recorded during the experiment, and their survival rates were determined at the end of the trial. Growth performance such as feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR) was calculated according to the following formulas:

FI = Average of the total feed given to each experimental group during the study

FCR = Feed intake / Weight gain (where Weight gain was calculated as Final mean weight - Initial mean weight),

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

SR (%) = 100 x (Number of total crab - Number of dead crab) / Number of total crab.

Monitoring of molting frequency

The crabs were checked daily and shells in the containers were recorded for determining the numbers of molts in the experimental groups. According to the recorded data, the mean molting frequency, n: the number of molted individuals; (MMF) = $[(n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + \ldots\ldots + (n_k \times k)]$ / total number of crab (Chen and Chen, 2003).

Carapace coloration measurement

Color measurements were taken from all crabs at the beginning and end of the experiment in order to obtain coloration data. Measurements were taken from the carapace region of the crabs on a flat surface with a colorimeter (Color Muse, Variable Inc., Tennessee, USA) (Dang et al., 2021). The measurements were achieved on top surface (4 mm) of carapace of each crab. The CIELAB color space parameters (CIE, 1976) were recorded as lightness (where 100% stands white and 0% stands black), redness (where +a* stands for red and −a* stands for green) and 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 (±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

The growth performance of marbled crab reared in different salinity levels is given in Table 1. Final mean weight (FMW) of the 25‰ group was significantly higher than the 5‰ and 15‰ groups ($P<0.05$). Final mean carapace length of the 5‰ groups was lower than 25‰ group ($P<0.05$). The highest feed intake was obtained in the 25‰ group ($P<0.05$). Specific growth rates (SGR) of the 25‰ and the 35‰ groups were significantly higher than the 5‰ group ($P<0.05$). Feed conversion ratio (FCR) of the 25‰ group was statistically higher than the 5‰ group ($P<0.05$).

Mean weights (MW) and mean carapace lengths (MCL) during 12 weeks and final survival rates (SR) of marbled crab fed on different salinities are shown in Figures 2, 3 and 4. The MW and MCL of the 5‰ group were lower than the others. The SR of the 15‰ (93.33%) and 25‰ (90%) groups were significantly higher than the 5‰ (63.33%) group ($P<0.05$).

Table 1. Growth performance of marbled crab reared in saltwater with different salinity levels for 12 weeks

	5‰	15‰	25‰	35‰
Initial mean weight (g)	0.79±0.01	0.85±0.03	0.77±0.02	0.72±0.02
Final mean weight (g)	1.79±0.06^{a}	2.25±0.09^{bc}	2.54±0.14^{d}	2.41±0.08^{cd}
Initial mean carapace length (cm)	1.08±0.03	1.14±0.02	1.08±0.02	1.05±0.01
Final mean carapace length (cm)	1.49±0.04^{a}	1.63±0.07^{ab}	1.74±0.06^{b}	1.68±0.05^{ab}
Feed intake (g)	0.75±0.03^{a}	1.21±0.08^{b}	1.64±0.11^{d}	1.47±0.09^{c}
Specific growth rate (%/day)	0.97±0.12^{a}	1.16±0.08^{ab}	1.42±0.08^{b}	1.44±0.10^{b}
Feed conversion ratio	0.75±0.07^{a}	0.86±0.11^{ab}	0.93±0.05^{b}	0.87±0.09^{ab}

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

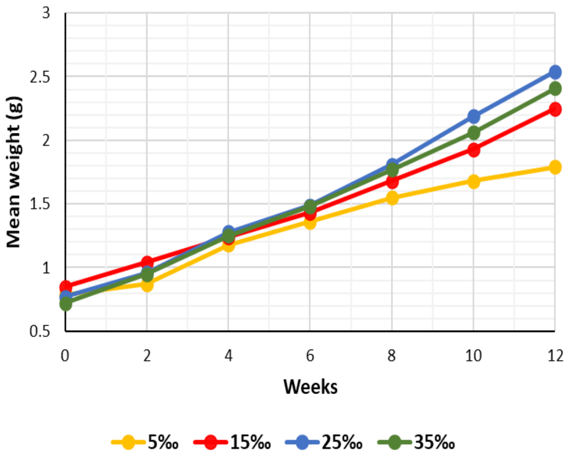

Figure 2. Mean weights of marbled crab during 12 weeks of feeding on different salinities

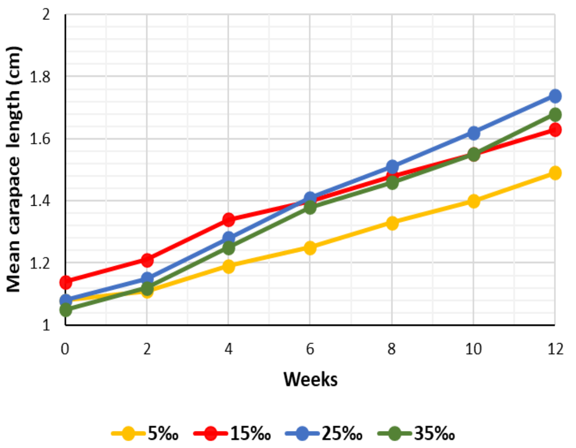

Figure 3. Mean carapace lengths of marbled crab during 12 weeks of feeding on different salinities

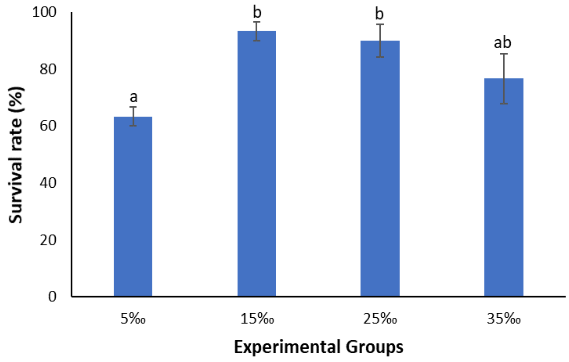

Figure 4. Survival rates of marbled crab fed on different salinities

Molting parameters of marbled crab reared in different salinities are presented in Table 2. Mean molting frequency (MMF) of the 25‰ group and mean molting number (MMN) of the 15‰ and 25‰ groups were significantly higher than the 5‰ group (P<0.05). According to the number of molts, the highest number of individuals in terms of groups was recorded as 8 (2 times) in the 5‰, 12 (3 times) in the 15‰, 11 (4 times) in the 25‰ and 7 (2, 3 and 4 times) in the 35‰ groups.

Carapace coloration parameters of marbled crab in different salinity levels are listed in Table 3. There was no significant difference in the final lightness (*L**) of the experimental salinity groups (P>0.05). Final lightness (*L**) of the 5‰ and 15‰ were significantly lower than their initials (P<0.05). Final redness (*a**) of the 25‰ group was the highest among the experimental groups (P<0.05). No significant difference was noted between the initial and final parameters of the redness (P>0.05). Final yellowness (*b**) of the 25‰ group was markedly higher than the 5‰ and 15‰ groups (P<0.05). Initial yellowness (*b**) of the 25‰ and final yellowness (*b**) of the 5‰ and 15‰ groups were significantly lower than their final and initial parameters, respectively (P<0.05).

Table 2. Molting parameters of marbled crab reared in saltwater with different salinity levels for 12 weeks

		5‰	15‰	25‰	35‰
Crab number (*n*)		19	28	27	23
Number of molting	**1**	4	4	1	2
	2	8	8	5	7
	3	6	12	10	7
	4	1	4	11	7
Mean molting frequency		2.19±0.21[a]	2.58±0.11[ab]	3.16±0.13[b]	2.80±0.18[ab]
Mean number of molting		14.00±2.08[a]	24.00±0.58[b]	28.33±1.20[b]	21.67±3.33[ab]

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

Table 3. Carapace coloration of marbled crab reared in saltwater with different salinity levels for 12 weeks

	5‰	15‰	25‰	35‰
Initial lightness (*L)**	42.10±0.21^{B}	40.66±0.33^{B}	41.15±0.16	43.07±0.20
Final lightness (*L)**	36.25±0.54^{A}	33.78±0.39^{A}	40.47±0.25	41.83±0.49
Initial redness (*a)**	8.02±0.15	7.88±0.12	8.18±0.09	7.93±0.11
Final redness (*a)**	7.96±0.13^{a}	8.11±0.07^{a}	8.35±0.17^{b}	8.09±0.05^{a}
Initial yellowness (*b)**	15.67±0.21^{B}	16.13±0.32^{B}	15.37±0.19^{A}	15.88±0.25
Final yellowness (*b)**	10.26±0.18$^{a, A}$	12.37±0.41$^{a, A}$	18.93±0.65$^{b, B}$	15.98±0.32^{ab}

Different lowercase and uppercase letters in the same line and the same column indicate statistically significant differences (P<0.05) among the groups, respectively.

DISCUSSION

The present study showed that marbled crabs (*Pachygrapsus marmoratus*) fed in different salinities survived at 15‰ and 25‰ with higher levels. However, the final mean weight (FMW), feed conversion ratio (FCR) and mean molting frequency (MMF) were enhanced at the 25‰, while specific growth rates (SGR) of the 15‰ and the 35‰ groups were higher. In addition, the final redness (*a**) and final yellowness (*b**) of the crab's carapace were improved at the 25‰ salinity level.

Salinity has important in the growth and nutrition of crab species (Fisher, 1999; Nurdiani and Zeng, 2007; Castejón et al., 2015). Fisher (1999) declared a negative correlation between growth performance and salinity of blue crab (*C. sapidus*). In this study, growth depending on optimum salinity level was determined as 25‰ in marbled crabs and they showed a lower growth rate at the further salinity level (35‰). But, as an important issue on this topic, salinity is considered a vulnerable environmental factor for the larval development of crab species and it is possible to say that previous studies focused on this way (Bas and Spivak, 2000; Baylon and Suzuki, 2007; Nurdiani and Zeng, 2007; Ikhwanuddin et al., 2012). For instance, Jantrarotai et al. (2002) stated that the survival rates of orange mud crab (*Scylla olivacea*) to the stage of megalopa from zoea were recorded as 13.16, 22.19, 8.25 and 7.08 percent at 28‰, 30‰, 32‰ and 34‰, respectively. Larvae advanced to the next stage (megalopa) with higher survival at 30‰ than other salinity levels. Therefore, in crustaceans, the survival rate could have been affected by the feeding due to their cannibalistic behaviors. However, salinity tolerance is higher in larger sizes of crabs. Rahi et al. (2020) recorded that even survival at 0‰ in the orange mud crab (*S. olivacea*). Nevertheless, the authors reported that crabs fed at salinity levels of 10‰ and 20‰ had higher FMWs. In the present study, the FMW of the crabs reared at the 25‰ was markedly higher than that of the 5‰ and 15‰ salinity levels. This can be explained by enhanced growth performance at the optimum salinity level for the species. The SGR and FCR results have proved this situation. Both parameters were enhanced by the optimum salinity level (25‰).

In previous research conducted by Cházaro-Olvera and Peterson (2004), the authors studied the different salinity levels (5‰, 15‰ and 25‰) on two *Callinectes* species (*C. rathbunae* and *C. sapidus*) and they stated no significance in molting period of *C. rathbunae*, while the duration was lessened with the further salinity levels in *C. sapidus*. Though, it can be said that the intermolt duration of blue crab (*C. sapidus*) was affected by salinity. A similar result was reported in a study (salinity levels of 5‰, 10‰, 20‰, 30‰ and 40‰) carried out with mud crab (*Scylla paramamosain*) (Gong et al., 2015) as in the *C. sapidus*. Ruscoe et al. (2004) noted a wide range of salinity tolerance in intermolt duration of the 18.43 mg crablets of mud crab (*S. serrata*) and they advised that these resistant features were considered the species a potential nominee for cultivation. In contrast, an ornamental marine crab species, *Stenorhynchus seticornis*, was reared at 30, 35 and 40‰ salinity levels, but the authors declared the salinity affected the percentage survival of the zoeal stages (Hernández et al., 2012). In the present study, marbled crabs (*P. marmoratus*) have successfully molted even at low salinity levels. For this reason, it is thought that this species can temporarily adapt to extreme salinities in aquarium conditions.

The marbled crab is one of the common intertidal decapods in the Mediterranean Basin (Flores and Paula, 2002), which can be considered a potential aquarium animal due to its high population and its attractive colors of the body (Karadal and Öndes, 2018). The colors of the crab show considerable variation from violet brown to black with a yellowish-brown with marbled form and there is also great patterning in crab species (Ingle, 1997; Caro, 2018). Salinity-related coloration changes in crabs are a study limited. In previous research, McGaw and Naylor (1992) stated a shore crab (*Carcinus maenas*) shows a range of carapace coloration from green to orange through its molting period and orange crabs are less tolerant to salinity decreases over this duration. It is known that the color difference due to this molting period is a natural physiological change, and the difference in salinity tolerance between two colors can be explained in relation to the vulnerable period in the duration of molting. In this study, unlike the previous research, no major color change was observed in the marbled crabs' inter- or during the molting period. However, a statistically significant color change was noted, which did not affect the visual coloration regarding the different salinity levels. It can be said that this statistical color difference is not a natural change and is a physiological response of the animal depending on external influences i.e., water parameters, lightning. Furthermore, Caro (2018) underlined various factors such as starvation or salinity are related to carapace coloration in several crabs. This raises the issue that changes in other water parameters, not only depending on salinity, may be effective on carapace coloration of the marble crabs, and it is recommended to increase studies on carapace coloration in crabs due to external factors.

CONCLUSION

According to the growth, molting and coloration data evaluated in the study, it was revealed that these parameters increased up to 25‰, which was recorded as the optimum salinity level, and started to decrease at the level of 35‰ salinity. In conclusion, it is recommended to maintain the salinity at 25‰ in the rearing conditions of the marbled crab. While growth and molting are significant parameters in carcinoculture, coloration is an important criterion for potentially identifiable species in aquarium fisheries. Therefore, rearing studies that can improve coloration along with growth are of great importance. Further studies are needed to reveal the potential properties of this species in marine aquariums.

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AUTHORSHIP CONTRIBUTION

Onur Karadal: Conceptualization, Investigation, Data curation, Writing- original draft preparation, reviewing and editing.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

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

DATA AVAILABILITY

All data generated and analyzed during this study are presented in this article.

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