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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Survival rate and restricted growth of marbled crab (*Pachygrapsus marmoratus*) in different salinity levels

Mermer yengecinin (*Pachygrapsus marmoratus*) farklı tuzluluk seviyelerinde yaşama oranı ve sınırlı büyümesi

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Abstract: The semi-terrestrial marbled crab (*Pachygrapsus marmoratus* Fabricius, 1787) is one of the most common species in the intertidal belt of rocky shores of the Mediterranean Sea. This study aimed to understand survival and restricted growth of marbled crab in different salinity levels (0, 5, 15, 25, 35‰) and dry area (D). Crabs were collected from rocky shores by hand in İzmir in January 2018. Specimens (initial mean weights of 0.82±0.01 g and *n*=180) were randomly distributed to 10-L aquariums with three replicates and they were not fed during the experiment. Results indicated that the survival times of crabs showed significant differences in salinity levels (P<0.001). All the individuals in the dry area and 0‰ salinity level died within the first 2 months. For the other salinity levels, all specific growth rate values decreased at minus on 84th day. Forty percent of crabs in aquariums that represented 15‰ salinity level were survived until the 126th day and the experiment was ended in the 140th day when the mortality of the last crab (0.73 g) occurred. In conclusion, the optimal salinity level for the survival and restricted growth of *P. marmoratus* was determined as 15‰ and 25‰, respectively. Future surveys would need to investigate the effects of different food types on survival and growth of this potential ornamental crab species of marine aquariums.

Keywords: *Pachygrapsus marmoratus*, salinity, survival rate, starvation, aquarium

Öz: Yarı karasal bir tür olan mermer yengeci (*Pachygrapsus marmoratus* Fabricius, 1787), Akdeniz'in kayalık kıyılarında bulunan gelgit zonundaki en yaygın türlerden biridir. Bu çalışma, mermer yengecinin farklı tuzluluk seviyelerinde (‰0, 5, 15, 25, 35) ve kuru ortamda (K) hayatta kalmasının ve sınırlı büyümesinin anlaşılmasını amaçlamıştır. Yengeçler, Ocak 2018'de İzmir'deki kayalık kıyılardan el ile toplanmıştır. Bireyler (ilk ortalama ağırlık 0.82±0.01 g ve *n*=180), üç replikat olmak üzere 10-L akvaryumlara rastgele dağıtılmış ve deney sırasında beslenmemişlerdir. Bulgular, yengeçlerin hayatta kalma sürelerinin tuzluluk seviyelerine göre anlamlı farklılık gösterdiğini belirtmiştir (P<0,001). Kuru ortamda ve ‰ 0 tuzluluk seviyesindeki tüm bireyler ilk 2 ay içinde ölmüştür. Diğer tuzluluk seviyeleri için, tüm spesifik büyüme oranları, 84. günde eksiye düşmüştür. Akvaryumlarda ‰15 tuzluluk seviyesindeki yengeçlerin yüzde kırkı 126. güne kadar hayatta kalmış olup, çalışma son yengeç (0,73 g) ölümünün meydana geldiği 140. günde sona ermiştir. Sonuç olarak, *P. marmoratus*'un hayatta kalma ve kısıtlı büyümesi için optimal tuzluluk düzeyi sırasıyla ‰15 ve ‰25 olarak belirlenmiştir. Gelecek çalışmalar, deniz akvaryumlarının bu potansiyel süs yengecinde hayatta kalma ve büyüme üzerine farklı besin türlerinin etkilerini araştırmalıdır.

Anahtar kelimeler: *Pachygrapsus marmoratus*, salinite, hayatta kalma oranı, açlık, akvaryum

INTRODUCTION

With the development of the aquarium systems, the aquarists have interested in marine aquariums. The first marine aquarium was established in 1846 with only coral and seaweed species (Kisling, 2000). Personal saltwater aquariums have spread in the early 1950's (Vitko, 2004). Currently, 10% of pet ownership in the U.S. have freshwater and 2% of have saltwater aquariums (APPA, 2018). About 1500 fish species have used in marine aquariums and 20-24 million fish have sold per year during the last decade (Türkmen et al., 2011). Besides the fish, invertebrate species such as shrimps and crabs have become more popular in marine aquariums (Calado et al., 2003a). The most commonly traded decapod species in the marine aquarium sector were remarked as Caridean shrimps. Brachyuran and Anomuran crabs were following this group (Penha-Lopes et al., 2005). It has been reported that four crab species belonging to Gecarcinidae and Sesarmidae families were sold in the aquarium sector in Turkey (Türkmen and Karadal, 2012a). Karadal and Öndes (2018) suggested that 35 crab species were found in the costs of Turkey, which may be considered as suitable decapods in marine aquariums.

The larval development of some crab species such as *Pachygrapsus marmoratus*, *Mithraculus forceps*, *Uca tangeri*, *Pisa tetraodon* (Rodríguez and Jones, 1993; Rodríguez, 1997; Cuesta and Rodríguez, 2000; Penha-Lopes et al., 2005) were investigated in the laboratory. Calado et al. (2003b; 2007; 2008) also studied the culture and rearing system designs of marine ornamental crabs. Effects of some physical parameters (temperature, light intensity, dissolved oxygen) on crab species were reported in previous studies (Gardner and Maguire, 1998; Hamasaki, 2003; Paschke et al., 2010) in the controlled conditions. In addition, feeding characteristics and starvation were examined on some freshwater and marine crabs, including *Eriocheir sinensis* (Wen et al., 2006), *Perisesarma bidens* (Türkmen and Karadal, 2012b), *M. forceps* (Penha-Lopes et al., 2006; Figueiredo et al., 2008), *Scylla serrata* (Suprayudi et al., 2002; Ruscoe et al., 2004), and *Portunus pelagicus* (Redzuari et al., 2012).

Many studies indicated that some biological and ecological characteristics of crabs such as, survival (Romano and Zeng, 2006; Nurdiani and Zeng, 2007), geographical distribution (Bryars and Havenhand, 2006), gonadal development (Fisher, 1999; Long et al., 2017), growth (Castejón et al., 2015), oxygen consumption (Winch and Hodgson, 2007), feeding behaviour (Shentu et al., 2015), and limb regeneration (Stueckle et al., 2009) were influenced by salinity. In addition to the impacts of salinity, some studies noticed that the combined effects of salinity and temperature on the development of crab species (Blaszkowski and Moreira, 1986; Paula et al., 2003).

Pachygrapsus marmoratus, commonly known as the marbled rock crab or marbled crab, which is one of the most common grapsid species in the intertidal belt of rocky shores throughout the Mediterranean Sea, Black Sea and North-eastern Atlantic coasts of Southern Europe (Flores and Paula, 2002a; Dauvin, 2012; Vinagre et al., 2012; Deli et al., 2015). This semi-terrestrial crab cannot be considered an opportunistic feeder and its diet mainly consists of limpets, barnacles, mussels, and algae (Cannicci et al., 2002; Silva et al., 2009).

Previous studies on marbled crab were related to the distribution and population structure (Cannicci et al., 1999; Flores and Paula, 2002a; Silva et al., 2009; Aydın et al., 2014; Arab et al., 2015; Sumer et al., 2016), relative growth (Protopapas et al., 2007), feeding habits (Cannicci et al., 2002; Cannicci et al., 2007; Silva et al., 2009) and reproductive ecology (Flores and Paula, 2002b). However, this species has been little investigated in the tank or aquarium to date (Sciberras and Schembri, 2008; Madeira et al., 2012; Coquereau et al., 2016). There has been no comprehensive study on the survival rates without food and salinity tolerances of this crab species. The first objective of the present study was to compare the survival times of marbled crabs in different salinity levels without feeding in the aquarium. The second objective was to determine the restricted growth rate of marbled crabs at the aquarium conditions.

MATERIAL AND METHODS

Collection of crabs and experimental conditions

Marbled crabs (*Pachygrapsus marmoratus*) were collected from rocky shores by hand in Urla, İzmir (38° 22′ 17″ N, 26° 45′ 44″ E) in January 2018. Crabs, which had same moult stage (hard carapace) and did not have limb loss, were selected for the experiment. A total of 180 crabs were transferred to aquariums in the Education & Research Unit of Faculty of Fisheries, İzmir Kâtip Çelebi University, Turkey. The experiment was performed in 10 L plastic aquariums filled with 6 L of marine water with different salinity levels. Ten crabs (initial mean weights of 0.82±0.01 g) were randomly placed per each aquarium with three replicates (Figure 1). Five different salinity levels (0, 5, 15, 25, 35‰) and dry area (D) were compared to assess information on survival times and restricted growth. Each aquarium contained 4-cm-diameter PVC pipes as the refuge and a stone, which was placed in the center of the aquarium. The water temperature of aquariums was maintained as 17.6±0.4ºC (same temperature value of the sampling area). Salinity, temperature, dissolved oxygen and pH (Hach HQ40D Portable Multi Meter) were checked in each aquarium daily. Dissolved oxygen was 9.53±0.01 ppm, pH was 8.28±0.02 and photoperiod was held at 12:12 (light/dark). The water in all aquariums was changed once a week with the ratio of 20%.

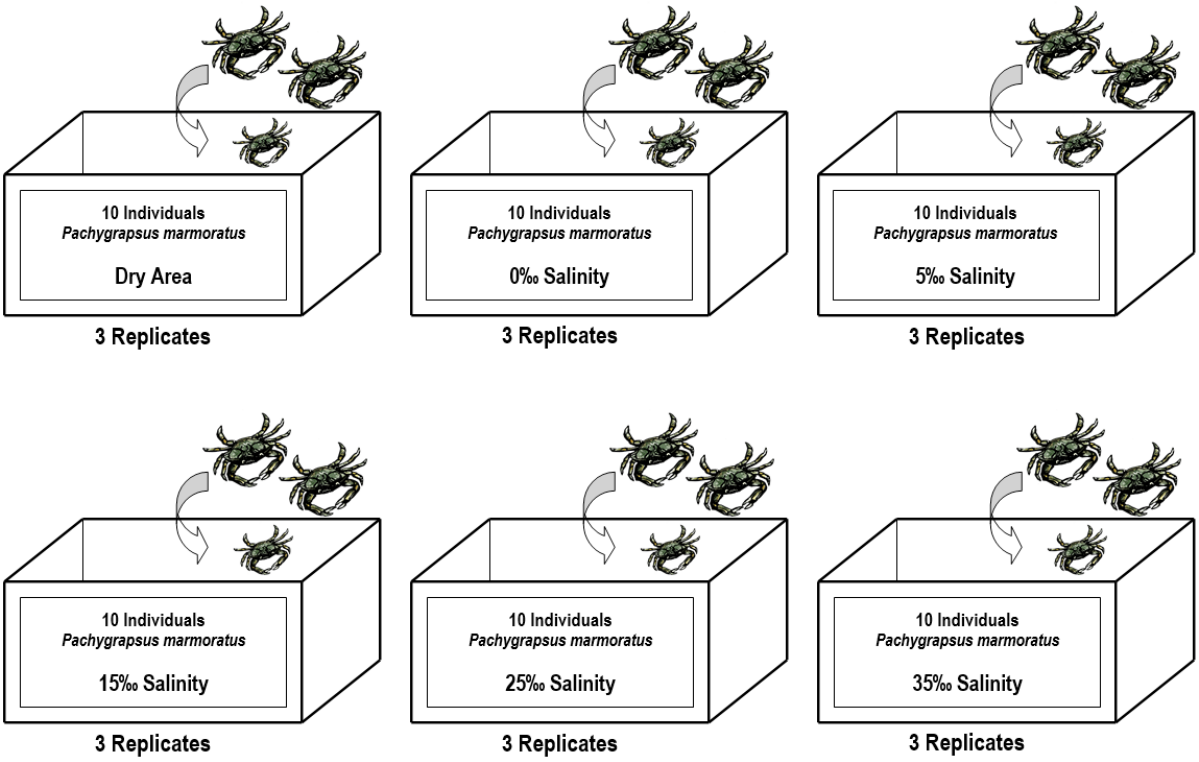

Figure 1. Design of the experimental study

Evaluation of restricted growth and survival rate

Weight and length measurements were carried out biweekly during 140-days experiment. All animals were dried with a paper towel to remove water before 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 vernier caliper. All aquariums were checked daily and molting, weight, carapace length and carapace width of dead crabs were noted. The specific growth rate (SGR) was calculated as following equation: SGR = 100 x [(*Ln* final crab weight) - (*Ln* initial crab weight)] / experimental days.

Data analysis

All data sets were examined to verify normality, independence, and homogeneity of variance. All data were subjected to one-way analysis of variance (ANOVA) when the interaction between the factors is found differences, Duncan's multiple range test was used to rank groups using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) software (Zar, 1999). All data were presented as the "mean±standard error" from all replicates.

RESULTS

The lowest survival rates of crabs were observed in the aquariums without water (Figure 2). The survival rate of crabs with 0‰ salinity level was lower than other salinity levels (5%, 15‰, 25‰, and 35‰). Fifty percent of crabs in aquariums at the salinity levels of 15‰ and 25‰ were survived more than 100 days (Figure 2).

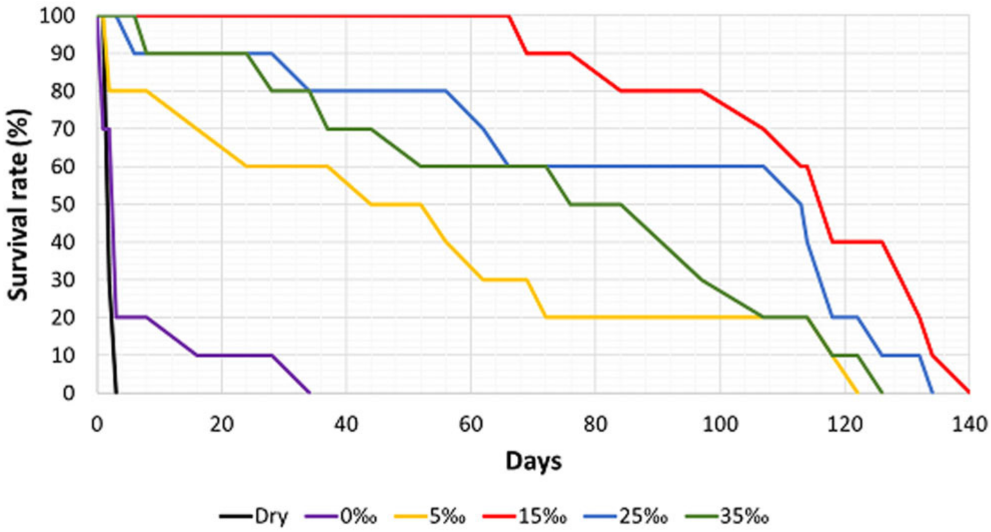

Figure 2. Survival rates (%) of marbled crabs during 140 days period in different salinity levels

The marbled crabs survived 2.0±0.5 days at 17.6 °C without water and food. Our results indicated that the highest survival time was determined as 140 days at 15‰ salinity. Moreover, 25‰, 35‰ and 5‰ salinity levels exhibited longer survival times than the salinity level of 0‰ (Figure 3). The survival times of crabs showed significant differences in salinity levels (ANOVA, P<0.001, F= 20.061).

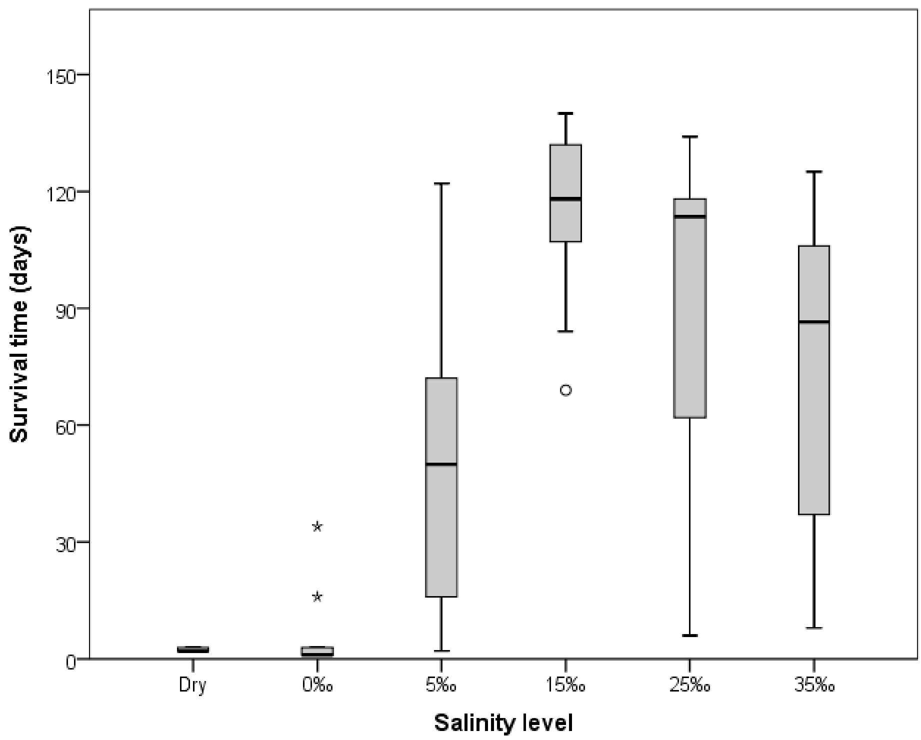

Figure 3. Survival time (days) of marbled crabs in different salinity levels

Weight and growth changes of marbled crabs in different salinities during 140-days of the experiment were detailed in Table 1. The continuous data were not presented in D, 0‰, 5‰, 25‰ and 35‰ groups because the mortalities were observed throughout the trial. On the 14th day, mean weights (MW) and specific growth rates (SGR) of 25‰ were significantly different from 5‰ (P<0.05). MW of 25‰ was different from 5‰ and the lowest SGR was found in 5‰ group on the 28th day (P<0.05). The highest and the lowest MW were observed in 25‰ and 5‰ groups, respectively (P<0.05) and no statistical differences was found in SGR on 42nd and 56th days (P>0.05). The first minus growth rate was observed in 5‰ group on the 56th day. MW of 25‰ was different from 5‰ (P<0.05) and SGR of 25‰ and 35‰ groups were decreased to minus on the 70th day. All SGR values were seen at minus and MW of 25‰ was different from 5‰ on the 84th day (P<0.05). MWs of 15‰ and 25‰ were significantly different from 5‰ (P<0.05) and no differences was observed in SGR (P>0.05) on the 98th day. Only three groups (5‰, 15‰ and 25‰) survived on 112nd day and MW of 25‰ was different from 5‰ (P<0.05). Four individuals of 15‰ lived until 126th day and last individual (0.73 g) died on the 140th day.

Table 1. Mean weights (MW, g) and specific growth rates (SGR, %/day) of the starved marbled crabs in different salinity levels during 140-days

Days		D	0‰	5‰	15‰	25‰	35‰
0	MW	0.82±0.01	0.82±0.01	0.81±0.01	0.82±0.01	0.82±0.02	0.81±0.01
14	MW			0.81±0.01[a]	0.84±0.01[ab]	0.88±0.02[b]	0.84±0.01[ab]
	SGR			-0.03±0.09[a]	0.21±0.12[ab]	0.50±0.05[b]	0.23±0.10[ab]
28	MW			0.80±0.01[a]	0.86±0.01[ab]	0.91±0.03[b]	0.86±0.01[ab]
	SGR			-0.09±0.03[a]	0.11±0.01[b]	0.23±0.04[b]	0.18±0.02[b]
42	MW			0.81±0.01[a]	0.87±0.01[b]	0.93±0.02[c]	0.88±0.01[b]
	SGR			0.06±0.09	0.12±0.02	0.22±0.11	0.12±0.01
56	MW			0.79±0.02[a]	0.87±0.01[b]	0.94±0.02[c]	0.88±0.01[b]
	SGR			-0.14±0.09	0.02±0.00	0.03±0.01	0.04±0.01
70	MW			0.72±0.09[a]	0.88±0.01[ab]	0.91±0.01[b]	0.87±0.01[ab]
	SGR			-0.76±0.72	0.04±0.03	-0.21±0.10	-0.07±0.02
84	MW			0.69±0.09[a]	0.85±0.01[ab]	0.90±0.01[b]	0.87±0.01[ab]
	SGR			-0.26±0.08	-0.23±0.04	-0.07±0.04	-0.07±0.01
98	MW			0.68±0.07[a]	0.83±0.01[b]	0.90±0.02[b]	0.83±0.01[ab]
	SGR			-0.08±0.20	-0.14±0.02	-0.01±0.20	-0.34±0.01
112	MW			0.68±0.05[a]	0.81±0.01[ab]	0.88±0.04[b]	
	SGR			-0.03±0.26	-0.24±0.01	-0.21±0.15	
126	MW				0.78±0.01		
	SGR				-0.26±0.04		
140	FW				0.73		

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

The mean weights of marbled crabs tended to reduce on different days (Figure 4). Groups of D and 0‰ did not live until the 14th days. Mean weights showed a decrease for 5‰ on 42nd day, for 25‰ and 35‰ on the 56th day and for 15‰ on the 70th day. In addition, moults were observed on 27th, 42nd and 93rd days (one individual for each day) in the 25‰ group.

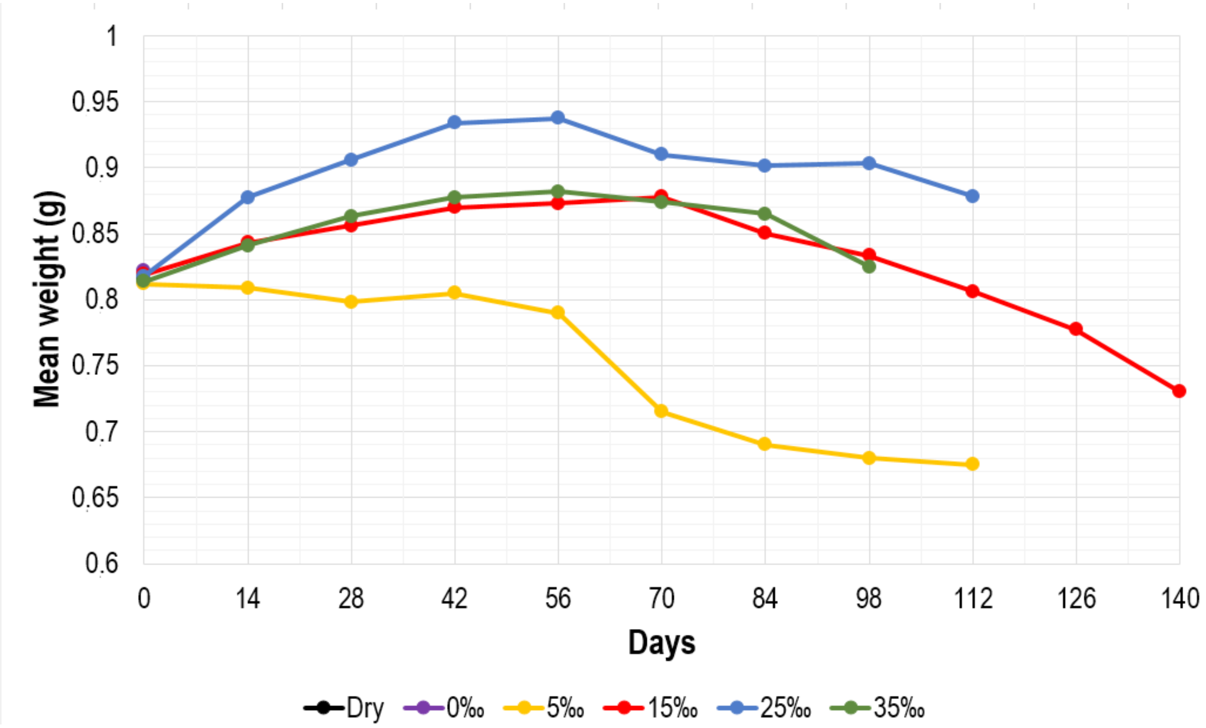

Figure 4. Changes of average body weights (g) of marbled crabs during 140 days experiment

The data of the carapace lengths (CL) and carapace widths (CW) of the experimental groups were given in Table 2. The final mean carapace length (FMCL) and final mean carapace width (FMCW) of 25‰ showed the highest value among all groups (P<0.05). FMCL of 15‰ was significantly higher than D, 0‰, 5‰ and 35‰ groups (P<0.05). FMCW of 5‰ was significantly lower than 0‰, 15‰, 25‰ and 35‰ groups (P<0.05).

Table 2. Initial mean carapace lengths (IMCL, mm), final mean carapace lengths (FMCL, mm), initial mean carapace widths (IMCW, mm) and final mean carapace widths (FMCW, mm) of the starved marbled crabs in different salinity levels during 140-days

	D	0‰	5‰	15‰	25‰	35‰
IMCL	7.40±0.60	7.45±0.60	7.20±0.50	7.65±0.33	7.80±0.24	7.55±0.33
FMCL	7.65±0.76^{ab}	8.05±0.80^{b}	7.38±0.88^{a}	8.50±0.43^{c}	9.21±0.29^{d}	8.07±0.53^{b}
IMCW	8.00±0.68	8.10±0.69	7.85±0.61	8.20±0.35	8.35±0.27	8.05±0.33
FMCW	8.20±0.84^{ab}	8.70±0.86^{bc}	8.00±0.94^{a}	9.10±0.49^{c}	10.00±0.36^{d}	8.64±0.55^{bc}

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

DISCUSSION

The present study showed that *Pachygrapsus marmoratus* individuals survived 2.0±0.5 days at 17.6 °C without water and food. Similarly, this species was lived 5 days at 20 °C without water, and more than 1 week at 12 °C (Pora, 1939; Vernet-Cornubert, 1958). Our results indicated that the highest survival time was determined as 140 days at 15‰ salinity. Moreover, 25‰, 35‰ and 5‰ salinity levels exhibited longer survival times than 0‰. Similarly, Costlow (1967) noticed that eggs and larvae of blue crabs (*Callinectes sapidus*) exhibited higher survival rates in higher salinity levels.

Salinity also plays an important role in the development and growth of crab species (Fisher, 1999; Castejón et al., 2015). Intermolt duration of *C. sapidus* was affected by salinity (Cházaro-Olvera and Peterson, 2004). Furthermore, Long et al. (2017) investigated the impacts of salinity on the gonadal development of Chinese mitten crab (*E. sinensis*) and reported that gonadosomatic index (GSI) showed a significant increase at salinity levels of 12‰ and 18‰ with compared to lower salinities of 0‰ and 6‰. Fisher (1999) declared that there was a negative correlation between salinity and growth of *C. sapidus*. In the present study, the optimal salinity level for the restricted growth was determined as 25‰. Crabs showed lower growth rate at the dry area and low salinity level (5‰).

Nutritional stress affects various important parameters in crustaceans, such as growth performance, energy metabolism or nutrient deposition. The growth increased for a while in this study, even though the crabs were left hungry. Then, growth rates of all groups decreased to minus on 84th day. Comoglio et al. (2008) reported the similar situation in southern king crab (*Lithodes santolla*) which survived at 12 days without feeding. This previous study has been pointed out that the minus growth rate observed on the 9th day. In contrast, Wen et al. (2006) declared that continuous weight loss in *E. sinensis* for 70 days. Energy metabolism of crustaceans is difficult to put on a standard schedule (Oliveira et al., 2003). The metabolic activities of many crabs decreased during starvation and they consume protein, glycogen and lipid sources (Oliveira et al., 2004; Wen et al., 2006). Differences in the consumed organic material may determine the rise and fall in restricted growth. Furthermore, the mean weight of crabs which represented at 25‰ salinity level was moderately higher than all other groups during the study. This differentiation can be explained by molting in this group. Small increments were found in carapace lengths and carapace widths from initial to final measurements in this study. Some previous studies (Penha-Lopes et al., 2006; Figueiredo et al., 2008) related to the ornamental crab (*M. forceps*) were supported to results of this present study.

It should be noted that salinity influences the feeding behaviour of crabs. For instance, Shentu et al. (2015) reported that at the low salinity levels (e.g. 5‰), crabs exhibited a decreased food intake. Little is known about the feeding of *P. marmoratus* in the aquariums or tanks. Cuesta and Rodríguez (2000) reported that the fifth zoeal stage of *P. marmoratus* cultured at 26 °C, *Artemia* sp. nauplii were used as a bait and it was reared in 24 days. *P. marmoratus,* is one of the most abundant intertidal crab species of the Mediterranean Basin (Flores and Paula, 2002a), which can be considered a potential aquarium animal due to the high population and its attractive colours of the body. Colour varies from violet brown to almost black with a marbled yellowish-brown pattern (Ingle, 1997).

In conclusion, this study suggests that dry area and low salinity levels (e.g. 0‰ and 5‰) result in the low survival rates in *P. marmoratus*. The optimal salinity levels were determined as 15‰ for survival and 25‰ for restricted growth.

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