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Investigation of Population Characteristics of Rapa Sea Snail (*Rapana venosa* Valenciennes, 1846) in Çamburnu Bay (Trabzon)

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ABSTRACT

In the present study, some population characteristics of Rapa Sea snail (*Rapana venosa*) samples collected from the coastal zone of the Eastern Black Sea were investigated. For this purpose, Rapa whelk samples were collected by snorkeling from depths of ~7-13 m in Çamburnu Bay (Trabzon) in the year 2020. Morphometric measurements such as shell size (shell length and width) and operculum size (aperture length and width) as well as total body weight were measured using a calliper with an accuracy of ±0.01 cm and a digital balance with an accuracy of ±0.01 g, respectively. Metal levels [zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb)] in muscle and hepatopancreas tissues of gastropods were measured by inductively coupled plasma atomic emission spectroscopy (ICP-OES). It was observed that 48.76% of the sampled sea snail population was female and 51.24% was male. The mean shell length-shell width, aperture length-aperture width, and snail total body weight were determined as 35.6-27.2 mm, 26.5-11.7 mm and 14.6 g, respectively. In this study, the relation showing the relationship between the total shell length and the total body weight of the sea snail was calculated as $W=0.0034*SL^{2.63}$. The least accumulation was observed at the level of lead and cadmium, when the metals in the sea snail muscle tissue were examined. On the other hand, when the metals in the hepatopancreas tissue were examined, excessive accumulation was observed at the level of zinc and copper. In addition, a positive correlation was determined between the metal accumulation in the tissues and the wet weight of the unshelled snail and the aperture length, respectively.

Rapa Deniz Salyangozu (*Rapana venosa* Valenciennes, 1846) Populasyon Özelliklerinin Araştırılması, Çamburnu Koyu (Trabzon)

Araştırma Makalesi

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ÖZ

Bu çalışmada, Doğu Karadeniz kıyısal alandan toplanan deniz salyangozu (*Rapana venosa*) örneklerinin bazı populasyon özellikleri araştırılmıştır. Bu amaçla 2020 yılı içerisinde Çamburnu Koyu (Trabzon) ~7-13 m derinliklerden şnorkel ile Rapa salyangozu örnekleri toplanmıştır. Kabuk boyutu (kabuk boyu ve genişliği) ve operkulum boyutu (ağız boyu ve genişliği) ile toplam vücut ağırlığı gibi morfometrik ölçümler ±0.01 cm hassasiyetli kumpas ve ±0.01 g dijital terazi ile ölçülmüştür. Gastropodların kas ve hepatopankreas dokularındaki metal düzeyleri [çinko (Zn), bakır (Cu), kadmiyum (Cd) ve kurşun (Pb)], indüktif eşleşmiş plazma atomik emisyon spektroskopisi ile ölçülmüştür (ICP-OES). Örneklenen deniz salyangozu popülasyonunun %48,76'sının dişi, %51,24'ünün erkek bireylerden olduğu gözlenmiştir. Ortalama kabuk boyu-kabuk genişliği, ağız boyu-ağız genişliği ve salyangoz toplam vücut ağırlığı sırayla 35,6-27,2 mm, 26,5-11,7 mm ve 14,6 g olarak belirlenmiştir. Bu çalışmada deniz salyangozunun toplam kabuk boyu ile toplam vücut ağırlık ilişkisini gösteren bağıntı $W=0.034*SL^{2.63}$ olarak hesaplanmıştır. Deniz salyangozu kas dokusundaki metaller incelendiğinde en az birikim kurşun ve kadmiyum düzeyinde gözlendi.

Diğer yandan hepatopankreas dokusundaki metaller incelendiğinde çinko ve bakır düzeyinde aşırı birikim gözlendi. Ayrıca, dokularda metal birikimi ile salyangozun kabuksuz yaş ağırlığı ve operkulum boyutları arasında pozitif bir ilişki tespit edilmiştir.

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1. Introduction

Rapa whelk *Rapana venosa* (Gastropoda: Muricidae) have many stands in a broad range of temperature, salinity, oxygen survivability, generalist feeding behaviour and high fecundity. That's why they have been successful nautical invaders over time (Harding and Mann, 1999; Scarabino et al., 1999; Pastorino et al., 2000). Gastropods, an important group in macrobenthic fauna, are distributed in aquatic habitats. Therefore, they are used in risk assessment of aquatic environmental quality and are consumed by carnivorous fed (crustaceans, fish, birds and mammals etc.) and saprophyte in other trophic levels (Salánki et al., 2003). Rapa whelk usually feeds on other Mollusca species such as oysters, mussels, and clams (Harding and Mann, 1999; Savini et al., 2002; Savini and Occhipinti Ambrogi, 2006; Harding et al., 2007; Kasapoğlu, 2021). The first periods of such gastropod life cycles, which are peculiar to temperate waters, developed very rapidly (Harding et al., 2008). Its consumption and export as food also started to be important whilst it is one of the biggest pests of mussel and oyster beds in the Black Sea (Yücel et al., 2013). The majority of its production (64.5%) is obtained from the Eastern Black Sea Region. According to the Turkish Statistical Institute (TUIK, 2021) reports, sea snail has been one of the stocks exploited by Turkish fishermen since 1986. It has been stated in various studies that the habitats where sea snails can best survive in the Black Sea are sandy, algae, sandy-algae and muddy environments, ranging from 0-100 m depths (Düzgüneş et al., 1988). They live in deep waters in winter and coastal zones during April-October (Karayücel, 1992). Global aquaculture production is increasing rapidly to meet the increasing consumption-based demand as human beings turn to fisheries for economic and ecological reasons (FAO, 2018). Likewise, coastal regions provide vital habitats for aquatic life sustainability (Constantin et al., 2015). However, changes in environmental pollution affect these ecosystems negatively and impact the growth and development of living beings there. Knowing the differences and variability in body sizes of living creatures in aquatic systems is significant in recording scientific reports and determining various aspects of aquatic population dynamics (Erzini, 1994). Domestic and industrial wastes, which occur due to irregular population growth, cause an increase in the level of metal pollution in the seas (Zhou et al., 2001). Heavy metal concentrations in marine organisms vary depending on water temperature, salinity, age, size and feeding habits of the animal (Eastwood and Couture, 2002; Göksu et al., 2005). Accordingly, some small shellfish, such as molluscs, can accumulate heavy metals and other toxic substances in their tissues up to 100000 times their level in the water (Avelar et al., 2000). This study aims to make various evaluations about some morphological parameters and heavy metal levels of sea snails sampled from Çamburnu Bay in Trabzon.

2. Material and Methods

This study was carried out in Çamburnu Bay, Trabzon (40^o 55′33.35 N′′ - 40^o 12′ 11.43′′ E) of the Eastern Black Sea Region in September 2020 (Figure 1).

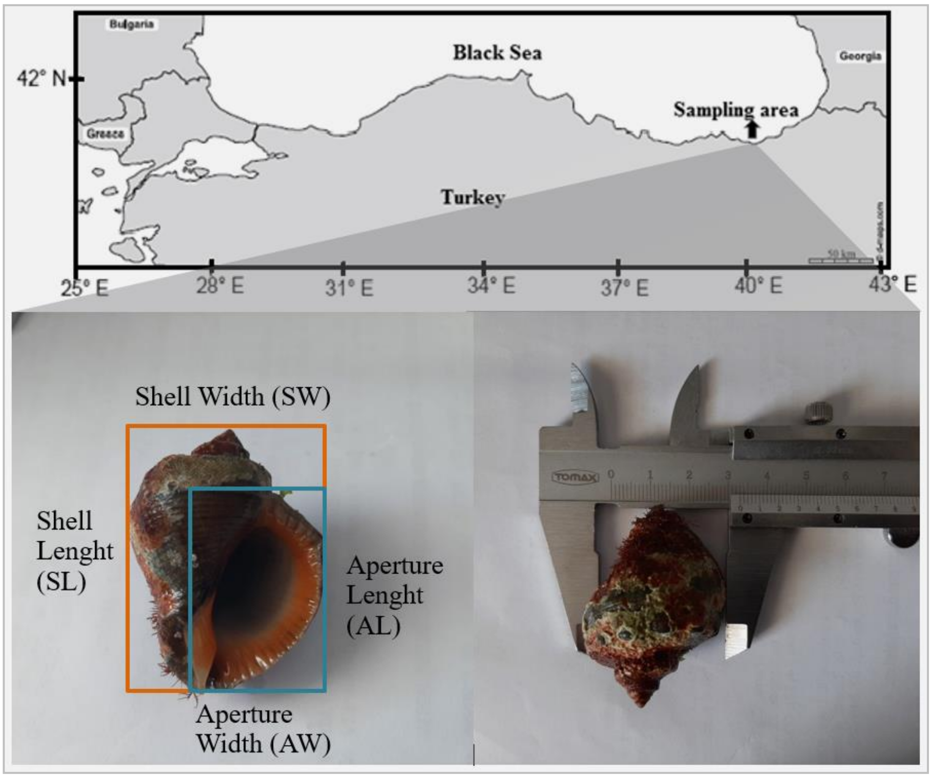

Figure 1. Sampling area and shell size measurements of a sea snail (♂) in Çamburnu Bay, Turkey.

Rapa whelk (n:121) were generally sampled in a sandy-muddy area from green macroalgae canopy (*Ulva sp*., *Ceramium sp*.) habitat. Sea snails were sampled manually by daytime snorkeling from depths of ∼7-13 m in Çamburnu Bay. Then, the collected samples were quickly placed in an insulated box containing ice and stored in a deep freezer (-20^0 C) until the analysis time. Rapa whelk was identified in the field. The scientific name of samples was rechecked according to Molluscabase (2021). Morphologic measurements including shell length, shell width, aperture length, aperture width, total body weight and unshelled weight were performed using a calliper with an accuracy of ±0.01 cm and a digital balance with an accuracy of ±0.01 g, respectively (Figure 1). The gonads of the snails whose shells were removed were examined under a simple hand lens and sex determination was made. The yellow colour at the terminal end of the internal organs was determined as the female indicator of the snail, and the brown colour was determined as the male individual indicator of the snail and noted (Erik, 2011; Sağlam et al., 2015). Muscle and hepatopancreas tissues of snails were dissected (n=121), and their heavy metal analysis in tissues was measured by ICP-OES (Inductively coupled plasma-optical emission spectrometry). All chemicals used in heavy metal analysis are of analytical quality and concentrated 69% HNO_3 and 60% $HClO_4$ (Merck) were used in the experiments. Working standards for inductively coupled ICP-OES analysis were prepared from proper standard solutions containing 1000 ppm for each element tested (PerkinElmer, USA).

Each tissue sample was heated in an oven at 105 °C for 18 hours. A weight of 0.5 g of each snail sample was treated with a mixture of 3 mL of 69% HNO_3 and 1 mL of 60% $HClO_4$ to induce acid digestion and carried out at 200 °C. An aliquot of 5 mL of 1% HNO_3 was added to each tissue sample and filtered through 0.45 µm filter paper and made up to 25 mL with deionized water and stored in polyethylene tubes at +4 °C until analysis (Massadeh et al., 2016). The recovery percentages of the device for Zn, Cu, Cd, Pb were 98.10%, 97.70%, 91.40% and 95.05%, respectively. The lobster hepatopancreas (TORT-2) was used as standard reference material. The length and weight relationships were determined by using the equation W=*a*xL^b, where W was the total wet weight (g), L was the total length (cm), *a* and *b* were the relationship constants (Ricker, 1975). Whether the slope was different from *b*=3 (isometric or allometric growth type) was determined by Pauly's *t*-test (Pauly, 1984). Data were evaluated using SPSS software (SPSS Statistics V 27.0.1.0, IBM, Corp., USA) and Bio-Vinci software (V 3.0.9., BioTuring Inc., San Diego, CA, USA). Statistical analyses of data were carried out using descriptive analysis for morphologic measurements, and the Pearson correlation test was applied after the normality test was verified (Sokal and Rholf, 1995).

3. Results

The results showing the morphometric characteristics (gender, min-max value, growth type) and L-W relationships (*a*, *b*, R^2 and SE(*b*)) were estimated for the snails are given in Table 1. Tissue dissection was performed for essential and non-essential metal analyses of a total of 121 snails collected from Çamburnu Bay in September 2020, and predation was witnessed on one specimen (Figure 2).

Figure 2. First report of predation on sea snail (♀) by a kind of hail crab in Çamburnu Bay, Turkey.

The relationships between the shell's total weight and shell length, between the total weight and aperture length, between the shell length and aperture length, and between the shell width and aperture width in sea snails were shown in Figure 3. The mean values with standard errors for heavy metals of each tissue were estimated for both genders and were given in a bar graph (Figure 4). It was found that 48.76 % of the collected sea snail population was female, while 51.24 % was male (Table 1). For all the sea snails sampled from Çamburnu Bay, the arithmetic mean and standard error of estimation values of the shell length, shell width, total body weight, aperture length and aperture width were obtained as 35.6±0.02 mm, 27.2±0.01 mm, 14.6±0.01 g, 12.0-39.4 mm, 0.80-19.5 mm, respectively. Minimum and maximum values of the shell length, shell width, total body weight, aperture length and aperture width were calculated as 17.5-51.1 mm, 7.00-42.9 mm, 1.0-26.75 g, 7.0-42.9 mm, 0.80-16.5 mm for the female snails, and 17.5-52.8 mm, 8.90-43.8 mm, 2.5-31.64 g, 8.9-43.8 mm, 2.30-19.5 mm for the male snails. The coefficient of determination R^2 values for the female and male sea snails were 0.89 and 0.88 (P<0.01), respectively. The values of *b* and standard errors of *b* for the female and male snails were found as 2.89, 2.37 and 0.03, 0.02 (Table 1), respectively. The total length-weight relationships of the female and male snails were found as follows: $W=0.0038*TL^{2.89}$ for the females, and $W=0.0030*TL^{2.37}$ for the male sea snails. In this study, negative allometric growth was determined for both genders, since the *b* value was obtained to be less than 3 (see Table 1).

Table 1. Biometrical data and estimated shell length (SL) - total weight (TW) relationships of *Rapana venosa* sampled from Çamburnu Bay (Turkey) in 2020-2021 (n=121).

	N (%)	SL (mm) x̄ ±SEM Min-Max	SW (mm) x̄ ±SEM Min-Max	TW (g) x̄ ±SEM Min-Max	AL (mm) x̄ ±SEM Min-Max	AW (mm) x̄ ±SEM Min-Max	Relationship Parameters a	*b*	*SE(b)*	R^2	GT
♀	59	34.8±0.14	28.1±0.01	12.9±0.11	28.1±0.01	9.90±0.01	0.0038	2.89	0.03	0.89	A-
	(48.76%)	17.5-51.1	7.00-42.9	1.0±26.75	7.0-42.9	0.80-16.5					
♂	62	36.4±0.13	26.3±0.01	16.15±0.1	26.3±0.01	13.4±0.01	0.0030	2.37	0.02	0.88	A-
	(51.24%)	17.5-52.8	8.90-43.8	2.5-31.64	8.9-43.8	2.30-19.5					
∑	121	35.6±0.02	27.2±0.01	14.6±0.01	26.5±0.01	11.7±0.02	0.0034	2.63	0.02	0.88	A-
	(100%)	17.5-52.8	7.00-43.8	1.0-31.64	7.0-43.8	0.80-19.5					

♀, female; ♂, male; ∑, all; N, number of specimens; x̄ ±SEM, arithmetic mean± standard error of the mean; Min-Max, the range of variability of the linear and mass indices; *a*, *b*, coefficients of the equations; SE*(b)*, standard error of estimation; R^2, coefficient of determination; GT: growth type, A-: negative allometry.

When we compared the total weight in both genders with the aperture lengths and shell length, we found a significant positive correlation (*P*<0.01; 0.92; 0.97; Figure 3). A significant positive correlation was also determined (*P*<0.01; 0.93) between the shell length and aperture length for both genders. However, we did not observe the same relationship between shell width and aperture width (P>0.05; 0.16; Figure 3). In addition, the shell rate of the snails was 74.56%, and the net meat rate was 15.44%. Pearson correlation coefficient was significant (*P*<0.01; 0.96) between the shell weight and the unshelled weight of the snails. The highest accumulations of zinc, copper, cadmium and lead were determined in the hepatopancreas tissue, while the least accumulations were observed in the muscle tissue (Figure 4). A positive correlation coefficient was determined between the unshelled body weight of the snails and the total zinc, copper, cadmium and lead levels in the muscle and hepatopancreas tissues (P<0.01; 0.87; 0.79; 0.95; 0.96). Additionally, a positive correlation coefficient was found between the aperture length and total zinc, copper, cadmium and lead accumulation in tissues of sea snail (P<0.01; 0.72; 0.82; 0.85; 0.83).

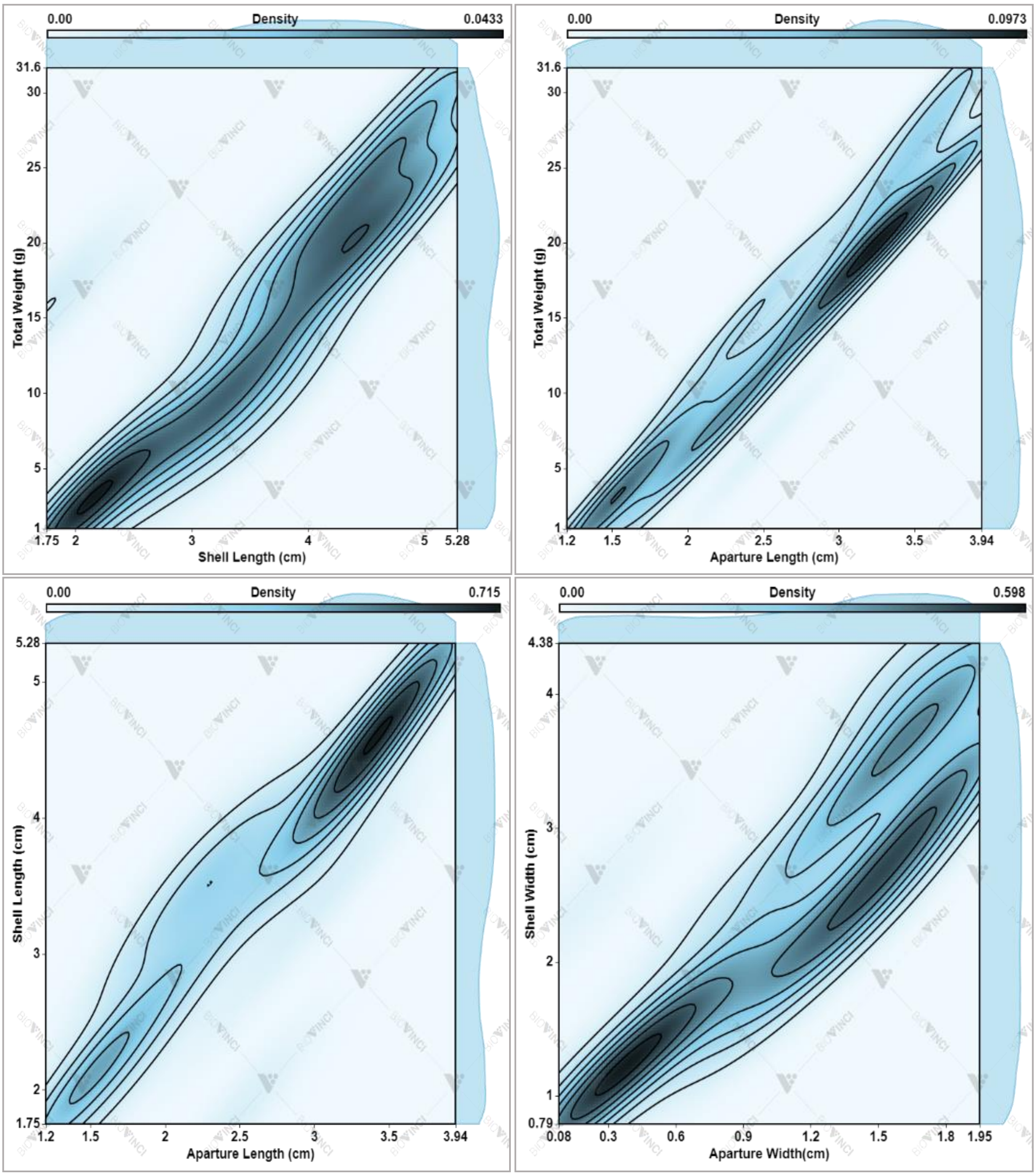

Figure 3. The relationship between morphometric size measurements of sea snails (for both genders) in the 2D- Density plot map.

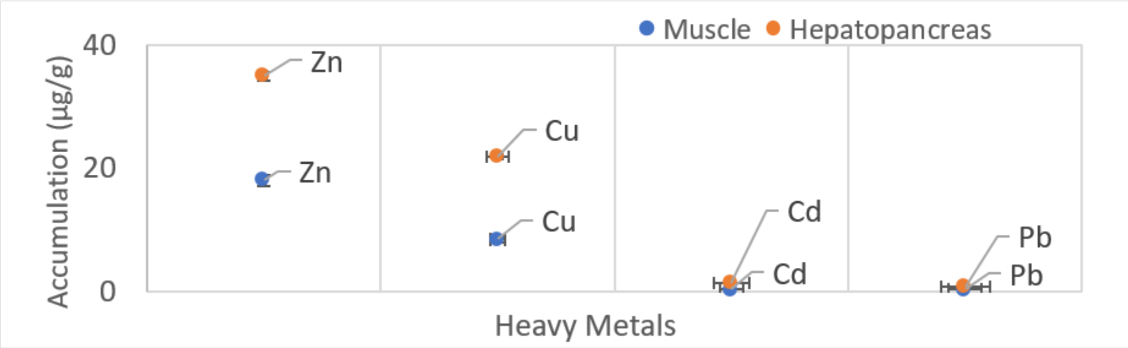

Figure 4. The mean value and standard deviations of heavy metal accumulations in muscle and hepatopancreas tissues of both genders.

4. Discussion

Studies examining the biochemical composition and body parameters of living beings together are becoming increasingly important in terms of time and space under the influence of changing environmental conditions. In this study, male individuals of sea snail specimens, which had reached sexual maturity, were determined to be 2.48 % more than female individuals (P>0.05), and the female - male ratio in the *R. venosa* population was found to be 1:1.05. In two studies conducted in the Eastern Black Sea region of Turkey, the male and female ratio were determined as 1:1.6 and 1:1.15 in the snails sampled, and the male ratios were observed as dominant (Sağlam, 2003; Sağlam et al., 2015). This slight abundance on behalf of males may indicate possible imposex as reported in the Black Sea side of Romania (Micu et al., 2009). It was determined that the sea snails in Sevastopol Bay reach a shell length from 20 mm to 40 mm in their first year, and individuals with a shell length of 35-78 mm reached their first reproductive maturity at the age of two. It was also reported that the breeding time of the sea snail was between July and September, and the spawning time lasted from May to November (Ciuhcin, 1984). It was pointed out that the shell rate of the sea snails in the Eastern Black Sea was 62.27% and the net meat yield was 17.21% (Düzgüneş and Feyzioğlu, 1994). The net meat yield of sea snails was found to be 19.50% in another study conducted in the Eastern Black Sea region (Koral and Kıran, 2017). The total length and weight measurements of Rapa snails sampled from the Bulgarian coast of the Black Sea, Central Black Sea (Sinop) were measured as 70-92 mm and 80-172 g, 64.9 mm and 46.1 g, respectively (Prodanov et al., 1995; Erik, 2011). In the present study carried out on the coast of Çamburnu, the shell rate was 74.56%, and the net meat rate was 15.44%. In addition, the average length of the species was determined as 35.6 mm and the weight as 14.60 g, while the growth type was found as negative allometric (Table 1). This situation can be explained by the fact that the size and weight of the snails and the changes in the shell and meat yield might have varied depending on different regions. The total length and total weight relationship parameters of the snails in the Sinop-Central Black Sea was reported as $W=0.0002*TL^{2.87}$ (Erik, 2011), whereas it was reported as $W=0.0001*TL^{2.93}$ in Laizhou Bay (Wu, 1988), which revealed that the b coefficient was rather high compared to the present study. The determination coefficient (R^2) was found in this study as 0.88, which indicates a strong relationship. This was slightly lower than other studies in which values were reported as 0.93 and 0.98 (Wu, 1988; Erik, 2011).

It was observed that the examined heavy metals accumulated the least in the muscle tissue and the most in the hepatopancreas tissue, in this study. The zinc, copper, cadmium, and lead concentration values measured in the sea snails were observed higher in the hepatopancreas than in the muscle by 1.94-fold, 2.60-fold, 4.48-fold and 2.48-fold, respectively. Hepatopancreas tissue is a very efficient organ in the uptake and storage of heavy metals. In particular, the hepatopancreas is the main regulatory organ and the primary storage and detoxification site of metals (Al-Yousuf et al., 2000). It is extremely important to monitor contaminants in regions, where sea food and nutrition is intense. Lead accumulation in the hepatopancreas of the gastropods was investigated, and a low prevalence of imposex in the gastropods in Bacoor Bay was reported (Su et al., 2015). Metal accumulation in snail tissues is at low levels, since the area is a protected area and pollution and industrial establishments were not common. It is also known that water temperature plays an important role in metal accumulation because it increases the metabolic rate (Yılmaz, 2003). In mollusca, acceptable copper level is reported as 10-30 μg/g, zinc level 40-100 μg/g (Wagner and Boman, 2004), 1 μg/g wet weight for cadmium and lead (EU, 2001). In another study, the acceptable limit for crustaceans was determined as 0.10 μg/g for copper, 50 μg/g for zinc, 0.10 μg/g and 1.50 μg/g wet weight for cadmium and lead, respectively (Mol et al., 2005). In this study, it was observed that the total zinc, copper and lead levels were within acceptable limits and did not pose a risk, while the total cadmium level slightly exceeded the acceptable limits.

In this study, a significantly positive relationship was determined between the unshelled wet weight and aperture length of the snails with metal accumulation in tissues ($P<0.01$). This situation can be explained by the metabolic rate of the creature until sexual maturity in the first two years and the excellent chance of uncompetitive feeding. On the other hand, it has been reported in the literature that there are other upper trophic related species that feed on this species, which results in the transfer of the current heavy metal level to such upper trophic species (Harding, 2003; Carranza et al., 2011; Bonelli et al., 2016). Given that heavy metal accumulations are observed more in small organisms depending on their metabolic structure (Boyden, 1974), sea snails are important bioindicators for monitoring heavy metals because they feed on a variety of mussels, oysters and other bivalves (Kos'yan, 2013) and thusly, absorb and accumulate heavy metal at the trophic level. In this way, they may provide heavy metal transport to the upper trophic organisms that feed on them (Figure 2) (Harding, 2003; Carranza et al., 2011; Bonelli et al., 2016). A similar situation was witnessed in this study, as well (e.g.: Figure 2). Similarly, it has been stated that sea snails are destructively damaging the benthic ecosystem of the Black Sea as they are an invasive species (Bat and Öztekin, 2016), However, over time, they have also started to fulfil the role of intermediate chain at the trophic level and have created a niche for themselves (Figure 2) (Harding, 2003; Carranza et al., 2011; Bonelli et al., 2016). According to the data we have obtained, it has been observed that the metal levels in the tissues of *R. venosa* collected from the marine coastal area of the Çamburnu nature reserve are low considering the acceptable metal levels.

5. Conclusions

Significant morphological differences were observed between *R. venosa* in different habitats evinced in the literature. The difference in their ecological conditions is reflected, first of all, in the dimensions of their shells, total weight, aperture dimensions and unshelled body weight. The main ecological factors that determine the morphological measurements and heavy metal amounts of the Rapa snails in different habitats are the potential prey they feed on and the diversity of their prey. The most important feature of the sea snail is that it consumes other trophic-level aquatic species such as mussels and oysters due to its carnivorous feeding feature. Mussels constitute an essential part of the zoobenthic fauna in the Black Sea, and these mussel beds are the food source of commercially important demersal fish. The decrease in mussel beds also affects the feeding environment of demersal fish. In this study, metal accumulation in muscle tissue was found to be less risky compared to the hepatopancreas. However, this situation creates a paradox: the hepatopancreas tissue is softer and more preferable to consume compared to the muscle tissue (Watabe et al., 2000), and thus, the transport of heavy metals into the predators of *R. venosa* becomes more possible. With this regard, it becomes of vital importance to regularly monitor metal accumulation studies in rapanas at regular intervals in terms of monitoring marine pollution.

Statement of Conflict of Interest

Author has declared no conflict of interest.

Author's Contributions

The contribution of the author is 100%.

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