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## Biological parameters and feeding behaviour of invasive whelk *Rapana venosa* Valenciennes, 1846 in the south–eastern Black Sea of Turkey

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### PEER REVIEW

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#### Comments

The paper is of broad interest for scientists working in invasive species and fisheries in the region and around the world. The methodology used in the manuscript is correct, the presentation of results is appropriate. Details on Page 445

### ABSTRACT

**Objective:** To determine length–weight relationships, growth type and feeding behavior of the benthic predator Rapa whelk at the coast of Çamburnu, south–eastern Black Sea.

**Methods:** Rapa whelk was monthly collected by dredge sampling on the south–eastern Black Sea at 20 m depth. The relationships between morphometric parameters of Rapa whelk were described by linear and exponential models. The allometric growth of each variable relative to shell length (SL) was calculated from the function  $Y=aSL^b$  or  $\log Y=\log a+b\log SL$ . The functional regression b values were tested by t–test at the 0.05 significance level if it was significantly different from isometric growth. The total time spent on feeding either on mussel tissue or live mussels was recorded for each individual under controlled conditions in laboratory.

**Results:** The length–weight relationships showed positive allometric growth and no inter–sex variability. Body size in the male population was significantly higher than in the individuals of the female. All characters in males and females showed a trend towards allometry rather than isometry. While the total time spent feeding increased with increasing prey size the total time that *Rapana venosa* spent feeding decreased with increasing Rapa whelk size. The total average feeding time needed by Rapa whelks was 160 min. But they took 310 min on live mussels in 27–28 °C in the laboratory conditions.

**Conclusions:** Length and weight relationships, growth type, total time spent feeding of this species were explained in details for this region. It would be useful to sustainable management in the south–eastern Black Sea of Turkey. The results about the feeding behaviour of this species will contribute to the understanding of the role of this species within the ecosystem.

### KEYWORDS

*Rapana venosa*, Rapa whelk, Length–weight relationships, Growth type, Feeding behaviour, Black Sea

## 1. Introduction

Rapa whelk, *Rapana venosa* Valenciennes, 1846, (*R. venosa*) is a predator native of Asian waters around Japan, China and Korea. Rapa whelks were first discovered out of its native range in Novorossiysk Bay in the Black Sea in the mid–1940s and spreaded throughout Adriatic[1], North America, Chesapeake Bay[2] and South America[3] in the recent decades. Ballast water transport of the planktonic larval stage between habitats is the most likely vector of

introduction across traditional zoogeographic boundaries. The Asian Rapa whelk can tolerate wide ranges in salinity and temperature and combine with currents, has a high potential for spreading into invaded areas.

Rapa whelk reaches sexual maturity at 40 mm size[4]. This whelk is a predatory gastropod feeding mostly on bivalves and themselves can be preyed by lobsters, crabs, sea stars and various fishes, such as cod, dogfish and rays[5]. There are no major predators of invasive adult *R. venosa* in the Black Sea and the population has become very large and

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destructive to native marine life<sup>[6]</sup>. This predator has been responsible for the destruction of native oysters, scallops, and mussels populations<sup>[6,7]</sup>.

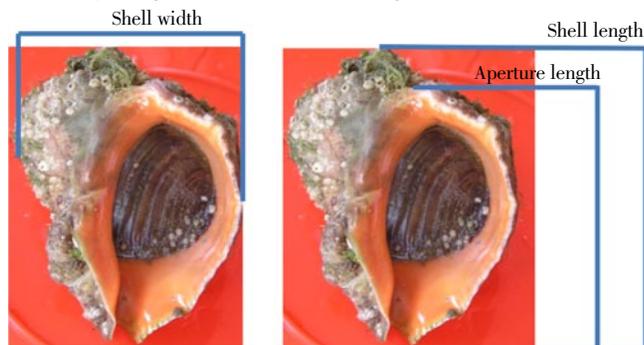
Previously, there have been some studies on biological parameters (mean length and weight) and length–weight relationships<sup>[8–11]</sup> and prey preferences, consumption rates, feeding periodicity, daily food consumption and gastric emptying<sup>[2,12–15]</sup> of Rapa whelk. The present study aims to determine length–weight relationships, growth type and total time spent on feeding of Rapa whelk in the Black Sea. This study is the first report about total time spent on feeding of Rapa whelk.

## 2. Material and methods

The study was carried out along the coast of Camburnu, south–eastern of Black Sea. Whelks were captured monthly between January and August 2000 by one 10 min haul with dredge (3.8 m in length, and 0.6 m height, with a 1 m, 40 mm mesh sized net bag) towed by a 8 m fishing boat at approximately 20 m depth, 700 m off coast. The sex individual whelk was identified based on the color of the gonad (yellowish for females and brownish for males).

### 2.1. Length–weight relationships

Shell length, shell width and aperture length were measured with a Vernier calliper to the nearest 0.01 mm. shell length was measured from the apex to the end of the siphonal canal (Figure 1). Soft parts were weighted (shell–free body weight) to the nearest 0.01 g.



**Figure 1.** Measurement of shells in Rapa whelk: shell length, shell width and aperture length.

The relationships between these morphometric parameters were described by linear and exponential. The relationship between shell length (SL, cm) and total weight (TW, g) was expressed by the equation:  $TW = aSL^b$ .

Where  $a$  and  $b$  are the coefficients of the functional regression between TW and SL. To confirm if the values of  $b$  obtained in the linear regression were significantly different from the isometric value ( $b=3$ ), a  $t$ -test ( $H_0, b=3$ ) with a confidence level of 95% was applied, expressed by the equation<sup>[16]</sup>:

$$t_s = (b-3)/SE_b$$

Where  $t_s$ = $t$ -test value,  $b$ =slope and  $SE_b$ =standard error of

the slope ( $b$ ).

The 95% confidence interval,  $CI$  of  $b$  was computed using the equation:

$$CI = b \pm (1.96 \times SE)$$

Where  $SE$  is the standard error of  $b$ . All the statistical analyses were considered at significance level of 5% ( $P < 0.05$ ).

### 2.2. Feeding behavior

Whelks (about 30 whelks) were maintained in fiberglass tanks (115 cm×115 cm×50 cm) supplied with continuously flowing seawater. Rapa whelks were allowed to acclimatise for 1–2 weeks and after that were placed in individual aquaria (30 cm×30 cm×30 cm), with water circulation and were fed mussel tissue or live mussels, *Mytilus galloprovincialis* (*M. galloprovincialis*). Feeding behaviour of whelks was observed during 24 h in each experiment.

In each experiment, each of the whelks was starved for 48 h and then presented a single meal of fresh mussel tissue, previously weighed to the nearest 0.01 g. The total time spent on feeding from the start of the feeding (from touch the shell of the prey with their foot) until end of feeding (to leave empty shell) on mussel tissue or live mussels was recorded for each individual. Effects of predator (*R. venosa*) size (71, 78 and 82 mm), prey (*M. galloprovincialis*) size (0.97, 1.35, 1.70 and 2.46 g), temperature (20 °C and 25 °C) and prey type (mussel tissue and live mussels) on the total time spent feeding were studied. At the end of the experiment shell length of each whelk was measured and the soft tissues of the animal were removed from the shell and weighted using a digital scale with a precision of 0.01 g.

## 3. Results

### 3.1. Biological parameters in Rapa whelks individuals

The parameters of each morphometric variable of females and males are presented in Table 1. The size of the Rapa whelk samples (1581 individuals) ranged from 13.5 to 95.8 mm shell length while the mean length, weight, shell free body weight and SW were about 52.85 mm, 27.73 g, 8.74 g and 20.10 g, respectively (Table 1). All characters measured were significantly larger in the males ( $n=340$ ) than in the females ( $n=280$ ) ( $P < 0.05$ ; Table 1).

**Table 1**

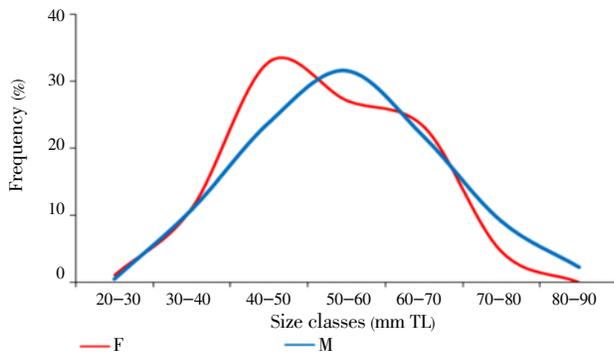
Morphometric parameter values of male and female Rapa whelk (means±SE).

Parameters	Female	Male	Both	Min–Max	$P$
Shell length (mm)	51.81±0.564	55.48±0.573	53.82±0.410	21.80–90.0	<0.05
Total weight (g)	25.48±0.945	31.74±0.942	28.91±0.711	1.41–122.8	<0.05
Body weight (g)	7.05±0.254	10.17±0.337	8.75±0.231	0.47–50.22	<0.05
Aperture length (mm)	39.27±1.047	43.32±0.734	40.98±0.500	28.40–57.20	<0.05
Shell width (mm)	36.36±0.553	39.80±0.457	38.18±0.393	8.80–57.00	<0.05
Shell weight (g)	18.44±0.723	21.57±0.641	20.16±0.504	0.30–74.91	<0.05

Both: all individuals; Min–Max: minimum and maximum;  $P$ : statistical significance).

### 3.2. Length frequency distribution

The structure of the sampled population according to the length of females and males is presented in Figure 2. The shell length frequency distribution differed significantly between females and males ( $t$ -test,  $P<0.05$ ).



**Figure 2.** Total length frequency distributions of male and female individuals of Rapa whelk from January to August 2000 (F: female, M: male).

### 3.3. Length weight relationships

Allometric analyses of weight–length relationship were done for both males and females to determine the growth type. Regression analysis showed that the slope ( $b$ ) was significantly different from 3 ( $t$ -test,  $P<0.05$ ) in both females and males, indicating a positive allometric growth pattern for both sexes ( $b_{\text{females}}=3.262$ ,  $b_{\text{males}}=3.195$ , Table 2).

**Table 2**

Changes in morphometric measurements for female and male Rapa whelks.

Parameters	Sex	a	b	R <sup>2</sup>	SE(b)	t-test	CI 95% for b	P	Growth type
TW=aSL <sup>b</sup>	F	0.00006	3.262	0.94	0.0482	67.659	3.168–3.356	<0.05	+A
	M	0.00008	3.195	0.95	0.0423	75.605	3.112–3.278	<0.05	+A
BW=aSL <sup>b</sup>	F	0.00006	2.926	0.82	0.0825	35.469	2.764–3.088	>0.05	I
	M	0.00004	3.054	0.90	0.0567	53.858	2.943–3.165	>0.05	I
SW=aSL <sup>b</sup>	F	0.00002	3.480	0.91	0.0650	53.511	3.353–3.607	<0.05	+A
	M	0.00004	3.242	0.92	0.1410	28.187	2.966–3.518	>0.05	I
AL=a+bSL	F	-4.434	0.875	0.96	0.0284	30.861	0.819–0.931	<0.05	-A
	M	-4.441	0.869	0.89	0.0578	15.029	0.756–0.982	<0.05	-A
SW <sup>*</sup> =a <sub>1</sub> bSL	F	-3.438	0.790	0.93	0.0208	37.942	0.749–0.831	<0.05	-A
	M	-3.155	0.777	0.90	0.0242	32.107	0.730–0.824	<0.05	-A

a and b, parameters of the equation; R<sup>2</sup>: coefficient of determination; SE(b), standard error of b, M, male; F, female; Slope patterns are: +A, positive allometry; -A, negative allometry; I, isometry (H<sub>0</sub>: b=1 for Y=a<sub>1</sub>bX, b=3 for Y=aX<sup>b</sup>). SL: shell length, TW: total weight, BW: body weight, AL: aperture length, SW: shell weight, SW<sup>\*</sup>: shell width.

### 3.4. Morphometric variables versus shell length

The parameters of the equation of each morphometric variable versus shell length of females and males are presented in Table 2. Shell weight and aperture length were linearly related to shell length. Weight, shell free body weight and shell weight were exponentially related to the shell length.

In females, total weight and shell weight revealed a positive allometric relationship (+A,  $t$ -test,  $P<0.05$ ), while shell width and aperture length had a negative allometric relationship (-A,  $t$ -test,  $P<0.05$ ). Body weight had an isometric relationship (I) with shell length ( $P>0.05$ ) (Table 2).

In males, total weight had a +A relationship while the aperture length and shell width had a -A relationship ( $t$ -test,  $P<0.05$ ). Body weight and shell weight increased isometrically with shell length ( $P>0.05$ , Table 2).

Regression analysis (comparison of slopes between sexes) revealed that three morphometric characters, (total weight, body weight and shell weight) were significantly different between females and males ( $t$ -test,  $P<0.05$ , Table 2).

### 3.5. Feeding behaviour of Rapa whelk

According to laboratory observations, Rapa whelk opens the shell of the prey (*M. galloprovincialis*) by pulling apart the valves with the pressure exerted by Rapa whelk’s foot and by the excretions of digestive mucous. After inserting their proboscis between the valves of the bivalve, Rapa whelk eats all mussel tissue using the radular apparatus. There were mucous excretions on the shell of mussel after end of feeding.

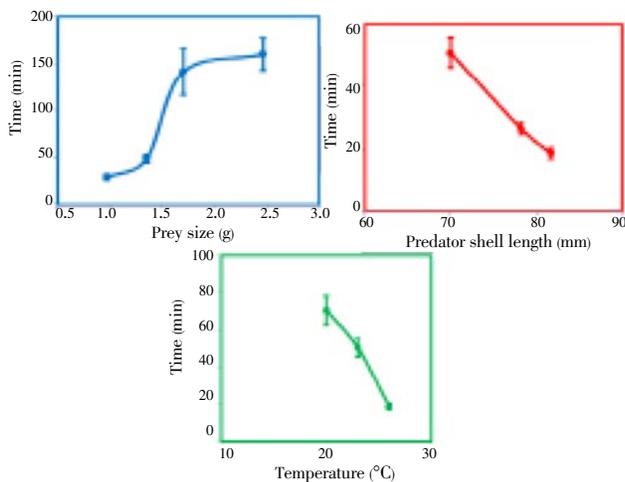
The whelks used in the feeding trials ranged from 61 to 89 mm in shell length. The total time that Rapa whelk spent feeding decreased with increasing of Rapa whelk size. There was a significant difference in feeding time among experimental individuals of different sizes ( $P<0.05$ ). The total time spent feeding also increased with increasing prey size ( $P<0.05$ ) (Table 3, Figure 3). The time from the starting of feeding to the meal was fully consumed decreased with the increasing temperature. Approximately 1 g mussel tissue was consumed in 18.5 min at 26 °C, and 70.6 min at 20 °C on average (Table 3, Figure 3).

**Table 3**

Characteristics of predator (Rapa whelk) size, prey size, prey type and temperature on feeding of wet mussel tissue, *M. galloprovincialis*.

Variables	T (°C)	N	L (mm)	PW (g)	Time (min)
Prey size	28	14	66.0±0.78	0.97±0.02	28.9±3.24
	26	18	65.1±0.84	1.35±0.04	48.8±4.29
	28	7	65.6±0.70	1.70±0.11	141.4±24.95
	28	13	67.5±0.67	2.46±0.16	160.4±17.27
Rapa whelk size	24	12	70.0±1.15	0.97±0.04	50.8±4.76
	25	17	78.2±0.82	0.96±0.02	26.4±1.85
	26	15	81.7±0.89	0.96±0.01	18.4±1.72
Prey type	28	13	67.5±0.67	2.46±0.16	160.4±17.27
	27	11	71.7±0.58	14.08±0.09*	309.6±27.81
Temperature	20	24	74.2±0.70	0.97±0.01	70.6±7.79
	23	13	70.6±1.19	0.97±0.04	50.8±4.85
	26	15	77.9±0.89	0.96±0.02	18.5±1.64

T: temperature; N: sample size; SL: shell length of Rapa whelk; PW: prey weight; Time: from start of feeding until the end; \*live mussels with shell.



**Figure 3.** Influence of predator size, prey size and temperature on the total time ( $\pm$ SE) spent on feeding on wet mussel tissue. Time from start of feeding to the end of the meal (mean $\pm$ SE).

The live mussels of (14.08 $\pm$ 0.09) ( $n=11$ ) g weight, having directly determined wet tissue weight of (2.34 $\pm$ 0.19) g, was offered to Rapa whelk, (71.7 $\pm$ 0.58) mm ( $n=11$ ) shell length (Table 3). The whelk took about 310 min to consume live mussels, and 160.4 min to consume wet mussel tissue (2.46 g) at 27–28 °C. It spent more energy and time to open the shell of the live mussels.

## 4. Discussion

### 4.1. Biological parameters

Larger shell length ranges were reported for North Atlantic–USA (103–149 mm)[2], for Adriatic Sea (101–106 mm)[9], for Argentina–Uruguay (28–120 mm)[10] and for Korea (38.8–140.6 mm)[11] than the Black Sea specimens in Turkey (24–96.4 mm) [8], in Bulgaria (40–115 mm)[17], in Romania (35–80 mm)[18] and in the present study (14–96 mm). This differences may be caused by lack of sufficient food and space for high Rapa whelk population, overfishing and different fishing gears used to catch it.

The  $b$  values in both male and female of Rapa whelks in length and weight relationships showed positive allometric growth in the present study. This positive allometric relationship is also found in other studies in different areas like Adriatic ( $b=3.21$ )[9], the Rio de la Plata estuary, like Argentina–Uruguay ( $b=3.39$ )[10] and the west sea of Korea ( $b=3.21$ )[11]. But the other researchers reported a negative allometric growth for this species in Black Sea–Bulgaria,  $b=2.81$ [17], in Black Sea–Turkey,  $b=2.77$ [8], in Chesapeake bay, USA,  $b=2.43$  and in Black Sea–Romania,  $b=2.56$ [19,20]. These differences between studies might have occurred in the different prey abundance, competition with native species and different levels of fishing exploitation (high in Black Sea, low in Atlantic), reflecting to the size structure of Rapa whelk catches.

### 4.2. Feeding Behaviour

Rapa whelks didn't damage the shell of its prey in this

study. In a similar way, it was reported that Rapa whelks produce their own toxins for paralyzing their prey (on bivalve species) and eating them with the aid of its soft proboscis without boring the prey shells[21]. Nevertheless, small Rapa whelks (<35 mm) feed by drilling through the bivalve shell, whereas large Rapa whelks (>35 mm) can attack and consume bivalves without leaving a drill-hole[22] or leaving slight predation marks on the edge of bivalve shells[10]. All of this variable feeding behaviours are reflecting the great plasticity of the Rapa whelks, which in turn can explain the great success of the species invading new areas.

In the present study, Rapa whelks (67.5 mm in shell length average) ate  $\sim$ 2.5 g *Mytilus* tissue in an average of 160 min (2.7 h). But *Neptunea* (70 mm in shell length) ate  $\sim$ 3 g *Mytilus* tissue in an average of about 32 h (from 19 h to 48 h) in 12–13 °C[5]. Rapa whelks consume the mussel faster than the *Neptunea*. Also *Neptunea* is a local gastropod against which Rapa whelk is competing. Invasive species are more severe competitors than local species. The whelk (*Buccinum isaotakii*) spent longer time in feeding on polychaetes (18.5 min) and sardine (18.2 min) preys while they spent little more than 1 min feeding on shrimp and squid for 1–day starvation period[23]. These results showed that the preys with shell like mussel were consumed faster than polychaetes and fish preys by whelks.

The estimated average food requirement for Rapa whelk was 1.2 g (Northern Adriatic), 0.68 g (Argentina), 0.2–0.3 g (South-eastern Black Sea) and 0.88 g (Uruguay) mussel tissue per day [12–14,24]. The approximately 1 g mussel tissue used in our experiments is higher than daily food requirement for *Rapana*.

In most of the locations and experiments mentioned, reflecting the high feeding plasticity of the species and also the different environmental conditions faced by this invasive species. In conclusion, the high success of this invasive species worldwide is clearly supported by its feeding behavior; therefore an appropriate management of the species in the region should include more population data and feeding behavior as presented here.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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### Comments

#### Background

The manuscript deals with the feeding behavior and the relationships between biological variables of an invasive species (*R. venosa*) in the Black Sea. New datas regarded

in this subject are needed for the understanding of the consequences of the worldwide invasion of *R. venosa*.

### Research frontiers

The study is focused on the potential effects and capability of adaptation of an invasive species to new environments, particularly regarding feeding behavior of an exotic marine gastropod.

### Related reports

There are many antecedents regarding the ability of this invasive species in dealing with local preys and how they managed to predate upon distinct bivalves worldwide. All of them are properly cited in the bibliography of the manuscript.

### Innovations and breakthroughs

The data is of great interest for the international scientific community worldwide.

### Applications

Data presented in the manuscript can be of great interest for people in charge of management of natural resources, since this species exploit natural resources of commercial interest, like mussels, oysters and clam banks.

### Peer review

The paper is of broad interest for scientists working in invasive species and fisheries in the region and worldwide. The methodology used in the manuscript is correct, presentation of results is appropriate.

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