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Chapter

The Predatory Marine Gastropod *Rapana Venosa* (Valenciennes, 1846) in Northwestern Black Sea: Morphometric Variations, Imposex Appearance and Biphallia Phenomenon

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Abstract

The presented chapter will show the results of long-term researches (2004–2013), concerning the study of variability of the size-mass relationships in mollusks *Rapana venosa* from the northwestern part of Black Sea (Odessa region, Zmiyiny Island, Danube Delta, Karkinitsky, and Tendra Gulfs) and near the eastern coast of Crimea (Sudak Gulf). The comparative evaluation has been made on the relationships between the total mass of the mollusks, mass of its soft body, and wide shells on the one hand and the size of animals (shell height, diameter, and thickness) in each study area on the other hand. Furthermore, the study of the appearance of the imposex in female mollusks (the small “penis” presence), “biphallia phenomenon” in male individual, and potential dependence of the occurrence of such anomalies on the ecological state of the marine coastal areas will be presented.

Keywords: Mollusca, Gastropoda, marine rapa whelk, *Rapana venosa* (Valenciennes), morphometric characteristics, size-mass relationships, imposex in female mollusks, biphallia phenomenon, northwestern Black Sea, Ukrainian shelf

1. Introduction

The Asian marine gastropod *Rapana venosa* (Valenciennes, 1846) [= *Rapana thomasi* Crosse, 1861 [1]] was introduced in the Black Sea accidentally, probably from the Sea of Japan with the ship ballast waters. The first finding of this mollusk in Novorossiysk Bay (the Caucasian coast, eastern part of the sea) was recorded in 1946 [2]. Since 1959, the *Rapana* individuals were also discovered in the northwestern part of the Black Sea (NWBS) and in the summer of 1971, on the Odessa coast, at first in after-storm deposits. Live specimens were encountered here in 1974 [3]. Today, the results of fundamental researches on the ecology and functional morphology of *Rapana* in the Black Sea are widely presented in literature. However, most of these publications describe mollusks from different biotopes of the eastern part of the Black

Sea from Crimean coast [4] to Caucasian coast of Russia [5–8] or the shelf of Turkey [9, 10]. Thus, morphometric characteristics of *Rapana* in the NWBS concern the animals which inhabit near Romanian [11, 12] and Bulgarian coasts [13, 14].

The purpose of our work was to study the variability of the size-mass relationships in *Rapana venosa* from the Ukrainian shelf of the NWBS (Odessa region, Zmiyiny Island, Danube Delta), attributed to small depths and strong freshwater influence of the runoff of the large rivers—Dnieper, South Bug, Dniester, and Danube—in contrast to animals inhabiting near the eastern coast of Crimea (Sudak Gulf). Furthermore, the appearance of the imposex in female mollusks (presence of the small “penis”) and “biphallia phenomenon” in male individual and tracking frequency of occurrence of these anomalies on ecological state of the marine coastal areas also were studied.

2. Material and methods

The living adult specimens of *Rapana venosa* were collected in 2004–2013 from the northwestern part of the Black Sea: near Odessa coast and neighboring areas, near Zmiyiny Island, Danube Delta area, Karkinitzky Gulf, Tendra Gulf, and from Ukrainian shelf of Crimea—in Sudak Gulf and near Tarkhankut Cape (eastern and western coast of Crimea) (**Figure 1**).

The animals were collected by diver on the rocks, stony bottom covered by mussels, on the sand, or on the sand with broken mussel shells. In the water area of the Odessa Sea Trade port, live specimens were collected on the surface of concrete breakwater constructions covered by mussel fouling. The empty *Rapana*'s shells were found in after-storm deposits, mainly on the Odessa coast. A total of 529 live specimens of mollusks and 155 empty shells for the whole period of research were analyzed (**Table 1**).

The height (H , mm), diameter (D , mm) of shells, and thickness of its border (Th , mm) were measured in all individuals. The total mass of the animal (M_1 , g), the raw mass of its soft body (M_2 , g), and shell mass (M_3 , g) were weighed. For



Figure 1. The Black Sea map and sampling areas of *Rapana venosa* near the Ukrainian shelf of NWBS, Odessa region, Zmiyiny Island and Danube Delta, and western and eastern coast of Crimea (Tarkhankut Cape and Sudak Gulf).

Sampling site	Data	Number of specimens	Depth (m)	Substrate
Odessa coast and adjacent areas	2004–2013	284 (l.s.)	3.0–10.0	R, S, S&BMS, MF
		126 (e.s.)	—	—
Odessa Sea Trade port	18.07.2006	20 (l.s.)	5.0–7.0	CPC
Zmiyiny Island	2004–2008	140 (l.s.)	7.0–12.0	R, S, S&BMS, MF
Danube Delta	21.05.2005	1 (l.s.)	21.0	M
Karkinitcky Gulf	10.09.2008	5 (l.s.)	14.0	S, S&BMS
	17.06.2011	3 (l.s.)	6.0–10.0	S
	17.06.2011	29 (e.s.)	—	—
Tendra Gulf	07.07.2005	3 (l.s.)	4.0–5.0	S
Sudak Gulf, eastern Crimea	04.05.2004	60 (l.s.)	12.0–13.0	S
Tarkhankut Cape, western Crimea	20.09.2009	13 (l.s.)	10.0–11.0	S

Note: Substrate: R, rocks; S, sand; S&BMS, sand with broken mollusks (mussels) shells; MF, mussel fouling on the bottom substrate; CPC, coast protecting concrete constructions (breakwaters and traverses); and M, bottom mud.

Table 1.

The sampling sites and collected materials: live specimens (l.s.) of *Rapana venosa* and its empty shells (e.s.) from the after-storm ejects on the marine coast.

some mollusks of the Odessa coast, the dry mass of the soft body (M_4 , g) was determined. The sex of the mollusks was determined by the presence or absence of the external genitals (penis): males, true females, and imposex females (with a small “penis”). Animal age (rounded up to a year) was calculated by the number of spawning marks on the shell [6].

The data of the morphometric parameters underwent standard statistical analysis (STATGRAPHICS Plus 5.0 program). Differences between the sampling sites in the measurements made were tested both by univariate and multivariate regression analysis of variance (ANOVA). On the basis of these data, statistically significant ($p < 0.01$) equations of mass-linear size of their shells from each of the study areas were drawn up.

3. Results and discussion

3.1 The morphometric features of *Rapana* in northwestern Black Sea (Ukrainian coast)

The height of shells (H) of the live specimens varied from 45.8 to 101.6 mm, its diameter (D) from 34.0 to 72.2 mm, and the thickness of the shell border (Th) from 0.9 to 4.2 mm. The total mass of the animal (M_1) was in the range of 20.6–173.9 g. The relation between the diameter, thickness of the shell, and the mollusk mass indices (total mass, raw and dry mass of the soft body, mass of shell), on the one hand, and the height of its shell, on the other hand, in each of the study areas of the Black Sea is described by the equation $y = a \cdot H^b$ (Table 2).

The mollusks from the NWBS were characterized by more elongated shells than specimens inhabiting the Crimean banks. The relation of shell height to its diameter (H/D) for *Rapana* near the Odessa coast, Zmiyiny Island, and in Sudak Bay was

Area	N	Min-max	ln a	b	r	R ²	SE
1	370	H = 45.80–101.60					
	370	D = 34.00–72.20	–0.6387	1.0661	0.986	97.30	0.051
	370	Th = 0.90–4.20	–3.9840	1.0609	0.835	69.76	0.202
	260	M ₁ = 29.05–173.87	–8.9635	3.0582	0.990	98.09	0.128
	260	M ₂ = 12.23–73.65	–13.0271	3.7951	0.979	95.96	0.234
	260	M ₃ = 13.90–100.52	–8.5490	2.7869	0.987	97.58	0.150
	54	M ₄ = 2.21–17.09	–14.0386	4.2661	0.910	94.02	0.156
2	60	H = 50.60–86.70					
	60	D = 36.50–69.30	–1.0625	1.1869	0.990	97.96	0.023
	60	Th = 1.40–4.80	–7.4876	1.9989	0.811	65.74	0.191
	60	M ₁ = 20.65–133.08	–9.2083	3.1332	0.971	94.26	0.102
	60	M ₂ = 4.05–37.89	–13.5531	3.8492	0.966	93.35	0.136
	60	M ₃ = 15.62 – 91.00	–8.6530	2.9078	0.948	89.82	0.129

Note: This and in **Tables 3 and 4**: N, number of specimens; H, D, Th, height, diameter of shell, and thickness of its border (mm); M₁, total biomass of the mollusk; M₂ and M₄, raw and dry mass of the soft body; M₃, shell mass (g); Min-max, the range of variability of the linear and mass indices; a, b, coefficients of the equations; r, coefficient of correlation; R², coefficient of determination (%); and SE, standard error of estimation.

Table 2.

The parameters of the equations ($y = a \cdot H^b$) of the relation between the diameter (D), thickness of the shell border (Th), and the main indices of the mollusk mass (M₁–M₄), on the one hand, and height of its shell (H), on the other hand, in the *Rapana venosa* from Odessa region of the northwestern Black Sea (1) and southeastern Crimean coast, Sudak Gulf (2).

equal to 1.7 ± 0.02 , 1.4 ± 0.02 , and 1.3 ± 0.01 , correspondingly. Similar differences were observed in the thickness of shell walls. In the Odessa area, the mean values in the relation of the height of the animals to their shell thickness (H/Th) were considerably higher than in those near the southeastern Crimean coast, 42.81 ± 0.64 and 26.02 ± 0.77 , correspondingly. However, the smallest thickness of *Rapana* shells was recorded near the Danube Delta [15]. Mollusk shell H was 82.7 mm, D = 53.2 mm, and Th was only 1.3 mm (H/Th = 63.6).

Typical shells of similar size mollusks (H = 80.1–82.7 mm) from the study areas in the NWBS and Crimean coast are shown in **Figure 2**. The relations of H/D and H/Th for these specimens of *Rapana* are from the near Danube Delta area (A) = 1.55 and 63.61, Odessa coast (B) = 1.52 and 45.22, and Sudak Gulf, eastern Crimea (C) = 1.23 and 19.38, correspondingly.

The comparison of relations of the thickness of shell border (Th, mm), diameter of shell (D, mm), and shell mass (M₃, g) on the one hand to shell height (H, mm) on the other hand has shown the existing differences between the mollusks in the study areas. The shells of the Crimean *Rapana* specimens are significantly thicker and heavier than NWBS mollusks (**Figure 3**). As a consequence, the relation of the total mass to shell mass (M₁/M₃) was lower for the Crimean mollusks than for *Rapana* in the NWBS, 1.5 ± 0.01 and 1.9 ± 0.01 , correspondingly. The relation of total mollusk mass to its raw body mass (M₁/M₂) was higher for *Rapana* from the Sudak Bay (3.6 ± 0.04) than for the animals from the NWBS (2.5 ± 0.03).

The shell mass (M₃) of *Rapana* near the Odessa coast and Zmiyiny Island was considerably lower than in specimens from Sudak, 51.5 ± 0.5 , 52.6 ± 0.4 , and $66.6 \pm 0.5\%$, respectively, of total mass (M₁) of the animal. However, a lower shell mass was observed in the near Danube Delta area—43.1% of the total animal mass

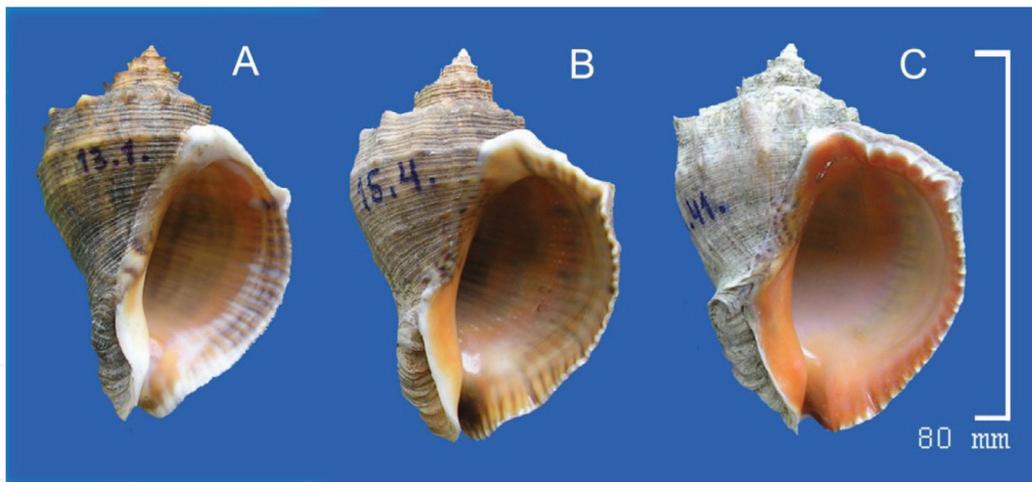


Figure 2.
General view of the shells of *Rapana venosa* (Valenciennes, 1846) [= *Rapana thomasiana* Crosse, 1861 [1]] from different areas in the Black Sea: near Danube Delta (A), Odessa coast (B), and Sudak Gulf, eastern Crimea (C).

($M_1 = 79.8$ g). It should be noted that for mollusks in the Kerch Strait (northeastern Crimean coast), this index was about 60% [16].

If we compare the diagrams of the dependence of the mollusk's soft body mass (M_2) on its total mass with shell (M_1) in NWBS and near the eastern coast of Crimea, a significant difference in the fatness of *Rapana* from these regions of the Black Sea can be clearly seen. So, the M_2 index was highest for the animals near the Zmiyiny Island and lowest for the mollusks in Sudak Gulf. The rapa whelk specimens near the Odessa coast occupied an "intermediate" position (**Figure 4A**).

At the same time, the proportion of shell mass in the total mass of the animal (M_3/M_1) was, on the contrary, the highest near the Crimean coast. Thus, it is necessary to pay attention to the almost complete coincidence of the trend lines in the graphs of the M_3/M_1 dependence for mollusks from Odessa and Danube regions of the Black Sea (**Figure 4B**).

In our opinion, a much thinner and less massive of *Rapana* shell in NWBS mollusks and near the Odessa coast, in particular, is ascribed to a low salinity in these areas. The mean of monthly salinities of coastal waters near Odessa, Sudak and Novorossiysk (place of origin of *Rapana* in the Black Sea) varies in the range of 11.70–15.73, 18.27–18.50, and 17.61–18.00 g/kg, accordingly [17]. In particular, near the Odessa coast, the average salinity of sea water in 1980–2009 was 13.8 g/kg, and in some abnormal years (2010), the average annual values of salinity could be reduced to 11.2 g/kg (in summer period 9.8 g/kg). It may suggest that the insufficient mineralization of sea waters stipulates a certain lack of building components for the mollusk shell.

At the same time, there are other opinions on the matter, excluding the direct dependence of the thickness and weight of the shells of mollusks on the level of salinity of the marine environment in the area of their habitat. According to I.P. Bondarev, comparative analysis of the thickness of the shells of *Rapana* from areas with different salinity (Azov-Black Sea basin) shows that the direct relationship between these parameters is absent [18]. This author believes that many factors may affect skeleton sizes in marine ectoderms, including in predation pressure, resource availability, energy acquisition, and the effort required to extract calcium carbonate (CaCO_3) from seawater. The main factor influencing the formation of thick-walled shell of the *Rapana venosa* from the Black Sea is the amplitude of the temperature fluctuations in the locality.

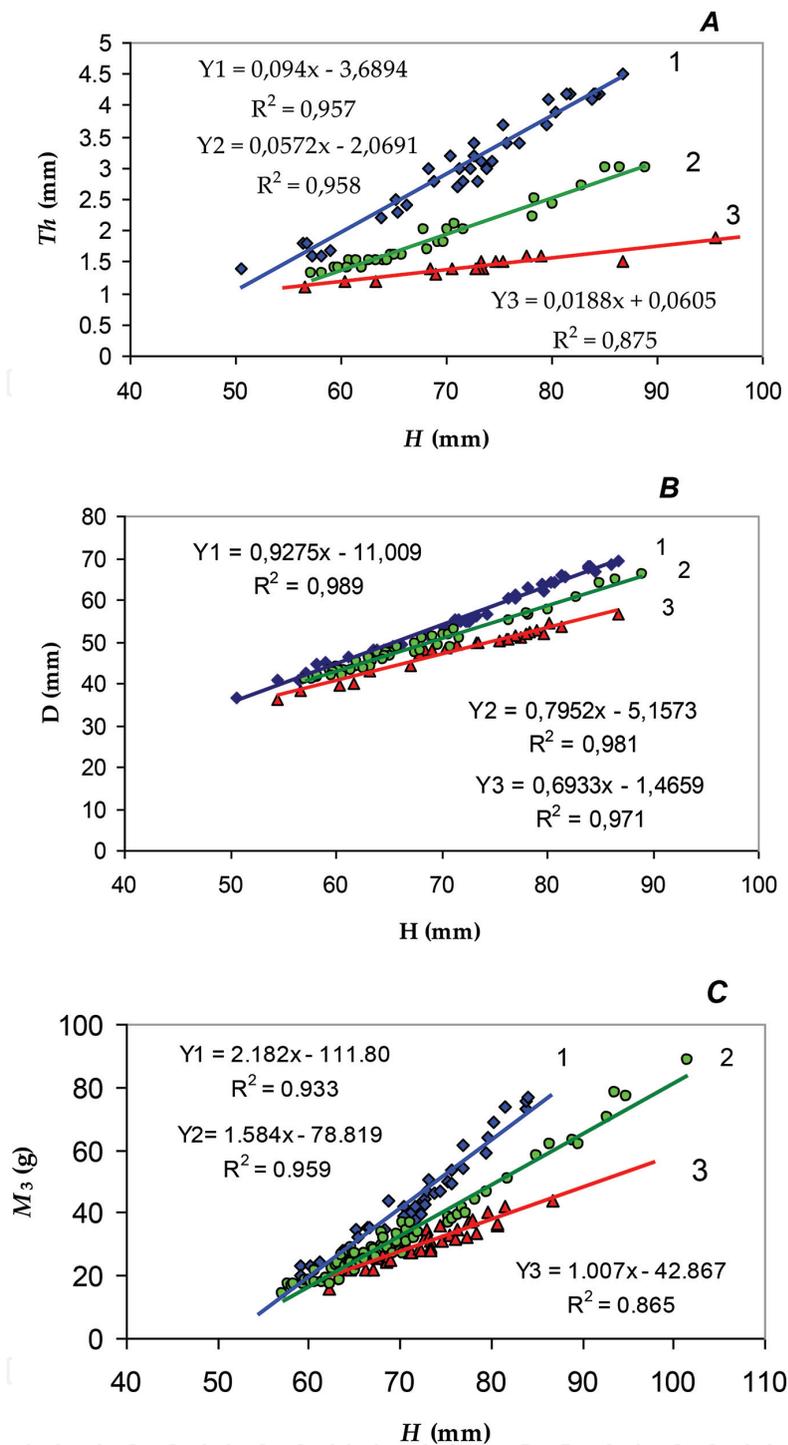


Figure 3.

The graphs of the relations of the thickness of the shell border (Th , mm) (A), diameter of shell (D , mm) (B), and the shell mass (M_3 , g) (C) to the height of shells (H , mm) in *Rapana venosa* from Sudak Gulf, eastern Crimea (—◆— 1), near Zmiyiny Island, Danube region (—●— 2), and near Odessa coast (—▲— 3).

There is also an opinion that the ecological conditions in the different biotopes defined, first of all, the size and thickness of *Rapana* shells. The main ecological factors determining the morphological characteristics of the rapa whelk in these biotopes are their specific diversity and the abundance and size of their potential prey, in the NWBS—mainly bivalve mollusks *Mytilus galloprovincialis*, *Anadara* sp., and *Chamellia gallina* [8].

At the same time, in our opinion, in the northwestern part of the Black Sea, the food base of *Rapana* is not the main limiting factor, determining the mass of the mollusk's shells and its morphometric features, including the thickness of its walls. Thus, in the Odessa region of the NWBS, the main food component for *Rapana* is

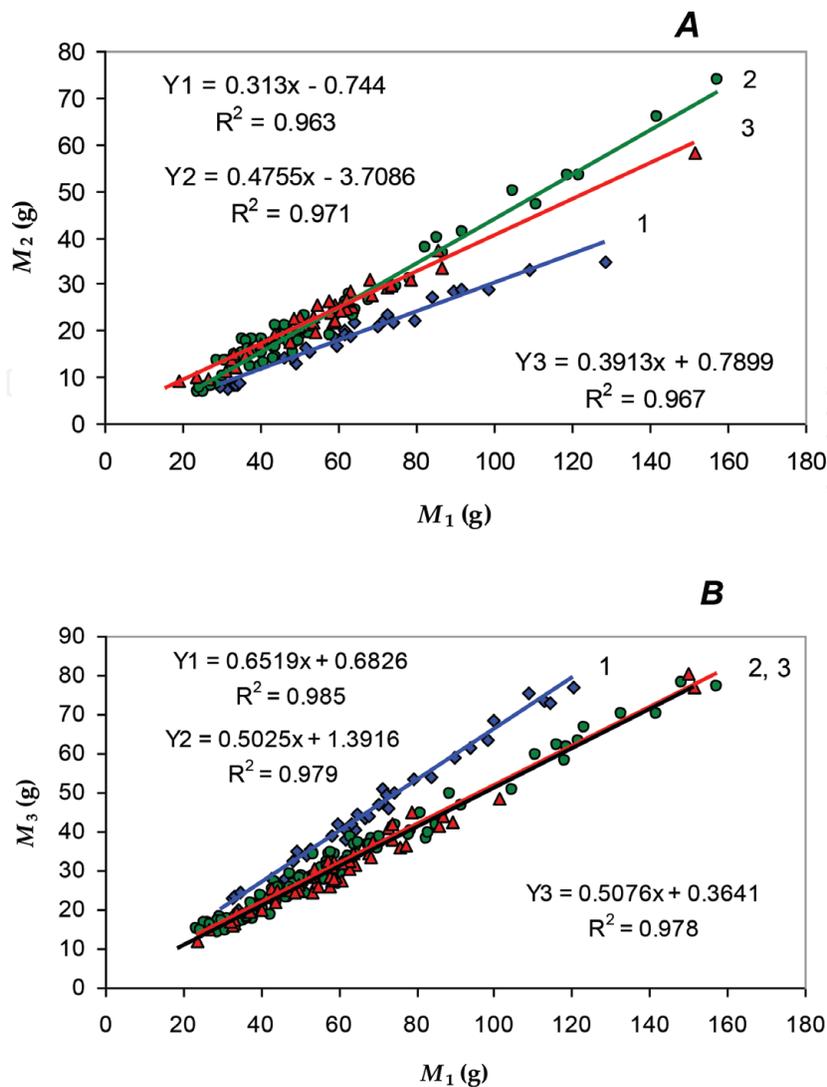


Figure 4.

The graphs of the relations of the raw mass of the soft body (M_2 , g) (A) and the shell mass (M_3 , g) (B) to the total mass of the animal (M_1 , g) in *Rapana venosa* from Sudak gulf, eastern Crimea (—◆— 1), near Zmiyiny Island, Danube region (—●— 2), and near Odessa coast (—▲— 3).

mussel *Mytilus galloprovincialis* (Lamarck, 1819). According to our observations, the density of this bivalve mollusk in benthic settlements (depth 3 m) near the Odessa coast can reach from 2.325 ± 0.417 to 2.725 ± 0.394 thousand spec./m² in the spring and summer and its biomass from 15.93 ± 2.81 to 16.56 ± 1.98 kg/m². At the same time, almost one third of the mussels in the settlements ($32.7 \pm 6.5\%$) were animals of the size group 30–40 mm.

The study of the consumption rates of rapa whelk in natural environment showed that this predatory mollusk most actively consumes the mussels from 30 to 40 mm length ($40.2 \pm 3.2\%$). On average, one predator with a shell height (H) 60–80 mm eats for a day one mussel 36.3 ± 1.5 mm long with a total mass 3.94 ± 0.56 g (1.48 ± 0.22 g raw mussel meat) [19]. Therefore, the *Rapana* in this region of the NWBS does not experience difficulties with obtaining food, which is confirmed by the high levels of the fatness of mollusks in comparison with the Crimean specimens in Sudak Gulf.

The main function of mollusks' shell is protection of soft organs of animal from outer biotic and abiotic subjections, including the external pressure increasing with depth. In this regard, it should be noted that the relatively small depths in the NWBS coastal areas (11–15 m near Odessa coast and 10–25 m in the Danube area than depths of more than 30–50 m near the Crimean and Caucasian coasts) are favorable for the survival of *Rapana* specimens with thin and more fragile shells. It

should be noted that in the 1970s, some researchers even proposed to refer the *Rapana* mollusks from the Odessa Bay to a separate ecological form, adapted to the local conditions differing from those of the eastern Black Sea [3].

The discovery in May 21, 2005, for the first time, a live adult male specimen of *Rapana* in the Danube Delta region can serve as confirmation of the further expansion of this mollusk in the NWBS. The mollusk was found 10 km from the mouth of the river (45°24.021'N; 29°53.022'E) at a depth of 21 meters on muddy bottom [15]. Prior to this case, there were no reports in the literature of the findings of *Rapana* in this water area experiencing a constant freshening effect of river flow. The extreme western point of the Odessa coast of NWBS, where we found in after-storm ejects the empty shells of this mollusk, is located on the sandy spit which separates the Tuzlovsky group of saltwater estuaries (Shagany, Alibey, Burnas) from the open sea (45°48.152'N; 30°06.154'E).

In comparison with the same size individuals from the southeastern coast of Crimea (Sudak) and the region of Zmiyiny Island, this specimen was more elongated. The ratio of the height of the shell to its diameter (H/D) in the animal found is 1.55. For comparison, in mollusks from the region of Zmiyiny Island and near the Crimean coast, this index is much lower (1.35 and 1.24, respectively). The shell of *Rapana* in the Danube region was characterized by a thinner wall ($Th = 1.3$ mm) and very less mass ($M_3 = 34.4$ g) (Table 3).

The final equations of the multiple regression analysis linking the basic mass parameters (M_1 – M_3) and linear size of mollusks (H and D) for *Rapana venosa* in the Ukrainian area of the NWBS are

$$\log M_1 = -7.763 + 1.0724 \cdot \log H + 1.8556 \cdot \log D$$

$$(n = 240; R^2 = 98.91; SE = 0.0938) \quad (1)$$

$$\log M_2 = -2.651 + 1.159 \cdot \log M_1 + 0.2551 \cdot \log H - 0.0071 \cdot \log D$$

$$(n = 206; R^2 = 96.99; SE = 0.1969) \quad (2)$$

$$\log M_3 = -0.973 + 0.7918 \cdot \log M_1 - 0.2541 \cdot \log H + 0.5792 \cdot \log D$$

$$(n = 212; R^2 = 98.47; SE = 0.1026) \quad (3)$$

Since the p -value in the ANOVA table is less than 0.01, these mathematical models show the statistically significant relationship between the variables at the 99% confidence level and can be used for determining the mollusk indices of the soft body and of the shell from this area without dissection of the animals. In addition, these equations can be used to predict the vital mass of mollusks (M_1) based on the size-mass characteristics of empty shells from after-storm ejects on the coast [20].

3.2 The imposex appearance in female specimens of *Rapana* from different marine coast areas on the Ukrainian shelf of Black Sea

The *Rapana venosa* is a bisexual marine mollusk. The males can be easily differentiated from females by outward copulative organ (penis) that is used for “capture” and holding of partners during mating. Its length is about 15–20 mm in the adult male. Sexual affinity can be visually determined by the height of the mollusk shell that is 30 mm higher for males. Females of *Rapana* with signs of masculinization (a phenomenon of “imposex”) that inhabit the muddy marine areas are an exception. Imposex females are characterized by the development of male characteristics, such as structure that looks like a small “penis.”

Many researchers have the opinion that this abnormality is caused by the exposure of those animals to organotin compounds (Ots), mainly tributyltin (TBT) and

Area (data)	N	H	D	Th	M ₁	M ₂	M ₃
1 (21.05.2005)	1	82.7	53.2	1.3	79.8	35.2	34.4
2 (06.08.2004)	4	81.0 ± 0.4	53.3 ± 0.5	1.9 ± 0.1	78.5 ± 0.9	36.1 ± 3.2	39.3 ± 3.3
3 (06.08.2004)	4	82.3 ± 0.9	60.8 ± 1.1	2.4 ± 0.1	104.9 ± 6.1	47.7 ± 2.6	52.4 ± 3.6
4 (04.05.2004)	7	82.9 ± 0.6	66.6 ± 0.5	3.9 ± 0.1	105.5 ± 4.1	32.3 ± 1.1	67.5 ± 3.4

Table 3.

The size-mass characteristics of *Rapana venosa* from Danube Delta region of the northwestern Black Sea (1), near the Odessa coast (2), Zmiyiny Island (3), and in Sudak Gulf, eastern coast of Crimea (4), 2004–2005.

triphenyltin (TPT), of the antifouling paints, used worldwide to cover the boats and other metallic constructions in order to prevent corrosion [21–23]. Owing to the environmentally deleterious properties of Ots, TBT-based antifouling paints were banned by the International Maritime Organization in 2008; however, these paints are still widely used [24]. In the last years, imposexed females of *Rapana* were found in the Northern Adriatic Sea [25] and on the Atlantic Ocean coast, Chesapeake Bay, USA [26]. Generally, imposex has been previously reported in more than 30 caenogastropods in South America [27].

In the Black Sea, this phenomenon has been discovered for the first time near Odessa coast, NWBS region [28]. Among the 135 specimens of female *Rapana* from NWBS, examined in 2004–2010 for the presence of the phenomenon of imposex (“masculinization” of females), we found 17 anomalous females (12.6%) with a small rudimentary “penis” no longer than 4–5 mm in length. All mollusks with signs of imposex were adult individuals, the age of which varied from 3 to 7 years.

The lowest occurrence of imposex among female *Rapana* was observed in the region of Zmiyiny Island—1.5% (1 specimen from 65 females examined). Near the Odessa coast, this index was 6.5% (7 imposex specimens from 61), and in the water area of the Odessa Trade Port, 100% of the females (9 specimens) had an underdeveloped small “penis.” As the manifestation of intersexuality in other species of gastropods of the Black Sea was most often found in polluted marine bays [4], the prevalence of masculinized females in Odessa port becomes clear.

According to their morphological characteristics, the imposex females distanced from males and typically female individuals. Thus, the total mass of the individual (M_1) and the mass of the shell (M_3) in the anomalous females were higher than in the same size animals of both sexes, and on the wet and dry mass of its soft body (M_2 and M_4), they occupied an intermediate position between these groups of mollusks (Table 4).

The results we obtained about the morphometric differences in abnormal females of *Rapana* support the opinion that imposex individuals represent a kind of “intermediate” between typical females and males, since the changes occurring with them can affect not only the external sexual characteristics of the animal (the presence of a rudimentary “penis” in particular) but also the structure of its internal organs [4].

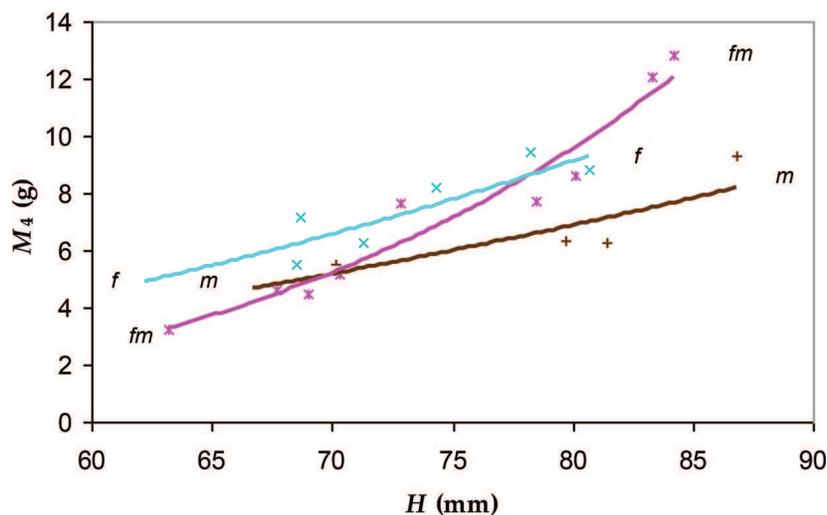
The clear differences in the graphs of the relations between the dry mass of a soft body of mollusks (M_4 , g) and the size of its shell (H , mm) in males and typical females of rapa whelks on one hand and females with signs of imposex on the other hand near the Odessa coast can serve as a visual confirmation of this opinion (Figure 5).

As seen in the figure, the trend line of the depending M_4/H in “abnormal” females with signs of masculinization (fm) occupies an intermediate position, distancing themselves from these dependency graphs for males (m) and typical females (f). Unfortunately, a small number of abnormal females (only 17

Sex	N	Lim H	Lim M	ln a	b	R ²	SE
Male	21	66.7–81.4	$M_1 = 37.22\text{--}92.75$	−9.567	3.150	91.48	0.106
			$M_2 = 15.81\text{--}38.22$	−17.322	4.795	97.60	0.134
			$M_3 = 19.60\text{--}49.67$	−9.263	2.969	65.83	0.134
			$M_4 = 4.96\text{--}9.22$	−7.595	2.193	68.57	0.154
Female	12	62.2–74.6	$M_1 = 32.25\text{--}58.47$	−8.517	2.913	88.13	0.085
			$M_2 = 13.60\text{--}23.85$	−8.854	2.775	62.76	0.141
			$M_3 = 16.92\text{--}32.58$	−7.137	2.452	61.94	0.127
			$M_4 = 5.36\text{--}9.47$	−9.355	2.635	64.25	0.141
Imposex female	12	63.2–84.2	$M_1 = 33.49\text{--}98.16$	−10.820	3.465	92.50	0.095
			$M_2 = 12.18\text{--}40.11$	−12.206	3.575	86.40	0.122
			$M_3 = 19.70\text{--}53.42$	−9.616	3.067	91.92	0.097
			$M_4 = 3.26\text{--}12.83$	−15.023	3.937	87.04	0.161

Table 4.

The parameters of the equations ($M = a \cdot H^b$) of the relation between the mass indices of mollusks ($M_1\text{--}M_4$), on the one hand, and height of its shell (H), on the other hand, in the same size male, female, and imposex female species of *Rapana venosa* in Odessa region of the northwestern Black Sea, July–August 2004–2009.

**Figure 5.**

The graphs of the relations between the dry mass of the soft body of mollusks (M_4 , g) and its shell height (H , mm) in *Rapana venosa* near the Odessa coast: m, male specimens (+); f, typically female individuals (X); and fm, imposex females with rudimentary small “penis” (X).

specimens) did not allow us to conduct a serious statistical analysis with a high probability of confirming or disproving this opinion about the “intermediate” place of imposex females of *Rapana* between typical female and male sexual groups in NWBS ecological conditions.

3.3 The first record of the biphallia phenomenon in male rapa whelk in the Black Sea

One of the varieties of imposexuality in anomalous females of Gastropoda is the appearance of more than one penial structure known as “double penis” or “biphallia.” The first record of it was made for imposex females of *Leucozonia nassa* (Caenogastropoda: Fascioliariidae) in southeast Brazil [29]. However, similar deviations in the development of outward copulative organs may occur in typical males



Figure 6. General view of male rapa whelk *Rapana venosa* with normal (left) and “double” penis (right) (A) and the normal male outward genital structure of the *Rapana* (left) and abnormal “double” penis (right) in mollusk found in stormy landings on the Odessa coast, northwestern Black Sea (Ukraine), January 11, 2008 (B).

of the gastropods. So, an adult male specimen of *Rapana venosa* with a double penis was accidentally found in storm deposits on the Odessa coast (46°27'48"N; 30°45'47"E) on January 11, 2008. The anomalous penis of this mollusk was divided approximately in the middle into two parts, which were similar in shape with typical “hooks” on the end (**Figure 6A**). The length of the main (basic) trunk of penis was about 20 mm, perpendicularly from the main trunk grew an “appendix” that did not exceed 10 mm length (**Figure 6B**).

The age of the whelk was more than 2 years; the height of its shell (H) was 64.4 mm and the diameter (D) was 44.7 mm. The total mass of the animal (M_1) was 44.23 g; the mass of its raw body (M_2) was 22.68 g, and shell mass (M_3) was 17.22 g. Before this finding, about 130 specimens of typical males of this mollusk from the northwestern part of the Black Sea were studied, without observing similar deviations in the penial structure. Therefore, the frequency of appearance of such anomaly in this area is less than 0.8% [30].

The cases of appearance of “imposex” in various marine gastropod species have been used in several areas around the world as a tool to monitor the contamination by organotin compounds. So far, imposex-related biphallic structures have been reported for females of *Leucozonia nassa* from Brazil [29] and *Hexaplex trunculus* from the Tunisian coast [31]. As the described case of finding the anomalous male, *Rapana venosa* has not been studied with the connection between the appearance of biphallia unit and the marine water quality in the region; the author can only state the conclusion of same analogy.

4. Conclusions

The predatory marine gastropod *Rapana venosa* (Valenciennes, 1846) in the northwestern Black Sea (NWBS) has certain morphometric features in contrast to mollusks from the southeastern Crimean coast. Its shell is more elongated, thinner, and less massive. As a result, the relation of the total animal mass (M_1) and the soft body raw mass (M_2) and shell mass (M_3) in the NWBS area is more evident than in the Crimean populations: $M_1/M_2 = 2.5 \pm 0.03$ and 3.6 ± 0.04 and $M_1/M_3 = 1.9 \pm 0.01$ and 1.5 ± 0.01 , respectively. The weight-size differences of the *Rapana* from NWBS may confirm certain morphometrical changes of the animals in different conditions of habitats in this area: low salinity and mineralization of the marine environment due to the strong freshwater influence of the river runoff (Danube, Dniester, South

Bug, and Dnieper), small depths in the coastal areas, etc. The evident morphometric features of *Rapana* in NWBS, the imposex appearance in females and “biphalia phenomenon” in males’ mollusks from sea waters subject to anthropogenic pollution, make it possible to consider them as a promising object for further study as an indicator of the ecological state of marine coastal areas.

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