

## Ecomorphological Analyses of Marine Mollusks' Shell Thickness of *Rapana venosa* (VALENCIENNES, 1846) (Gastropoda: Muricidae)

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**Abstract** Shell thickness of *Rapana venosa* was investigated from ecologically different places of the Azov – Black Sea basin. In the formation of the shell thickness there are two major trends: thickening with age, and inversely proportional to the size of the thickening of even-aged individuals of the same population. Shell thickness formation was analyzed in connection with biotic and abiotic environmental factors of influence. *R. venosa* ontogeny is not conducive to the rapid succession newly acquired characters in local populations. The formation of a thick shell is mainly the individual response of bions to the environment. Individuals' of the same type reaction is the cause of formation of specific conchological characters of separate populations or parts thereof. Mechanical impacts (e.g. damage by breaking predators and storm waves hitting on rocks) have no significant effect on the increasing of thickness of shells. Comparative analysis of the thickness of the shell of *R. venosa* from areas with different salinity shows that the direct relationship between these parameters is absent. *R. venosa* is capable of forming its own salinity medium in the mantle cavity, which is different from the external environment. The main factor influencing the formation of a thick-walled shell is the amplitude of the temperature fluctuations in the locality.

Shell thickness of *R. venosa* was considered as ecomorphological character in comparison with the same feature of shell-bearing mollusks from the World Ocean at different latitude zones. It is shown that high temperature gradient is one of the most important factors of forming a thick shell. The greatest show this intertidal species and ecomorphs of temperate and high latitudes, where the temperature gradients are most expressed.

**Keywords** Shell thickness; *Rapana venosa*; Molluscs; Ecology

### Introduction

The main function of mollusks' shell is protection of soft organs from outer biotic and abiotic subjections. The thickness is one of the most important characters of seashell safety. Resistance to breakage generally increases as the square of shell thickness increase (Young, Budynas, 1989), so that breaking resistance doubles for a 41% increase in thickness (Vermeij, 1993), thus providing a good return in shell strength for each unit of shell thickening (Watson et al., 2012).

Shell thickness is a quite particular characteristic, but the literature on its study is very versatile. The main investigating areas are as following: the formation and structure of the shell, the strength characteristics of the shell (as the protective function of the organism and the model of promising engineering and construction material), the reasons for the formation of a more or

less thick shells, and ability to use this feature for monitoring the marine ecosystem.

Biochemistry of marine mollusks' shell formation has been studied in general in the first half and in the middle of the 20th century (Manigault, 1939, Jodrey and Wilbur, 1955, Abolins-Krogis, 1958). These researchers demonstrated that the formation of shell is extracellular physical and chemical deposition process of calcium carbonate (most often aragonite, sometimes calcite) from extrapallial liquid organic matrix, which takes an active part mollusc mantle. In the epithelium of the mantle discovered enzymes that are also found in osteo areas of vertebrates. These enzymes are involved in calcium metabolism.

More recent studies complement these views. Mollusks use a highly cross-linked protein layer

(periostracum) and the epithelial cells of the mantle, the organ directly responsible for shell formation. They then elaborate a matrix within this space comprising various macromolecules. This matrix is the framework in which mineral forms. The major components of the matrix are the polysaccharide b-chitin, a relatively hydrophobic silk protein, and a complex assemblage of hydrophilic proteins, many of which are unusually rich in aspartic acid (Lowenstam and Weiner, 1989). The final stage of the process is the formation of the mineral itself within the matrix. Some of the acidic proteins are also occluded within the mineral phase as it forms. (Lowenstam and Weiner, 1989; Simkiss and Wilbur, 1989).

The mechanical advantage of the highly organized crossed-lamellar structure is an increased fracture resistance. The relatively weak and ductile interfaces together with a hierarchical laminated microstructure at several length scales make this possible through a combination of energy-dissipation mechanisms such as multiple cracking and crack bridging. Being biomineral aggregate, mollusc shells are about 1,000-times tougher, in comparison with these brittle minerals (calcite, aragonite), of which it is composed to at least 95%. This fact has attracted the attention of researchers involved in the creation of new structural materials for construction and other engineering applications (Barthelat et al, 2009).

The strength of shells and its thickness is linked direct quadratic equation, an additional shell strength gains due to peculiarities of its geometry. *R. venosa* shell last whorl with its widely open mouth could be approximated as a hemispherical dome. This case, peak stresses ( $\sigma$ ) induced by a compressive force ( $F$ ) applied at the shell's whorl can be quantified as:

$$\sigma = C \cdot F / t^2 \quad (1)$$

Where  $t$  is the thickness of the shell and  $C$  is a coefficient that varies with shell geometry, material properties and other factors (Young and Budynas, 1989). The presence of  $t^2$  in the denominator indicates that twice thicker shell will persist 4 time greater mechanical stress and an elevated risk of breakage, all else held equal.  $C$  declines rapidly as the shell's radius of curvature decreases that means morphologically plastic mollusk could produce equivalently strong, but thinner, shells by forming more highly domed

structures. Actually this effect could be realized with the shell size diminishing in dwarf forms.

Being ectosomatic organ, shell faster than the internal organs reacts to changes in the environment, which allows shelled mollusks to adapt to a wide range of outer conditions (Severtsov, 1939). Morphogenetic analysis of shell enables us to understand the reasons for the formation of more or less thick-walled shells and use this to monitor the environment on a local and global level, as proposed (Watson et al, 2012). Accordingly, this parameter can be used to reconstructions of paleo-environment.

Comparison of closely related species inhabiting in a wide range of environmental conditions - the only mean to determine the influence of the environment features on conchological characters. But it is always the possibility of error to take individual reaction species or group of taxa studied on the environment setting for the concept of the reaction. This kind of error can be avoided analyzing the reaction of one species, but of virtually no species of molluscs - cosmopolitans, which would conduct research in a wide range of environmental conditions. *R. venosa* is one of the very few species, which due to their tolerance to environmental factors could spread via human in almost all the Oceans in areas of freshening of water (Mann et al., 2004, Bondarev, 2010). Outside of a native inhabitant of the area freshened areas temperate western Pacific Ocean-*R. venosa* first appeared in the Black Sea (BS), which was discovered



Figure 1 Basic areas of *Rapana venosa* sampling (white stars) and water salinity (‰): 1- Romanian coast, 2- northwestern Black Sea shelf, 3- Mezhvodnoye Bank, 4- Eupatoria region, 5- Sevastopol vicinity, 6- Laspi Bay, 7- Karadag mountains coast, 8- Kerch Strait (BS) region, 9- South Azov

in the 1940s. In 1956 it was recorded in the Sea of Azov (AS), which is connected to the BS narrow Kerch Strait (Figure 1). Both BS and AS belong to the system of the Mediterranean Sea Atlantic Ocean, allowing *R. venosa* spread in freshened areas of the basins.

Discharging into small AM large rivers is the cause of lower (4.3-14 ‰) salinity than the BS, which for the most part of the coastal zone is 15-19 ‰. The presence of regions with different levels of salinity can trace the influence of this factor on the formation of more or less thick-walled shells. The variety of habitats suitable for *R. venosa* life led to the formation in the Azov-Black Sea basin of rapana metapopulation consisting of individual populations that differ in conchological parameters. And even within populations, there are groups which conchological peculiarities tailored to highlight individual ecomorphs differing the shell thickness among other characters (Bondarev, 2010).

In this paper, an analysis of the potential impact on the formation of *R. venosa* ecomorphs with different shell thickness of various environmental factors: the depth of habitat, water salinity and temperature, bottom ground type and potential predators. Analysis of the factors of influence allows determining the main cause of the formation of a more or less thick-walled shell, which is the main goal of this study. Tolerance to a wide range of environmental conditions makes *R. venosa* convenient object for studying the reaction of mollusc on these terms. Quite a large individual size makes it easy to measure parameters of mollusc with affordable means with minimal errors.

In the BS inhabited another muricid - sharply stenotherm species *Trophonopsis breviata* (Jeffreys, 1882), having boreal biogeographic roots and timed to zone of the cold intermediate layer (CIL) (Bondarev, 2011a, b). This species also has ecoforms differing in

shell thickness that allows for some analogies in the morphogenesis of this species and *R. venosa*.

Analytical work on the study of differences in the thickness of the shells of mollusks from different latitudinal zones (Watson et al, 2012) inspired the conduct in the now represented paper a more outlying analogies. These comparisons of different, sometimes quite far on phylogeny, shelled molluscs can identify some general trends in their ecomorphogenesis and to clarify the perspectives to use of shell thickness indexes in comparative studies for representatives from different geographical zones.

## 1 Results and Discussion

### 1.1 Variability and the general regularities of formation *R. venosa* shell thickness

Shell thickness in mature individuals of *R. venosa* is usually in the range 1.0-5.0mm (average - 1.5-3.0mm). Less than 1mm thickness is common in immature individuals, shell which consists mainly of an outer prismatic layer. After puberty, a thickening of the shell due to the deposition of internal plate-amorphous layers pigmented with reddish-orange color. Annual growth cycle comprises forming the outer layer, followed by growth of internal ones, so that by the beginning of spawning *R. venosa* shell has two-layered structured shell with completely formed outer lip.

Specimens with shell thickness exceeding 5 mm in the Black Sea are found sporadically, in the native part of the range so thick-walled shell is also quite rare and are marked only for a very large specimens, whose age is close to the limit for the species (Table 1). The maximum thickness of the shell of the studied sample was 6.5 mm for 139 mm individual 12 years of age. It is noteworthy that the average thicknesses of the BS mature *R. venosa* more than two times lower than the limit one, while the relative indexes - differ not so considerably (by 15-35%).

Table 1 Main morphological parameters of *R. venosa* shells with a thickness close to the limit of the Sea of Japan (JS) and the Black Sea (BS) and their mean values for the Black Sea metapopulation (line 4)

No	H (mm)	D (mm)	Th	Th/H	Th/D	Age	Region
1	168.7	134.2	6.5	0.04	0.05	14	JS
2	155	121.8	6.2	0.04	0.05	12	JS
3	139	100	6.5	0.05	0.07	12	BS
4	81.5	62.2	2.8	0.035	0.045	4	BS (mean)

Since its appearance in the BS in the late 1930s - early 1940s and to the end of the 1990s the population of *R. venosa* had a simple size-age structure. Relationship between the size and the age was characterized by close to linear. A similar pattern has the ratio of the size and thickness of the shell. Examples of such dependence are shown for some populations of the North-Western part of the Black Sea (NWBS) and the southern coast of Crimea (SCC) (Govorin, 2009). Many modern populations retained the original character of the relations, as shown in Figure 2. This applies to the populations that we call “typical” (with

the size of sexual maturity of more than 50mm) and “dwarf’s” populations – with the size of individuals that do not exceed 50-60mm, even reaching the age limit. However, the linear dependence of the characteristics of the shells of mollusks, even in such populations is not of a universal character, and has features at different stages of ontogeny. An example of such a typical population is the population of the BS area before the Kerch Strait (Figure 1, area 8). The main indicator ratios of individuals of this population are shown on Figure 2.

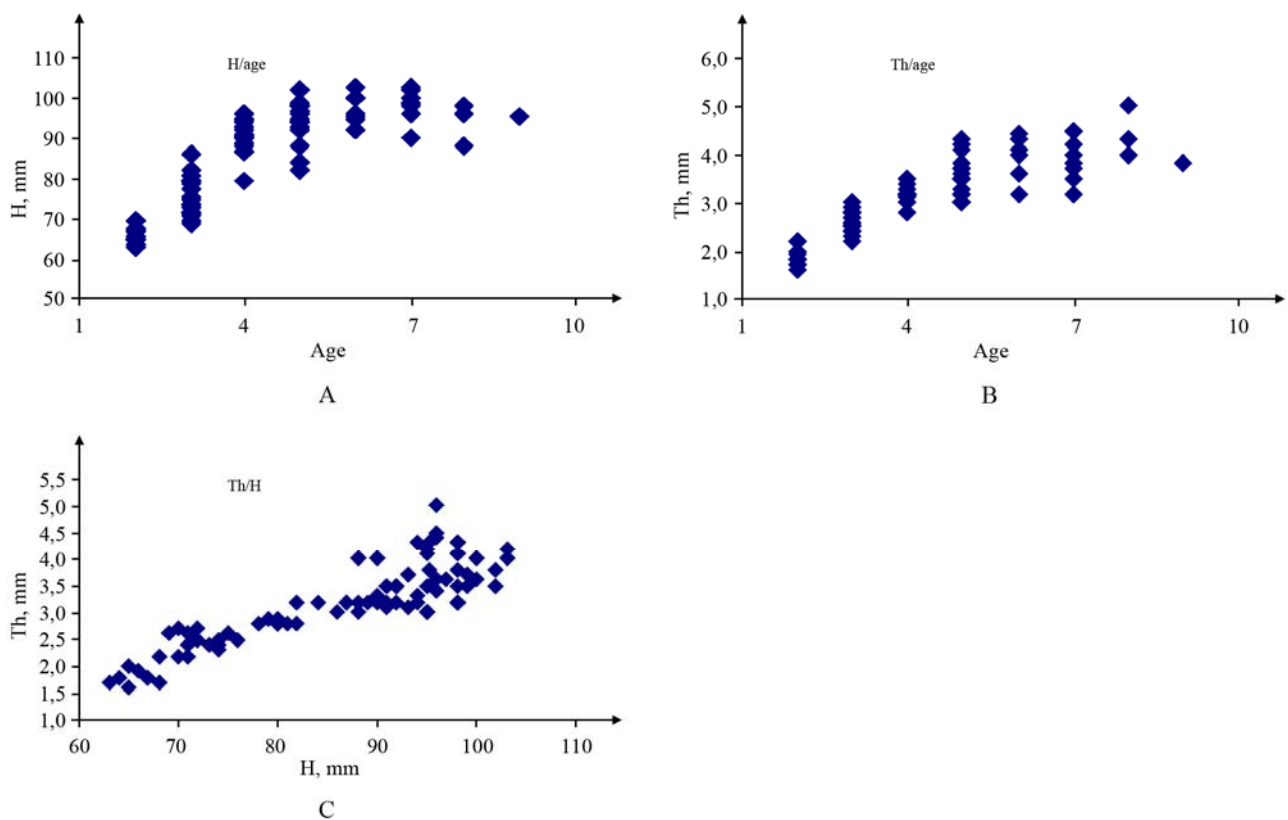


Figure 2 Comparison of shell indices *R. venosa* from typical contemporary (2012) population before the Strait of Kerch (area 8): A – H/age, B – Th/age, C – Th/H

Graph increasing in the height of the shell, depending on the age (Figure 2a), the nature of almost linear up to 5 years. In most populations of up to 90% and more are aged 5 years or less, so if not the full sample graph H / age will be quasi-linear. The graph in Figure 2a shows that to achieve a 5-year-olds gain shell height slows down. Annual line of the old-growth molluscs shells are very close, which sometimes makes it difficult to determine the age of (Bondarev, 2010a). The probability of reaching old age in *R. venosa* individuals not the largest size is higher, due to the

limitation of opportunities to support the growth of natural food resources, which reflects the right-wing plot. In mixed populations the oldest individuals more likely to be the representatives of the dwarf population’s group.

A similar but more straightforward character is a graph of the age of the shell thickness (Figure 2b). Before reaching the age of five the shells of most individuals markedly thickened. When individuals have stepped 5-year mark the annual growth rate of



thickening significantly reduced. Only a few individuals are able to continue to significantly thicken the shell, and usually it is not the largest size specimens for the population (Figure 2c). Thickening of the shell and its growth occurs most rapidly in the first years after reaching sexual maturity, what all three graphs in Figure 2. Comparison charts Th / H and Th / age gives grounds to say that the thickening is more related to age than to the size, which is confirmed by the analysis of similar plots for a mixed population (Figure 3). This is particularly evident in the large scatter of points in the right wing of the

graph, where the figures of the largest individuals. This reflects the fact that the intensive growing and largest specimens usually are not the oldest in the population.

In populations at the present stage of development, characterized by a low level of maintenance of the forage base, the maximum thickness of the shell was fixed at eight years of individuals. This fact was the basis for determining the age criterion when comparing possibilities of forming a thick shell, depending on the salinity (see below).

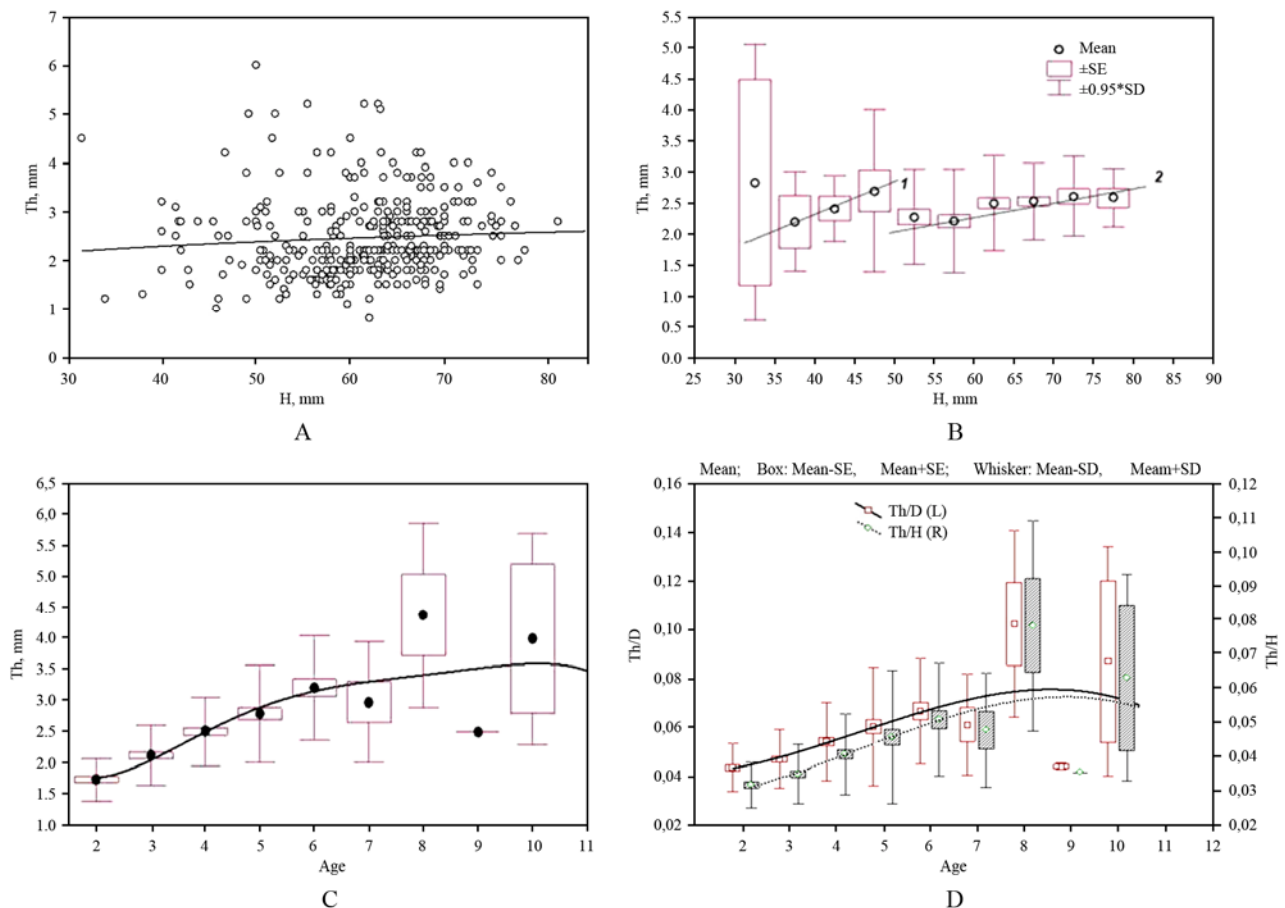


Figure 3 Dependence of *R. venosa* shell thickness indexes of the shell height and age for the mixed type population (Area 5)

Many of the Black Sea population in the last 15-20 years developed a mixed structure, when along with the typical form in the population there is quite an isolated dwarf component (Bondarev, 2010). One such population is the population of Blue Bay, near Sevastopol (Figure 1, area 5). In this population, except dwarf and typical forms are present and individuals that can be attributed to transitional. This

allows us to consider this population as a peculiar model of the Black Sea metapopulation. The fact that for this population environmental conditions are (quasi-) uniform, allows us to follow some general patterns of morphogenesis regardless of external factors.

When the field of points that define the dependence of the thickness of the size a homogeneous population is

quite consolidated (Figure 2a) and it is easy to trace tendencies of shell development, then for the mixed population the field points of analogous diagram forms a cloud (Figure 3a). In this case, the relationship Th - H can only be very roughly approximated by a line showing the relationship corresponding increase in both parameters. Marked correlation for Th/H (at  $p < 0.05000$ ) = 0.08, which means almost no correlation between these two parameters. If we take the data on *R. venosa* metapopulation in the BS in general, then this field will be more extensive and apparently even less structured. Application of Box and whiskers plotting (Figure 3b) allows to separate two principal population's grouping of Blue Bay - a dwarf (Figure 3b, line1) and the typical (Figure 3b, line 2), which develop a fundamentally similar way but distinguishable from the slope of the approximating line. Cluster analysis by the combination of *R. venosa* size - age parameters, made for a similar population of Crimea, Karadag region (Figure 1, area 7) showed the accuracy of the existence of the two principal forms in one and the same population (Bondarev, 2010). In fact, each sized group develops as described above for a typical population of Area 8 (Figure 2). The enlarged "box" in the size range up to 35 mm is obtained by the fact that some dwarf individuals can reach age limit for such a small size and a significant thickening occurs with little linear growth. Box and whiskers plots, showing the increase in the thickness of age shows that the mean and variance can not give a great spread for the population as a whole (Figure 3c). Marked correlation ( $r = 0.58$ ,  $n = 603$ ,  $p < 0.05000$ ) is much higher then with H (see above) and shows that age is much more useful character for correlations between different populations in shell thickness development investigation. On the right side of the graph increased scatter due to the small number of high aged specimens and the presence of individuals with critical shell thickness values. Furthermore, this variation reflects two trends in shells development - natural thickening with growth and reducing the aging physiological capabilities of the organism. The differences between the relative indicators of thickness (Th / H and Th / D) are age-leveled (Figure 3d), as in old age individuals growth in height is minimal, and the growth of shell is realized at the expense of the mouth wideness, which increases the maximum diameter. In the process of ontogenetic development *R. venosa* difference between the height and the maximum diameter decreases. *R. venosa*

individuals of different ages differ in habit (Bondarev, 2010), it is also important to keep in mind when comparing the populations in which the proportion of young and old individuals may be different.

The above data confirm that the comparison of the various populations of the thickness of shell is more correct to hold comparing the even-aged individuals than the same sized.

In the Black Sea exist populations, representatives of which have relatively higher shell spire (Govorin, 2009), which is confirmed by our data. A striking example of intraspecific differences by habitus in *R. venosa* individuals are shown in Figure 4.

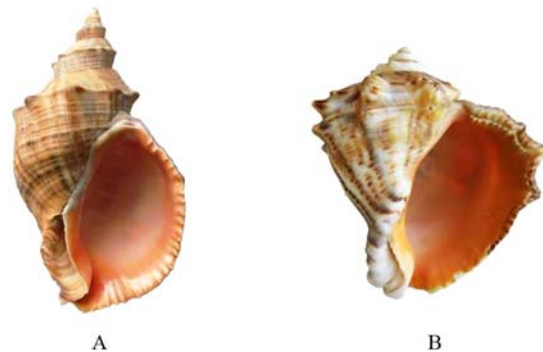


Figure 4. Two same age (8 years) specimens of *R. venosa* with the same D = 60mm, but different H and extreme habitus: A - H-105.8 mm, Th -3.3 mm, Th/H- 0.31, Th/D- 0.055; B - H-70 mm, Th/H- 0.06, Th/D- 0.07

Of course, intrapopulation and interpopulation, and the more inter-species relationship Th / D is not linear, but it is clearly more correlated than the ratio of Th / H. Thus, taking into consideration the significant differences observed in the habitus shells *R. venosa* (Figure 4) and other gastropods, subsequently more appropriate to use a measure of the relative thickness to diameter of the last whorl (Th / D). The latter indicator is less variable and more accurately reflects the strength characteristics of the shell which are connected not only with the thickness but also with a radius of curvature of the hollow hemisphere [Eq. (1)], which can be approximated by the last whorl of gastropods shell. Accordingly, specimens and species with various proportions of the shell can be more correctly comparable.

Within *R. venosa* populations appears at first sight paradoxical tendency for individuals of the same age

have a thick-walled shell with a smaller size. However, this phenomenon as the graphical statistical analysis (Ris.1.1.4) is the rule not the exception. This phenomenon is observed in all populations and at all age groups. This reversible dependency is more clearly expressed in mature enough (4-6 - year old) specimens (Figure 5) which have a sufficiently thick plated inner shell layer.

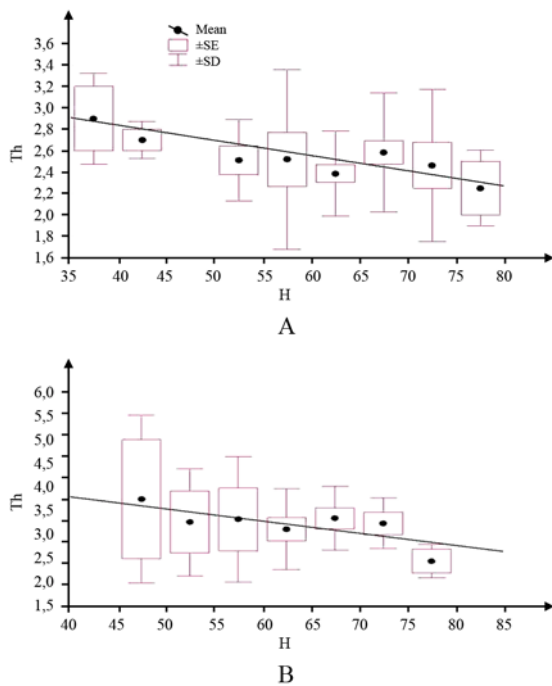


Figure 5 Th-H relations for *R. venosa* age groups: A - 4 year old specimens, B - 6 year old specimens

Mixed population includes varieties of color phenotypic line (Bondarev, 2013), as well as individuals differ in the shell shape. Gentle reversion trend between the size and thickness of shells of coeval individuals, noted in all phenotypically homogeneous and sex groups (Figure 6), that is between the most genetically close individuals, confirms that this relationship is not due to random comparison of heterogeneous material. The scatter of data in the graphs in Figure 5 is due primarily to the presence of populations of different size groups (dwarf and normal), and various phenotypic groups. For example, 3 out of the total sample (over 600 specimens) were chosen arbitrarily on the basis of various sizes among specimens with close phenotypic characteristics in one sex and age (Figure 6 A, B, C).

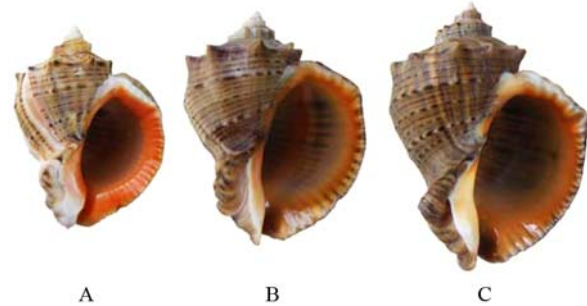


Figure 6 Coeval (5 years) *R. venosa* males the closest shell color and shape (habitus) phenotype with reversing the ratio of the H-Th, mm: A-52.0-5.0; B-63.5-2.7; C-70.0-2.5. Morphometric and weights of these specimens, as well as similar data for the population as a whole are presented in Table 2

Table 2 Dimension and weight data of *R. venosa* specimens (A, B, C), shown in Figure 6 and the population as a whole (P) data (min-max/mean); W- molluscs' general weight, Ws – shell weight, Wt – soft tissue weight

	H, mm	Th, mm	W, gram	Ws, gram	Ws/W	Wt, gram	Ws/Wt
A	52.0	5.0	26.7	20.0	0.75	6.7	2.9
B	63.5	2.7	43.3	28.4	0.66	14.9	1.9
C	70.0	2.5	56	34.9	0.62	21.1	1.7
P	38-87	1.5-5.5	24-126	14.3-76.7	0.6-0.78/ 0.67	9.7-49.3	1.6-3.72/1.85

Table 2 shows that the ratio of weights  $Ws / W$  and  $Ws / Wt$  decreases with increasing size which corresponds to the trends Th-H relations illustrated in Figure 5. In accordance with formula (1) smaller individuals, possessing greater curvature shell surface (factor C), have shell strength higher than individuals larger size the same thickness. The fact that smaller size individuals generally have "excessive" thickness

and the relative weight of shells than coeval larger individuals suggests that the thickened shell is not only a mechanical protection, but performs a different function. Such a function can be thermoregulation has been suggested (Bondarev, 2010). Shell thickness and size are offsetting characteristics. The large size and the associated total mass are directly recognized as the adaptive

characteristics for the maintenance of homeostasis in terms of the critical temperatures. Thick shell is obviously a thermostat, allowing smooth out external influence and to maintain a relatively stable temperature.

Plasticity in shell traits has been noted for intertidal gastropods appears to be closely linked to variation in growth rate. Both thicker shells and changes in shell shape can be associated with reduced growth rates. Differences in growth rates may arise because of differences in food availability or reduced feeding activity (Trussell, 2011).

There is no doubt that the lack of food leads to a slowdown in growth and the reduction in the limiting size of the animals. But the explanation of the formation of a thick shell in specimens of smaller size only by the lack of food can hardly be regarded as satisfactory, taking into account energy costs. Differences in diet can not explain the reverse trend indicators shell thickness and size for every age group of the population. Probably, there are molluscs' genetically determined relationship between the size of individuals and shell thickness developed during the evolution of the phylum. The implementation of these relationships provides homeostasis of the organism and helps it adapt to changing environmental conditions.

## 1.2 The influence of abiotic factors on the thickness of the shell

### 1.2.1 The depth of the sea

Depth through the water column pressure has almost no effect on physiological processes ranging *R. venosa* its habitat. However, a number of characteristics of the environment depend on the depth. First of all there are water salinity and temperature. The dynamics of the

water column can affect on mollusks directly, forcing to create different adaptations (Donovan et al, 1999), one of which may be thick-walled shell. Hydrodynamics largely determines the nature of the biocenosis associated with certain ground surfaces. The species composition and the degree of benthic biocenosis development determine *R. venosa* foraging possibilities of and, consequently, the growth. Soil conditions can also influence the formation of shells, as shown below.

### 1.2.2 Water salinity

Water salinity is an important factor for the spreading and activity of *R. venosa*, and for the possibility of the formation of the shell. Despite a wide range of tolerance to this parameter (Mann et al., 2004), the most comfortable *R. venosa* probably feels itself at salinity 15-22 ‰, in which the survival of its young is maximal (Mann, Harding, 2003). In the BS water salinity changes precisely in these limits, with an average of 18 ‰. As the molluscs get calcium for building shells of aquatic environment (food and water), it is logical to assume that between salinity of waters and the possibility of forming a thick shell there is a relationship. For the BS *R. venosa* direct link has been demonstrated by analyzing the thickness of the shells of mollusks from several areas NWBS with low salinity and one area of the Crimean South Coast with a salinity near normal (Govorin, 2009). To verify these findings, we analyzed the samples *R. venosa* of 8 areas of the Black and Azov seas, the salinity of which the most different (Figure 1). Data on the shell thickness compared with salinity in the areas of selection (Table 3).

Table 3 Parameters of shell of *R. venosa* 8-age individuals with maximum thickness from areas with different salinity. Numbers in brackets are the areas marked on the map Figure 1

Region	Romania Coast (1)	Karkinitzky Bay (3)	Sevastopol Vicinity (5)	Laspi Bay (6)	Kertch, BS (8)	Azov Sea (9)
S‰	15.0	18.3	18.0	18.2	17.5	12–13
Depth (m)	5–10	16–18	5	22–23	5–10	5–8
H (mm)	107.5	99.0	55.5	36	96.0	48.3
D (mm)	83.0	76.0	42.2	25.7	72.1	32.0
Th (mm)	4.5	4.2	5.2	2.0	5.0	5.0
Th/H	0.04	0.04	0.09	0.08	0.05	0.10
Th/D	0.054	0.055	0.136	0.065	0.069	0.156
Ecomorph	Typical	Typical	Dwarf	Dwarf	Typical	Dwarf



In the Azov-Black Sea basin the populations of *R. venosa* are usually found at a salinity of 12-20 ‰. Since the average salinity in the coastal zone of the Black Sea is 18 ‰, *R. venosa* has a circumbasin distribution.

In the AS distribution of *R. venosa* is limited to the southern part, under the influence of salinity regime of the Kerch Strait. In this sector, the AS salinity is about 12 ‰, and the approach to the Strait of Kerch, it increases to 15 ‰. The analyzed specimens from the AS were selected at salinity 12-13 ‰. It was found that the thickness of shells for specimens of this region is the same or higher than that of specimens from areas with higher salinity (except Sevastopol area), and the relative thickness is the maximum for AS-BS basin (Table 3).

Romanian coast (Area-1) is under the influence of the Danube River and the salinity in the surface layer of the water in this area could fall below 12 ‰ (in the spring). Specimens of the Area-1 (Figure 1) selected at salinity 15 ‰, but their maximum thickness indices are higher than those for another area of NWBS – Mezhdudnoye Bank (Karkinitskii Bay, Area-3), where the maximum salinity of the investigated areas (18.3 ‰) marked (Table 3).

As shown above, *R. venosa* thickness indices have greater correlation with the age than the size. Comparison same age specimens allow accurate analysis of the studied parameters in different sized ecoforms. The maximum age for the Black Sea *R. venosa* of our sampling is 16 years. Specimens over 8

years old were found single specimens, besides just for 8 year olds maximum thickness of the shell was marked in several populations. So an 8-year-old age criterion was adopted to compare the possibilities of forming a thick shell in different water salinity.

The maximum values of shell thickness for the area with average statistical salinity (Area-5) can be explained by the fact that the sample of individuals (>600 species) of the area is much more than other comparable (about 100 spec/area). It is clear that increasing the samples from other areas can correct maximal shell thickness data upwards.

Our data (Table 3), which can be considered representative and credible evidence that the indicators of shell thickness (both absolute and relative) is not related to the level of salinity in the locality. It should be borne in mind that the formation of shells of the same thickness dwarf specimens are large energy costs relative to the weight of the soft tissue than big sized ones. Therefore, the formation thick-walled shell of dwarf form under conditions of low (12-13 ‰) salinity particularly clearly shows that this factor is not relevant for *R. venosa* in their habitat in the BS-AS basin. The lowest thickness of the shell are also characteristic of dwarf population Laspi Bay (Area 6), which resides in a somewhat higher average salinity of 18.2 ‰ (Table 3, Table 4). Comparison of the shells representatives dwarf forms *R. venosa* (Table 4) also confirms the absence of a direct link between the level of salinity and the possibility of the formation of the shell.

Table 4 The range of variations in the characteristics of shells dwarf forms of *R. venosa* for mature individuals (3-8 years) of the population living on the sand bottom at different depths at different salinity and temperature range (min-max / mean). Numbers in brackets are the areas on the map schema (Figure 1)

Region (No)	Depth (m)	S‰	T°C	H (mm)	Th (mm)	Th/H	Th/D
Laspi Bay (6)	22-23	18.2	7-17	37-48.8/41.3	1.0-2.0/1.48	0.025-0.05/0.034	0.035-0.07/0.045
Sevastopol (5)	5-12	18.0	7-22	38-55.5/49.2	1.3-5.2/2.36	0.03-0.095/0.045	0.045-0.14/0.06
Azov (9)	5-10	12-14	1-26	30-48.339.1	1.8-5.0/2.9	0.05-0.10/0.08	0.06-0.16/0.12

Mollusks, like many other mineralizing organisms, including the vertebrates, are capable to isolate their own internal conditions of mineral formation from the environment outside (Simkiss and Wilbur, 1989; Veis, 2003). The difference between the salinity of the external environment and the internal cavities of the

body for molluscs is typically 1-3 ‰. Our measurements of salinity in the sampling area and *R. venosa* intrapallial fluid salinity made on 12 specimens collected in the Round bay (Sevastopol) showed the excess salinity of the mantle fluid compared with seawater. The maximum difference of

2.8 ‰ (17.58 ‰ and 20.38 ‰, respectively), that corresponds 13.75%. These studies show that *R. venosa* is able to effectively regulate intrapallial conditions that are different from the external environment. This fact is crucial in *R. venosa* adapting to different salinity and the possibility of forming a thick shell. Probably, the salinity is about 20 ‰ for *R. venosa* is more comfortable than the lower.

With increasing depth, salinity increases in the BS

(Sorokin, 1982), while *R. venosa* shell thickness decrease with depth. This is shown as the above data (Table 3, Table 4), and the measurements carried out on samples from other populations of relatively deep water (Table 5). Consequently, for the opportunity and necessity of forming a thick shell other environmental parameters responsible. Data from Tables 4 and 5 show that one of these parameters may be a temperature gradient in the mollusks' locality as discussed below.

Table 5 The range of variations in the characteristics of *R. venosa* shells (min-max / mean) mixed populations of mature individuals (3–6 years) of locations with close salinity level at various depths and at different temperatures. Numbers in brackets are the areas on the map 2.1

Region (No)	Depth (m)	S‰	T°C	H (mm)	Th	Th/H	Th/D
Karadag (7)	2–8	18.0	7–25	37–88.5	2.0–4.7/2.8	0.035–0.09/0.055	0.045–0.12/0.07
Karadag (7)	22	18.2	6.8–16	42–85.5/66	1.6–3.0/2.2	0.024–0.05/0.035	0.033–0.055/0.044
NWBS (2)	15–22	18.0	6.5–17	48.8–75	1.5–2.8/2.2	0.023–0.04/0.032	0.03–0.05/0.04

### 1.2.3 Temperature

Temperature affects the rate of biochemical processes in *R. venosa*, like all living things. *R. venosa* able to survive in a wide range of temperatures, but there are certain limits of vital activity and the optimal value. In its native Korean range, adult *R. venosa* demonstrate large scale annual temperature tolerance from 4°C to 27°C measured for the location (Chung et al., 1993). The upper thermal tolerance of the species occurs between 27°C and the summer maximum for Hong Kong 35°C (Liu (1994) by Mann et al, 2004). In the Sea of Azov, which is ice covered for 2 to 4 months of the year, *R. venosa* inhabits southern part with the least winter water temperature around 1°C (Goptarev et al., 1991). Thanks to a shallow depth (average 7 m) as early as May in the AS the water temperature reaches 15°C and reaches a maximum in July (32.8°C in some years in the coastal zone), usually not exceeding 26°C (Goptarev et al., 1991).

The temperature on the BS shelf is an inverse function of depth, falling from the water line up to the CIL, where the temperature throughout the year nearly constant and does not exceed 8°C. This boundary is located in the BS at a depth of 45-50m is the lower border of *R. venosa* distribution. *R. venosa* in winter burrows in loose ground and holds an inactive state in the cold period becomes active in the spring when the water is heated to 9-10°C and above. Special studies for the most comfortable life temperature for *R.*

*venosa* were not conducted. However, *in situ* observations in the BS with simultaneous temperature measurement can be concluded that the range of 15–20 °C is optimal for *R. venosa*, reflecting its biogeographical roots of temperate latitudes inhabitant. At these temperatures, *R. venosa* actively feed and reproduce, and in this temperature range are confined largest (commercial) concentrations.

During the summer in the BS warm-generated thermocline, located at depths of 20-30 m, sometimes descending to 40 m, within which the temperature decreases sharply from 15.7 to 9.3 °C (Sorokin, 1982). Thus the temperature a comfortable habitat for *R. venosa* limited the depth of 25-30 m even in summer. Therefore, despite the fact that the maximum biomass of the preferred object for *R. venosa* food - mussels located on the silt at a depth of 40m, *R. venosa* at these depths is very rare.

In the coastal zone to a depth of 5 m in the summer the water temperature is usually above 20°C (up to 25 °C). But when there is a phenomena of cold water rise with depth the temperature for several hours reduced by 10-15°C, sometimes making 8°C in high summer. This is characteristic of the local upwelling areas with a narrow (5-25 km) continental shelf (for example, the Crimean South Coast), and practically does not occur in the NWBS coastal zone where the width of the shelf up to 220 km. This may be one reason for the

differences in the thickness of the shells in these areas, as noted (Govorin, 2009) and shown according to our data.

As shown in the Tables 4 and 5 in the locations with the greatest range of temperatures average and maximum values of shell thickness have the greatest value. The fact that individuals from the AS, where annual temperatures are distinguished maximum contrast, have the maximum relative thickness of the shell, indicative of the fact that this environment parameter is most important for the formation of a thick shell. In addition, the population of the AS is the most isolated and therefore it has highest chance of inheritance characteristic of thick-walled shells. The highest shell thickness (including the maximal known individual thickness of 6.5mm) is typical for areas where temperature contrast arises not only due to annual seasonal cycle, and due to of short-term (several days) fluctuations caused by local upwelling. This fact suggests that the thickness of the shell serves for thermoregulation. Since the physiological process of forming the inner layer of shells in the (pre)spawning period is associated with an increase in temperature, it can be assumed that temperature gradient of water from cold to warm can be a "trigger" mechanism for the thickening of the shell. Recurrence these conditions several times during the season of active growth may contribute to thickening of the multiple launch mechanism and as a result - a more thick-walled shell formation. Thus, the new adaptive capabilities are implemented through already available physiological process.

#### 1.2.4 Bottom grounds

*R. venosa* found in all types of grounds, which are present on the shelf of the BS. It is noted that *R. venosa* specimens, who lives on the loose sandy soils have a more thin-walled shell, than individuals inhabiting firm rock surfaces. Exceptions are AS individuals, where *R. venosa* lives on soft bottom grounds, but the seasonal temperature regime the most contrast. Among the most likely causes of connection shell thickness with a certain type ground can be mentioned: the possibility of enhanced protection in the hydrodynamic impact of storm waves on a solid surface, the possibility of burrowing into loose ground for protection from predators or temperature fluctuations. The thicker shell as a way to resist

mechanical stress combines the first two points and discussed below. Possible reaction to temperature changes discussed above, however, the interaction rapana with a certain type ground evidence in favor of this.

The fact that *R. venosa* uses burying in the ground to maintain a stable temperature in the winter shown field observations. When short-term summer upwelling of deep cold waters that creates a sharp temperature gradient in the coastal zone, lowering the water temperature from 20-25 °C down to 8-10 °C, *R. venosa* burying in the ground and slowing of vital activity happen the same way in the winter. For *R. venosa* inhabiting the solid bedrock rapid response to sudden changes in temperature by burying is impossible. Maintaining homeostasis possible in this case if a thick shell which is capable of exposure to temperatures as smooth with excessive heating and cooling.

The benefits of having a thick shell for enhanced protection in the hydrodynamic impact of storm waves on a solid surface are obvious. Damage to shells on impact against a hard surface on its result is similar to damage caused by predators-breaking. But features of ontogeny rapana not contribute hereditary transmission of the protective function and forming populations corresponding characters, as shown below in consideration of damage predators.

#### 1.3 The impact of predators and parasites

Protection against damage of the soft body is one of the main functions mollusks shell and as shown above its strength properties depend on the thickness of a considerable extent. A number of studies show that there are dependencies between the pressure from predators and shells thickness of gastropods, which are the objects of feeding. Shell thickness has been shown (Hughes and Elnor, 1979) was increased in areas where muricid *Nucella* spp. coexisted with crabs, suggesting a protective function. Muricidae who themselves are predators, also fall into the category of victims of predators at higher trophic levels. These predators are most often the crabs and fishes (Hughes and Elnor, 1979; Palmer, 1979, 1985; Vermeij, 1993), and sea stars also (Donovan et al., 1999).

In the Black Sea there are four crab species from which only one could be potentially dangerous for *R. venosa*. It is warty crab (more known in the Black Sea area as rocky crab) *Eriphia verrucosa* (Forskål, 1775) which lives in shallow water, using as a refuge coastal rocks and rock scatterings which also inhabits the favorite prey item of *R. venosa* – mussel *M. galloprovincialis* Lamarck, 1819. Crabs can be seen on the sand "clearings" in a small distance from the rocks, where they collect carrion and prey on small bivalves - Cardiidae and Veneridae, which are object to *R. venosa* feeding also. *R. venosa* used rocky ground to attach the egg clusters during the spawning season. Thus *R. venosa* inhabiting or spawning in shallow water is syntopic with crabs and can be attacked by them.

On the example of the aquarium observations two species North Pacific muritcid (*Ceratostoma foliatum* (Gmelin, 1791), and *Nucella lamellosa* (Gmelin, 1791)) and their predator interaction, crabs use two basic strategies for extracting of the soft parts of the mollusks shell: breakage half and gradually chipping pieces of shell from the mouth outer edge (Donovan et al., 1999). Laboratory experiments have shown that crabs are capable of feeding on small specimens (Harding, 2003). Our aquarium observations support the crab *E. verrucosa* is able to break the pressure claws small (2.5 cm) thin-walled (1mm) shell *R. venosa*. The adult *R. venosa* has a large and a solid shell that crab is unable to break. A mature mollusk in the spawning period has a maximum thickness of the shell and on the edge of the shell sometimes formed a terminal varix-like axial fold (Figure 7A) which gives it additional strength. Immature rapana and individuals in the process of growth between spawning cycles have a thin outer edge of the shell (Figure 7F), which is able to break off the crab. A direct observation in the habitat of this process is not available, but the pattern of shells damage suggests the likelihood of such effects. *R. venosa* shell damages are usually located in the lower part of the outer edge between the siphonal canal and the middle of the outer lip where it has the least thickness and smallest curvature surface (Figure 7 arrow marks). After damage mollusk intensifies the shell growth on damaged spot and after a while, even heavily damaged edge becomes the normal form (Figure 7D, F). In this case, the total abnormal thickening of the shell as a

whole is not observed when in the affected area and its continuation shell can thicken. Sometimes this results in abnormal growth of shells in the affected area (Figure 6B), which is extremely rare. Most often can be observed the backlog in the growth of the shells on the affected area (Figure 7A), which is obviously a consequence of damage to the edge of the mantle area, responsible for the formation of the shell.

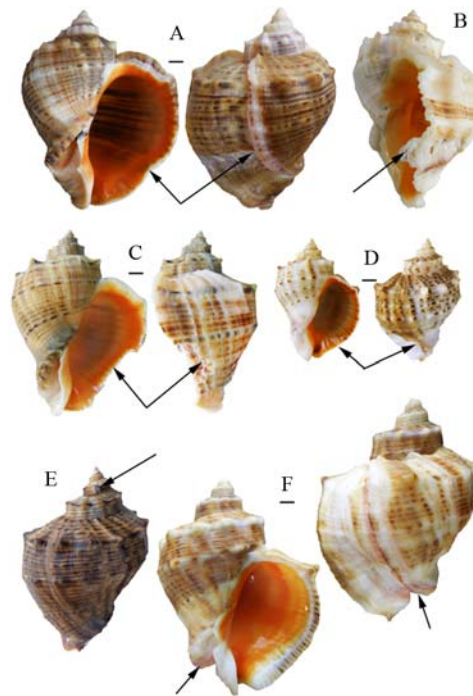


Figure 7 Regeneration of traumatism on *R. venosa* shells, H-Th (mm): A - growth retardation the edge of the mouth, 70.0-2.5, B- excessive growth with the mouth edge formation of the odontoid projection, 69.0-2.2 ("tooth" - Th-3.5mm), C - an intermediate step in the process of damage overgrowing, 63.0-2.3, D – complete restoration of the mouth edge, 42.3-1.8, E - damage on the juvenile stage followed by normal growth, 62.2-2.0, F - restoration of the normal mouth edge after severe damage of siphonal canal area, 76.0-2.2

*R. venosa* is capable to pull-in the soft tissue deep into the shell enough to place it deeper the terminal varix thickening of previous annual cycle (Figure 7A-right). This allows the mollusk to stay alive even with significant damage by predator of a new-generated shell thin edge. For large specimens rapana crabs do not represent a real threat. With its powerful, muscular foot effort which *R. venosa* able to reveal a large mussel or oyster, it is also able to grab a crab claw and theoretically cause autotomy last.



During the dozens of hours collecting and observing *R. venosa* in vivo has never been observed even attempt an attack on the mollusk by crab. Meantime feeding on other objects crabs constantly get in sight, from which we can conclude that *R. venosa* is not often the object of the attack crabs and not the traditional victim. Consequently, the crabs in the Black Sea are not a factor of formation of a thick shell. Mollusk-invader *R. venosa* is widely spread in the Black Sea, because also the lack of predators that could affect the population.

Juveniles and dwarfs can be captured mouthparts benthophagous large fish, for example - the sturgeon that live in the BS but are more common in the AS. Shells of dwarf form have the mouth edge breakage (“growth”) marks more often than typical individuals, even though the relative thickness and hence strength of their shells is significantly higher. Numerous damages (growth scars) on the dwarfs’ shell from the Sea of Azov, where stone crabs are not available, apparently are the result of the impact of benthophag-fishes. *R. venosa* size in the AS is limited food supply, but the thickness is not inferior to other areas. Indexes of the shell relative thickness in the area are highest for the Azov-Black Sea basin (Tables 4, 5). The presence of growth scars in dwarfs may be given another explanation. Their size is close to the size of the potential victims, so the use of muscular effort for opening victims by an individual with its shell on growth stage can lead to a breaking of the thin edge of the mouth with the shell of victims – bivalves (Figure 8). A small radius of curvature of the mouth of the dwarf *R. venosa* can be a negative factor that increases the risk of damage and the appearance of scar tissue growth.

Damage analysis shows that the change in thickness of the shell does not usually happen regardless of what stage of growth the damage received, and how badly it was. The figure shows specimen 6E, which as a result of damage to the juvenile stage (marked by arrow) had removal the axis of the column. But the mature shell has a normal (average) thickness for individuals from the same population of this size and age, as well as shells, damaged in adulthood (Figure 7A, B, C, D, F). Various shell-boring organisms: sponge *Pione vastifica* (Hancock, 1849) and the polychaetes *Polydora*



Figure 8 Azov Sea *R. venosa* (H-32.8mm, D- 22.8mm, Th-3.0mm) with growing scar on the ventral part of the last whorl, and its potential victim *Gouldia minima* (Montagu, 1803) 14.8x13.5mm; one scale

*ciliata* (Johnston, 1838) and *P. websteri* Hartman in Lousanoff et Engle, 1943 may affect some individuals as well as a significant part of the some population species. In this shell thickening occurs only in the place defeated shell-boring polychaetes, where mollusk forms a kind of blisters. Sponge *P. vastifica* can drill through *R. venosa* shell, hitting the entire surface, with the exception of the newly formed edge area. The fact that the sponge strikes already formed part of the shell, while in general negatively affecting the vital functions of mollusk, identifies the lack of effect of thickening shell with this action.

Poorly defined or missing effect inheritance of a thick-walled shell as a result of damages regardless of their nature is explained by peculiarities of *R. venosa* ontogeny. Immature (under 2 years) individuals always have a thin shell. In adult animals, each subsequent annual stage of the shell growth also begins with the formation of a thin upper layer (Figure 7F). In addition, *R. venosa* planktonic larval stage may last up to 80 days (Chuhchin, 1984). Spacing currents during this time also complicates the work of the mechanism of inheritance in the population. On the first place there is the individual adaptive response. The most isolated *R. venosa* population is AS one. In this population, an inherited traits transfer occurs most efficiently approaching to the species to direct development.

In species that lack planktonic larvae, such as muricid *Nucella lamellose*, *N. lapillus* and littorinid *Littorina obtusata*, gene flow between populations is thought to

be low and local adaptation high (Reimchen, 1982 and Vermeij 1982 by Trussell, 2011). Predator- induced increases in shell thickness have now been documented in three above noted marine gastropod species: (Trussell, 2011). Moreover, results and those of Appleton and Palmer (1988) and Palmer (1990) have documented phenotypic plasticity in three distinct geographic regions - New England, the Northeast Pacific, and the British Isles, respectively. These trends suggest that phenotypic plasticity in shell thickness may have evolved as a general strategy to the threat of crab predation (Trussell, 2011)

Meantime, wave-exposed snails raised without crabs also produced shells thicker (Trussell, 2011) As a result, caution must be used when interpreting morphological variation among populations and morphological change through time as evidence of microevolutionary change (Palmer, 1985b; Palmer, 1990; Trussell, 2011)

The large size and thick shell with its small aperture of sheltered shore gastropods are thought to be adaptations to the high abundance of highly motile predators, to crushing by stones, and to desiccation and heat stress (Kitching et al., 1966; Heller, 1976; Atkinson and Newbury, 1984; Johannesson, 1986 – all by Boulding et al., 1999). Unfortunately most of the evidence for the adaptive nature of these shell features is circumstantial and few field studies have investigated the mechanisms responsible for the differential survival of gastropods with different shell morphologies on wave-exposed and wave-sheltered shores (Boulding et al., 1999).

The heavy surf on wave-exposed shores is thought to select for small size whereas the high risk of shell-breaking predation on wave-sheltered shores is thought to select for increased shell size and thickness. In contrast to expectations, the smallest size-class of *L. sitkana* suffered significantly lower rates of predation than the largest size-class at one of the wave sheltered sites. The effect of shell thickness on predation mortality was as predicted from previous laboratory experiments. The thin-shelled littorinid species, *Littorina subrotundata*, suffered significantly higher rates of predation than two thicker-shelled species, *L. sitkana* and *L. scutulata s.l.*, at three of our four sites. Boulding et al (1999) conclude that the higher rates of

shell-breaking predation on wave-sheltered shores of the Northeastern Pacific selects for *L. sitkana* with thicker but not necessarily larger shells than those on wave-exposed shores.

In experiments a small difference in the shell thickness between *Littorina sitkana* and *L. subrotundata* was sufficient to affect their vulnerability of *L. sitkana* to the predatory shore crab, *H. nudus*. However such a small difference in shell thickness may be difficult to detect statistically in field-collected specimens. Raffaelli (1978) expected to see differences in shell thickness of *Littorina saxatilis* between habitats varying in wave-exposure and crab predation intensities but found no correlation (Boulding et al., 1999).

### 1.3.4 Comparative analysis

Another Black Sea representative of the family Muricidae, having circum-basin distribution is *Trophonopsis breviata*. This small-size (6-11mm) sharply stenothermic species which habitation is limited to the CIL position (45-50 - 120-130m) has the direct development of larvae (Bondarev, 2011). At the core of habitats (70-90m), where the temperature is stable throughout the year (7-8 °C), *T. breviata* has quantity peak. The mollusc shells here have a minimum thickness of 0.2-0.3 mm, while the border zone habitat, where temperatures can fluctuate upward by a few degrees, there are more thick shells (Figure 9). At depths less than 50 m, where the maximum temperature deviation (7-10 °C), the shell thickness varies from 0.2-0.6mm, with some large specimens reaching 0.8mm. In this zone, marked the second population peak of the species, due to the large number of young bivalves species more thermophilic than the main object of his nutrition - *Modiolula phaseolina* (Philippi, 1844), such as *M. galloprovincialis* and others which are also victims of *T. breviata*. The maximum size of individuals *T. breviata* also confined to this frontier zone (Bondarev, 2011). At depths greater than 120 m water temperature increases, reaching 8.6 °C in the 150m, shell thickness in the range 0.2-0.5mm. As the salinity of the depth of 50m to 150m depth gradually increases from 18.4-19.8 till 20.95-21.01 ‰ in winter-summer (Sorokin, 1982), and predators cracking the shell within the CIL are not available, the most likely cause of the increase in the shell thickness on the boundaries of habitat can be

assumed temperature gradients to sharply stenothermic species.

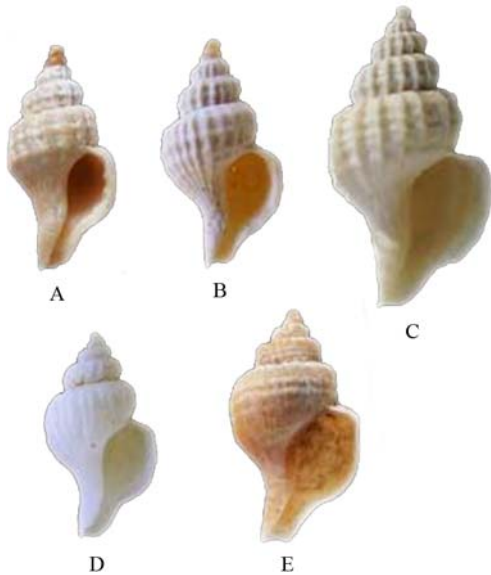


Figure 9 *T. breviata* ecoform specimens from different ecological sub-zones: A, B, C – thick shelled - from border, D, E – thin shelled - from central part of habitat. A – depth, 48m, H-7.7mm, Th-0.5mm; B – 50m, H- 8.0, Th - 0.4; C- 158m, H- 10.2, Th- 0.5; D - 91m, H- 7.2, Th- 0.2; E - 85m, H- 8.0mm, Th- 0.3mm

It is noteworthy that the fossil specimens *T. breviata* have a thick-walled and strongly sculptured shell than the typical individual core distribution area (Ilyina, 1966). That is, the fossils' shell characteristics are similar to those that have recent individuals from the borders of habitat belt. This is because the occurrence of *T. breviata* in the BS due to the formation of CIL on the shelf, which began about 3000 years ago (Bondarev, 2012). During this period, cold-water conditions on the BS shelf were underdeveloped and unstable, which corresponds to the boundaries of recent CIL conditions. Consequently conchological characteristics, in particular the shell thickness of *T. breviata* also were similar to the ancient ones.

Boreal biogeographical roots of *T. breviata* (Bondarev, 2011a, b) predetermine its sharp stenothermy and the need for adaptive response to even a slight rise in temperature, which is expressed in a thickening of the shell.

As mentioned above, increasing with a depth of the water salinity has no direct effect on *T. breviata* shell thickness. Moreover, in the depth range of 50 to 100 m, where the salinity increases from 18.4 ‰ till 20.28 ‰ (Sorokin, 1982), this species shell thickness is

reduced by half (max. 0.6mm vs. max. 0.3mm). Shown and discussed above data on the thickness of *R. venosa* shell also indicate the absence of a direct link between the salinity of the water in the habitat and the thickness of the shell. As the salinity is an important indicator for the life of the organism, affecting the osmotic processes within the organism and organism-environment system, it is necessary to consider the influence of the characteristics in more detail.

Increased by 13.75% (from 17.58 to 20.38 ‰) salt concentration intrapallial fluid of *R. venosa* compared with sea water habitat, obtained in our experiments, indicating a preference for this species of salinity higher than the average for the BS. But as *R. venosa* - a resident of desalinated marine areas that increase the salinity itself beyond in a certain limit can not only contribute to the shell formation, but on the contrary - to inhibit this process in general complex of physiological processes.

As the building material for molluscs shell is calcium carbonate, it is the calcium content of the water should determine the potential for the formation of the shell. There is a very definite opinion that the increase of calcium salts in the water up to certain limits (emphasis added - IB) results in thickening of mollusk shells (Wilbur, Owen, 1964).

Meantime, content of calcium ions in sea water are not always depends on the salinity of which is mainly formed by sodium and potassium salts. At that time, as fresh water salinity of more than 90% of the dissolved calcium and magnesium salts. Seasonal inflow of fresh water from coastal runoff and precipitation can change the salinity and the ratio of salts in seawater. The greatest impact of desalination has on the surface layer, but the fluctuations in the values in the near-bottom layer can also be significant. For BS and AS band changes in salinity and Ca<sup>2+</sup> in the bottom layer of water in some parts shown in the Table 6.

Table 6 The range of the Ca<sup>2+</sup> and salinity in the bottom layer of water in some parts of the FM and AM (according to data from Goptarev et al., 1991; Simonov et al., 1992)

Area of BS, AS	Ca <sup>2+</sup> , mg/L	S‰
Odessa Bay, BS	224-375	14.4-17.4
Yalta Bay, BS	217-269	15.8-19.0
Theodosia Bay, BS	247-257	18.2-18.3
Azov Sea	140-186	10.8-12.4

The Table 6 shows that in the Odessa Bay, where the salinity level decreased and *R. venosa* to (Govorin, 2009) have a thin shell (0.9-4.2 mm), the amount of  $\text{Ca}^{2+}$  not lower, and may be even higher than in the Southern Coast of Crimea areas (Yalta Bay and Theodosia Bay, located between the two regions - 6 and 7 map in Figure 1), where the salinity is higher and close to the average for the BS, and the shells of *R. venosa* by (Govorin, 2009) is more thick (1.4-4.8). Our data also confirm the presence of these phenotypic differences, but the reason as shown above, is not to differences in salinity and calcium content, but the differences in populations habitats temperature regime.

Most marine calcifiers can increase fluid pH and carbonate ion concentration at the site of crystal nucleation, which enables synthesis of shells and/or skeletons even when external seawater parameters are thermodynamically unfavorable for the formation of  $\text{CaCO}_3$  (Cohen and Holcomb, 2009). The energetic costs argument is based on the need to pump calcium ions from seawater to make skeletons (Wilbur and Saleuddin, 1983). This is achieved via membrane pumps and active transport across tissues. In molluscs the extrapallial fluid has a  $\text{Ca}^{2+}$  concentration that is higher than seawater, usually by 15–25% (Crenshaw, 1972; Wilbur and Saleuddin, 1983; Furuhashi et al., 2009). This percentage is close to the maximum value obtained by us from the difference in salinity (13.75%) for *R. venosa*. It can be assumed that in areas with low salinity (AS), this difference will be more. And the differences in the content of  $\text{Ca}^{2+}$  ions will not be less than specified in the above studies. No special studies to determine the optimal conditions for growth and the formation of *R. venosa* shells maximum thickness depending on the total salinity of the water and calcium content was carried out. However, we can already say that this relationship is not straightforward, and additionally must be influenced by other parameters of the water environment, the most important of which is the temperature regime.

The concentration of calcium ions as well as AS water salinity significantly lower than in the BS regions however, as indicated above, do not affect the ability of the formation rapana thick shell.

In the same steam the fact that freshwater mussels, for example - unidonids, are able to form quite a thick shell (2-5mm) with mineralization from 0.2 up to 1 ‰. However some species such as the famous European freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) prefers water with a low concentration of calcium 5-20 mg/L, and the most intensive growth it recorded in the calcium content of 6-7 mg/L (Alimov, 1981). Only some freshwater bivalves' optimal growth conditions are at a concentration close to the upper limit of calcium for their existence (about 100 mg/L). In most species, the optimum growth is shifted toward lower calcium concentrations (30-50 mg/L). The dependence of the constant growth in most unidonid species is inverted parabola with a maximum value corresponding to the 30-50 mg/L, and the minimum - 20 and 80-90 mg/L (Alimov, 1981).

Antarctic bivalve species *Laternula elliptica* (King and Broderip, 1831) had a size corrected shell over twice as thick as other Laternulid species, were measured in the study, depending on the thickness of the calcifiers shells from latitude habitat (Watsson et al., 2012). This species on the relative thickness of the shell had the thickest shell. It is possible to add that to the size of the uncorrected (absolute) of this mollusc shell thickness exceeds the other studied by Watsson et al (2012) laternulid species many times. Shell thickness of this species from our data up to 4 mm in shell length of 90 mm. Such anomalous for a family of indices cause additional interest because *L. elliptica* occurs mostly in areas exposed to lowered salinity (Fisher, 1985). It is assumed that in this infaunal genus, there is a clear selective advantage for thicker shells in the Antarctic species, which may offer protection from physical disturbance by ice (Watsson et al., 2012). *L. elliptica* lives mainly in depths less than 100 m, reaching a maximum strength at depths less than 20 m (Dell, 1990). Burrowing deep into the ground, *L. elliptica* inhabits the sandy silt in the sediment deposition zone, not glacial denudation. Also no shell can save mollusc from the kreshing force of the glacier. But regardless of the external causes, *L. elliptica* demonstrates the potential for the formation of a thick-walled shell in polar latitudes.

The main conclusions of the (Watsson et al., 2012) study is the following: shell thickness decreased with



latitude in buccinid gastropods (excepting the Australian temperate buccinid) and echinoids, but not brachiopods and laternulid clams. The variation in trends in shell thickness by taxon suggests that in some circumstances ecological factors may override latitudinal trends (Watson et al., 2012). Let us consider other examples of mollusks studied by Watson et al (2012), to the extent possible come nearer to the question of what kind of habitat conditions have a major influence on the formation of a thick shell.

Another "abnormal" species by (Watson et al., 2012) is *Cominella lineolata* (Lamarck, 1809) which had a significantly thicker shell than all other buccinids but the other temperate species, *Buccinum undatum* Linnaeus, 1758, and all polar species *Neobuccinum eatoni* (Smith, 1875), *Buccinum glaciale* Linnaeus, 1761, and *Buccinum* cf. *groenlandicum* Hancock, 1846) had thinner shells than the tropical species and the temperate *C.lineolata*. So, species living at low seawater temperatures had thinner shells than those from warmer habitats (Watson et al., 2012). There are two points identified as trends for example rapana: shells smaller individuals have a relatively thick-walled shell, shell species formed in conditions of greater temperature contrast accordingly thicker-walled, as well as the inhabitants of rocky grounds in relation to the animal on the loose bottom. *C.lineolata* - inhabitant of the intertidal zone of exposed rocky shores (Wilson, 1994) in temperate latitudes, where the daily and annual variation of temperature is quite contrasting. *Buccinum undatum* and *B. glaciale* do not have adaptation to life in the intertidal zone (Golikov, 1980). All other buccinid species compared also home of subtidal to the bathyal lower boundary the where temperature fluctuations are negligible. Size of *C.lineolata* individuals - 25mm – the least of all the species groups analyzed (Watson et al., 2012) and although the comparisons performed on the size-corrected data, the index used does not reflect the natural tendency of increasing the relative thickness of the shell size decreases. *Neobuccinum eatoni* with its size and empty habitat depth range is close to the above buccinids of the northern hemisphere high latitudes. Although this species is the most common but is not typical with its size (40-60mm, up to 86mm) from Antarctic Buccinidae. Except it rather large size (60-80 mm) has only

Neptuneinae *Antarctoneptunea aurora* (Hedley, 1916), living in the depths of 200-600 m. The other about 40 species of Antarctic buccinid have a size of 5-10 mm and only a few of them, 15-20 mm to 30 mm (Dell, 1990). And although among them there are known no one intertidal species and their habitats in the area of temperature fluctuations are small, but their size-corrected data would be much closer to those of the other analyzed buccinid, including tropical ones (*Phos senticosus*, *Cantharus fumosus*) and even an "anomalous" *C. lineolata*.

Buccinids of the northern hemisphere high latitudes found in the intertidal zone, for example - *Neptunea arthritica* (Valenciennes, 1858), *N.bulbacea* (Valenciennes, 1858), have thick (Th - 4.0-4.5mm, Th / D - 0.07-0.075) shell with a relatively large (up to 102 and 140 mm, respectively) size. For comparison *Neobuccinum eatoni* size 49 mm from our collection has a thickness of 0.6mm shells and Th / D - 0.02. Similar indexes are characterized by a number of members of the genus *Buccinum* of the Far Eastern seas deep shelf. In the northern hemisphere there are buccinid species with lower thickness of the shell, such as *Corneobuccinum lepidum* (Dall, 1918) which calcareous part of the shell is thin (<0.1 mm) layer under conchiolin periostracum. *Volutharpa ampullacea* (Middendorff, 1848)) also has the indices shell thickness smaller than that of the Antarctic *Neobuccinum eatoni* at similar depth range and close temperature conditions (0°C~6°C) in the habitat. The above-mentioned *N. arthritica* and *N.bulbacea* occur from the littoral, where fluctuations in temperature range from -1°C (winter) to +23°C (summer). The thick shelled species *N. arthritica* and *N.bulbacea* occur from the littoral where fluctuations in temperature range from -1°C (winter) to +23°C (summer) to the depths with near constant temperatures - 150m and 585m, respectively (Golikov, Kusakin, 1978). The thickness of their shells allows assuming that these species were formed in the littoral. Proof of this is that the maximum density (4.10 ind./M<sup>2</sup>) *N. arthritica* creates during the spawning season is in the intertidal zone.

Another species of (Watson et al., 2012) list is Antarctic limpet *Nacella concinna* (Strebel, 1908) which demonstrates great flexibility in this characteristic within a single genetically homogenous

circum-Antarctic population and is a good example of intraspecific differences in the thickness of the shell as an adaptation to the littoral environment. The limpet's intertidal and shallow subtidal individuals have shells 2-3 times thicker than those from deeper sites (Hoffman et al., 2010; Morley et al., 2010). According to our data (Bondarev, 2010b) not only the thickness but also the amount of *N. concinna* specimens in shallow water is much higher than in the subtidal zone. The highest abundance and biomass *N. concinna* confined to the lower boundary of the littoral zone, where they have maximum protection from predators (benthophagous fishes in the water and seagulls on the intertidal beach. In fish *Nototenia neglecta* stomachs the shells of semi-digested limpets were found as up to 35% of the whole content. Gulls eat the limpets whole and the shells are regurgitated later, often unbroken (Ralph and Maxwell, 1977). So thickness of the shell is a necessary adaptation to the littoral harsh environmental conditions, with its high-scaled temperature fluctuations - in the first place.

## 2 Conclusions

Many factors may affect skeleton sizes in marine ectoderms, including variation in predation pressure, resource availability, energy acquisition, and the effort required to extract calcium carbonate ( $\text{CaCO}_3$ ) from seawater (Watsson et al., 2012). However it should be borne in mind that between salinity, carbonate content and the thickness of the mollusks shells is no direct relationship. For each species there is an optimal salinity for shell growth. Most calcifiers capable effectively regulate the salinity and  $\text{Ca}^{2+}$  concentration in the liquid mantle with respect to the parameters of the surrounding water. For *R. venosa* excess salinity found to 13.75%.

Reducing the thickness of shells at increase salinity with depth, as shown by the example of the Black Sea species (*R. venosa* and *T. breviata*), indicates the priority of the temperature fluctuations of the factors of influence.

In the populations of *R. venosa* there are two opposing trends in the basin: an increase in thickness with age specific to individual development and the relative increase in thickness in individuals with smaller sizes in each age group. It can be argued that these trends are manifested in the interspecific level. In

comparative studies of geographical latitudes should be based not only on the systematically closely related species, but on the size - and ecologically similar ones.

Syntopic smaller sized individuals (and species of small size) tend to have higher rates of relative thickness than larger due to the thermoregulation or the possibility of mechanical damage including predators affect. "Excess" on the strength characteristics the relative shell thickness favors of thermoregulation.

The greatest temperature variations are typical for marine littoral zone. The inhabitants of this zone are characterized by the greatest relative thickness of the shell. In the BS and AS there is no littoral in the classical form as a zone between high tide and low tide. However, the living conditions in the shallow waters in some areas of the Azov- Black sea basin are close of World Ocean littoral conditions on the dynamics and temperature gradients. In these areas, *R. venosa* has the highest indicators of shell thickness. Burrowing mollusks in loose ground helps to soften the temperature gradients and partly compensates necessity of forming a thick shell.

When comparing the thickness of the shell fish populations and different types of the most versatile is the ratio of  $\text{Th} / \text{D}$ , which represents not only geometrical but strength characteristics.

It's correctly pointed out that both capacities for adaptation in rates of skeletal production and physiological (phenotypic) flexibility remain to be evaluated in high latitude marine species (Watsson et al., 2012). It must be added that the possible environmental and physiological causes and possible adaptations of the thickness of shells should be evaluated for all of species latitudes. As described above the range of this character values even within one species may vary considerably depending on environmental features in one geographical point to slightly different depths and different temperature regimes.

Obviously that the full grade analysis of shell thickness depending on the latitude or other large-scale studies should include as much many species of different environmental groups even within

one and the same family. It is necessary to take into account the ecological and evolutionary aspects of the analyzed taxa.

### 3 Materials and Methods

More the 2000 *R. venosa* specimens from different regions of the BS and AS (Figure 1) and 25 specimens from Sea of Japan (JS) examined. Depth range of sampling was 1-30 m. Most of the samples are collected author, but about 30% of the material from some areas and at depths of more than 10m collected by professional divers.

The bulk of the material obtained from the BS in the period from 2008 to 2012, a small part - in 1976-2000. Specimens of the JS gathered in the 1980s by divers. Gathering *R. venosa* specimens in the AS was made by dredge in 2006 and 2008 at depths of 5 to 10m.

Collection of samples carried out in areas with different salinity (Figure 1) on habitats with different bottom grounds and temperature conditions. It is possible to analyze the dependence of the thickness of the shell from a number of abiotic factors.

Gathering samples *T. breviata* implemented author and colleagues IBSS NASU from the board of RV "Professor Vodyanitsky" by box corer and dredging in 2008 to 2011 in the same areas as the collection of *R. venosa*, but at depths of 45-165 m, covering the area of this species inhabited.

To study the thickness of the shell in homogeneous environmental conditions has been selected base population inhabiting sandy ground biotope on the depth of 5-12 m in the Blue Bay of Sevastopol vicinity (Figure 1, area 5). In summer 2012 the author studied and collected *R. venosa* snorkeling in a mask and flippers at depths of 5-8 m. At these depths in the contact zone of sand and rocky outcrops *R. venosa* creates spawning concentrations in summer, facilitating mass gatherings. More then 600 specimens from this population were collected and analyzed. The choice of this population as a test for several reasons: the presence of a population of two size forms (typical and dwarf) and transitionally sized specimens, phenotypically different color lines (Bondarev, 2013) as well as variations with different shell shape. Such phenotypic diversity revealing some genetic differences, which suggests that the universal or

different reactions of the morphs on factors of influences in the field a quasi-uniform background environmental conditions.

The possible impact of predators was assessed by comparing the type of shells collected damage with published information from the literature with respect to *R. venosa* (Harding, 2003), some closely related species (Hughes and Elner, 1979; Donovan et al., 1999), and some other gastropods (Boulding et al., 1999; Palmer, 1979, 1985; Trussell, 1996).

Linear dimensions were recorded with dial calipers ( $\pm 0.1$  mm) for maximum size - shell height (H), the maximum width or diameter (D), height of the mouth (ha) and shell thickness (Th).

The measurements made in individuals with completed formation to the maximum thickening of the annual cycle edge of his lips. The greatest thickness (sometimes exceeding twice the average) outer lip of *R. venosa* shell has junction with the parietal area. The minimum thickness of the outer lips is in the transition zone to the basal part where the shell traumatized more often (Figure 7). Shell thickness (Th), we take the dimension that corresponds to the average thickness of the edge of the aperture. Measurement location is on shell outer lip between shoulder edge spiral rib and the next to him abapically as shown on Figure 10.

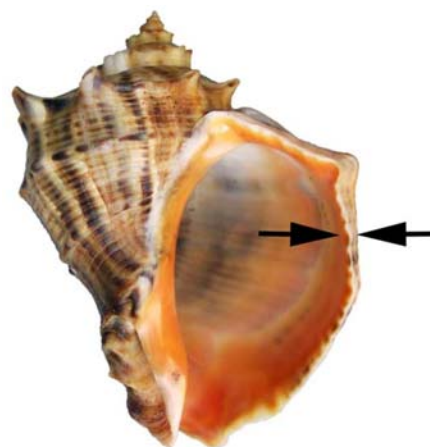


Figure 10 *R. venosa* thickness (Th) measurement points (shown by the arrows)

On the same site was measured thickness I. Govorin (personal communication), who has studied this parameter of *R. venosa* shell, depending on the salinity

of some BS areas (Govorin, 2009), that allows us to compare our results. The use in his and the present work relative to the height of shell thickness parameter (Th / H) increases the ability to compare our data.

Direct measurement of Th and D allows carrying out mass measuring quickly and obtaining statistically reliable data for comparative analysis of different populations and species.

Individual age *R. venosa* specimens determined based on annual spawning marks on the shell as described (Bondarev, 2010a).

The process of sampling was detected the depth, ground type, water temperature and salinity at the time of collection. For habitat range specified of temperature and salinity on seasonal and long-term data were taken from the oceanographic literature (Goptarev et al., 1991, Simonov et al., 1992). In collection sites salinity was determined by various traditional methods. In August 2013 we measured salinity in the Round Bay (Sevastopol) and intrapallial fluid salinity of 12 *R. venosa* specimens from the same place by conductimetry. The total error in the method for determining salinity and equipment does not exceed 2%.

For comparative studies of the shells characters from different regions of the World Ocean author used material from his own work shell collection and the of fund collection IBSS NASU.

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