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MARINE BIOLOGY

Determination of *Rapana venosa* Individuals' Ages Based on the $\delta^{18}O$ Dynamics of the Shell Carbonates

A. R. Kosyan and Zh. A. Antipushina
Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow
E-mail: kosalisa@rambler.ru

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Abstract—The results of the analysis of the stable oxygen isotopes content in shell carbonates for the purpose of the individual age determination of the *Rapana venosa* gastropod (Valenciennes 1846) are presented. The data acquired via this method agreed with the number of spawning marks on the shell surface. Additionally, the duration of the seasonal extrema can indicate the velocity of the shell's growth throughout the mollusk life, and the relative $\delta^{18}O$ value in the shell's near-apex increments, the season when a young shell comes out of its cocoon. It has been shown that the common method of growth rings counting at the operculum is unreliable for a shell's age determination. The distinguishing of modal classes is ineffective as well, since the corridor of the variations in size for coeval individuals is very broad. Apparently, such variable characteristics as the growth rate, the wall's thickness, and the relative weight are greatly dependent on the alimentary conditions, which are unequal even in the same biotope.

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INTRODUCTION

To assess the ecological state and dynamics of a population, one must know the individual ages of the organisms. Unfortunately, this is still an issue with respect to marine gastropoda. Such methods as the determination of the growth rate in an aquarium, finding of the coefficients in von Bertalanffy growth equation [17], and the examination of ultrastructures of otoliths and operculum [5, 15] are very laborous. Due to this, the most often used method is the distinguishing of modal classes in histograms of the size frequency distribution [11] through the counting of the increment rings at the operculum. The counting of rings is a simple but insufficiently reliable method based on the study by Santarelli and Gros [16] for gastropoda of the *Buccinum* genus. This method was also applied for the age determination of *Busycon* and some other neogastropoda [14]. The application of this method for rapana's age determination is complicated by the fact that the previous rings are skinned by a corneous layer in the bottom simultaneously with the formation of new rings along the upper edge of the operculum, whereas these rings are completely hidden in old individuals [3].

Another technically simple method of age determination is related to counting of the vertical marks on the shell; they are formed on the shells of many Bivalvia and Gastropoda during the winter growth stoppage [2, 6]. On rapana shells, these marks are formed as a result of the growth stoppage during the reproduction period and are called spawning ones. The character of the marks' formation and their correspondence to age are described in detail by Chukhchin [3]. In the normal state, the rapid growth in the first years of life is followed by gradual deceleration, [1, 3, 4] and the growth rates of old individuals are so slow that the marks are located very close to each other; hence, the precise determination of their number becomes impossible. Rapanas living in an unfavorable ecological environment have both decelerated growth rates [4] and often corroded shells, which also creates difficulties in count of the spawning marks. In addition to this, the external rim of a shell is easily damaged, for example, due to crab attacks [8], and traces of postattack recovering are often indistinguishable from spawning marks. Despite all these difficulties, the count of spawning marks on shells with one–two years added (beginning of the puberty age) is the most reliable field method for the age determination in the opinion of some authors [1, 3].

In the recent time, the analysis of the stable oxygen isotopes $^{16}O/^{18}O$ in the carbonate of shells has become more widely used for age determinations. It is known that the ratio between the stable oxygen isotopes $\delta^{16}O$ and $\delta^{18}O$ in organogenic carbonates depends on the water temperature. An increase in temperature reduces the fractionation between the isotopes, which leads to a decrease in the content of the heavier isotope $\delta^{18}O$ [7]. This method is most often applied to subfossil and modern Bivalvia for the reconstruction of the paleotemperature dynamics [12]. This isotopic analysis is much more rarely used for the determination of the individual age of gastropoda [15, 16]. The present work attempts to compare the results of the isotopic

The parameters of the collected shells (H is the height of the shell, D is the diameter of the shell, Nop is the number of rings at the operculum, Nsp is the number of spawning marks)

Spec. nos.	Depth of sampling, m	Date of sampling in 2009	Gender	H/D, mm	Total weight, g	Shell weight, g	Nop/Nsp	Age, yrs.
Tuzla spit								
1	3–5	30.06	?	91.5/70.6	108.2	54.0 (50)	5/4	6
2	3–5	30.06	?	52.9/36.8	25.9	14.8 (57)	5/4	6
Orlenok								
3	1	1.09	♂	36.1/28.0	9.0	7.4 (83)	5/5	6
Golubaya Bay								
4	20	12.06	♀	43.2/31.6	12.1	9.8 (81)	4/3	5
5	20	12.06	♂	41.3/31.0	17.7	14.7 (83)	5/5	7
6	20	12.06	♀	40.4/28.5	15.0	12.5 (83)	5/?	5
7	20	12.06	♂	41.4/28.4	12.5	9.9 (79)	4/?	5

Note: The share of the shell's weight of the total weight of an individual is given in parentheses.

analysis with the counting of the spawning marks in order to determine the individual age of rapanas.

MATERIALS AND METHODS

The studied material was taken in the Russian sector of the Black Sea in June and September of 2009. The sampling was performed in three biotopes that differ in terms of the ground and alimentary conditions: (1) at Tuzla spit (sand, the alimentary objects are large *Anadara* sp., *Chamelea gallina*, and *Mytilus galloprovicialis*, 100 specimens were sampled, 2 were taken for the analysis); (2) in the coastal zone of the All-Russian Children's Center Orlenok (rocks and sand, the alimentary objects are *Chamelea gallina* and *Anadara* sp. and small mussels, 90 specimens were sampled, one was taken for the analysis); (3) in Golubaya Bay of Gelendzhik city (rocks, the alimentary objects are small mussels, 160 specimens were sampled, 4 were taken for the analysis).

Shells were cleaned and then their height and diameter were measured, as well as the weights of an entire individual and an empty shell without the soft body of the mollusk. The sampling of the carbonate was performed with a 2–4 mm step along the upper part of the whorl (shoulder) starting from the edge of aperture and finishing by the apex. A microdrill 1 mm in diameter was used (Fig. 1e). For the analysis, carbonate weighings of 150 μg on average were used.

The analysis of the stable oxygen isotopes was performed via the standard technique [9]. The weighings were treated with concentrated orthophosphorus acid (H_3PO_4) at 50°C. The analysis of the isotopic composition of the released carbon dioxide was performed at the Thermo-Finnigan DELTA-V Plus mass spectrometer (Severtsov Institute of Ecology and Evolution). The analysis was implemented in a double-repeated manner; the total number of examined specimens is 390, and that of laboratory standards NBS-19 and MCA-7 is 60. The standard deviation for $\delta^{18}O$ did not exceed 0.045‰. All the results are given in delta $(\delta)^{18}O$, ‰ = (value of the specimen – value of the standard)/(value of the standard) × 1000 [7], where the correlation of the isotopes $\delta^{16}O/\delta^{18}O$ is given relative to SMOW.

RESULTS

Tuzla spit. The shells of both individuals (Figs. 1a–1d and 1f; table) contain four spawning marks that corresponds to the age of six years. In the graphs of the seasonal $\delta^{18}O$ dynamics for the shell of specimen no. 2 (Fig. 2), one can see six summer minimums (indicated by vertical solid lines) with spawning marks near them (vertical dashed lines). Thus, the age determined by the $\delta^{18}O$ dynamics coincides with that derived through counting of the spawning marks. The interpretation of the graph of the seasonal $\delta^{18}O$ dynamics for specimen no. 1 (Fig. 2) is complicated since the summer mini-

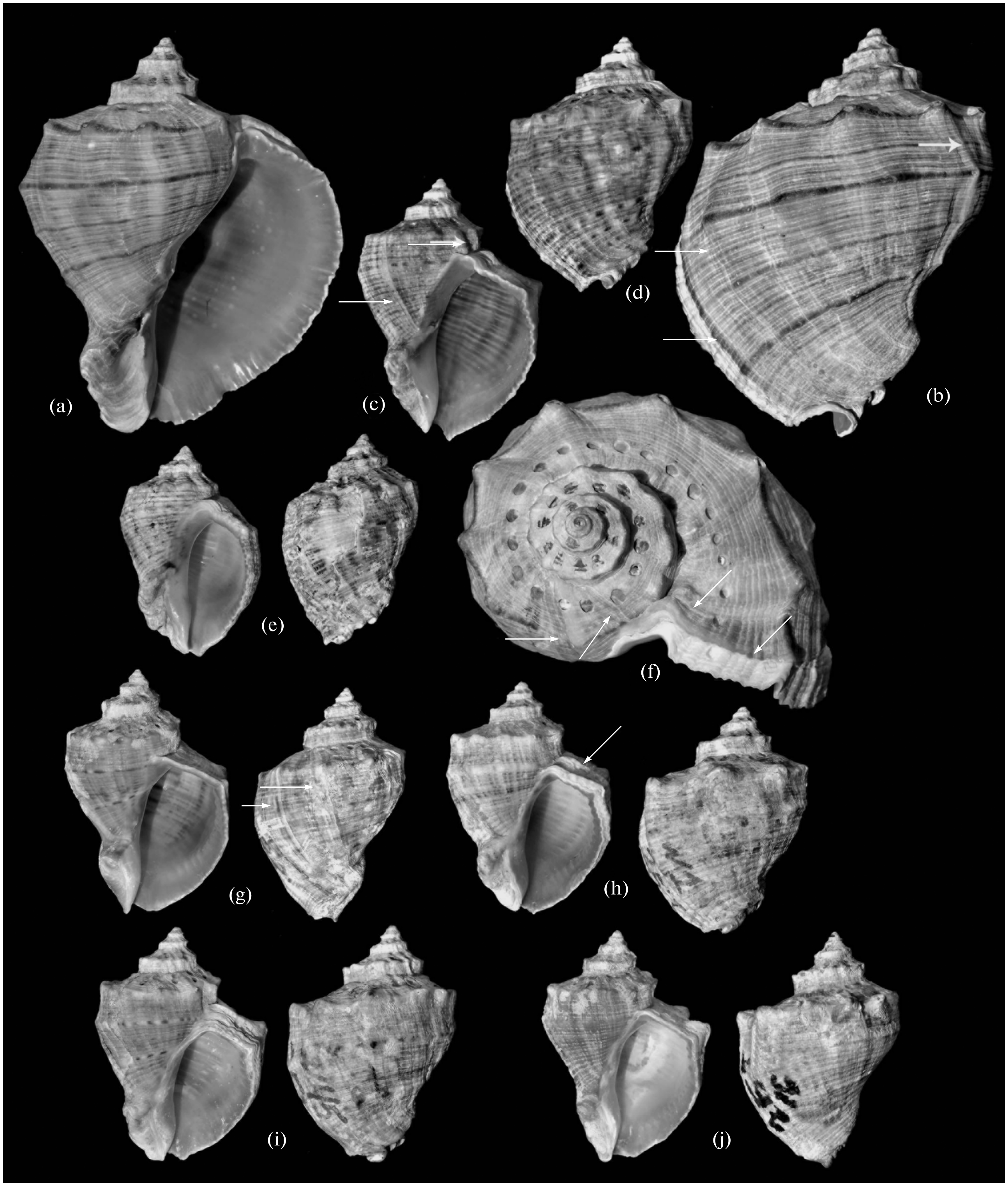

Fig. 1. Rapana shells used for the isotopic analysis of the carbonates: (a), (b) specimen no. 1 from Tuzla spit; (c), (d) specimen no. 2 from Tuzla spit; (e) specimen no. 3 from Orlenok; (f) example of the drilling points location on the shell of specimen no. 1; (g) specimen no. 4 from Golubaya Bay; (h) specimen no. 5 from Golubaya Bay; (i) specimen no. 6 from Golubaya Bay; (j) specimen no. 7 from Golubaya Bay. The arrows show the spawning marks.

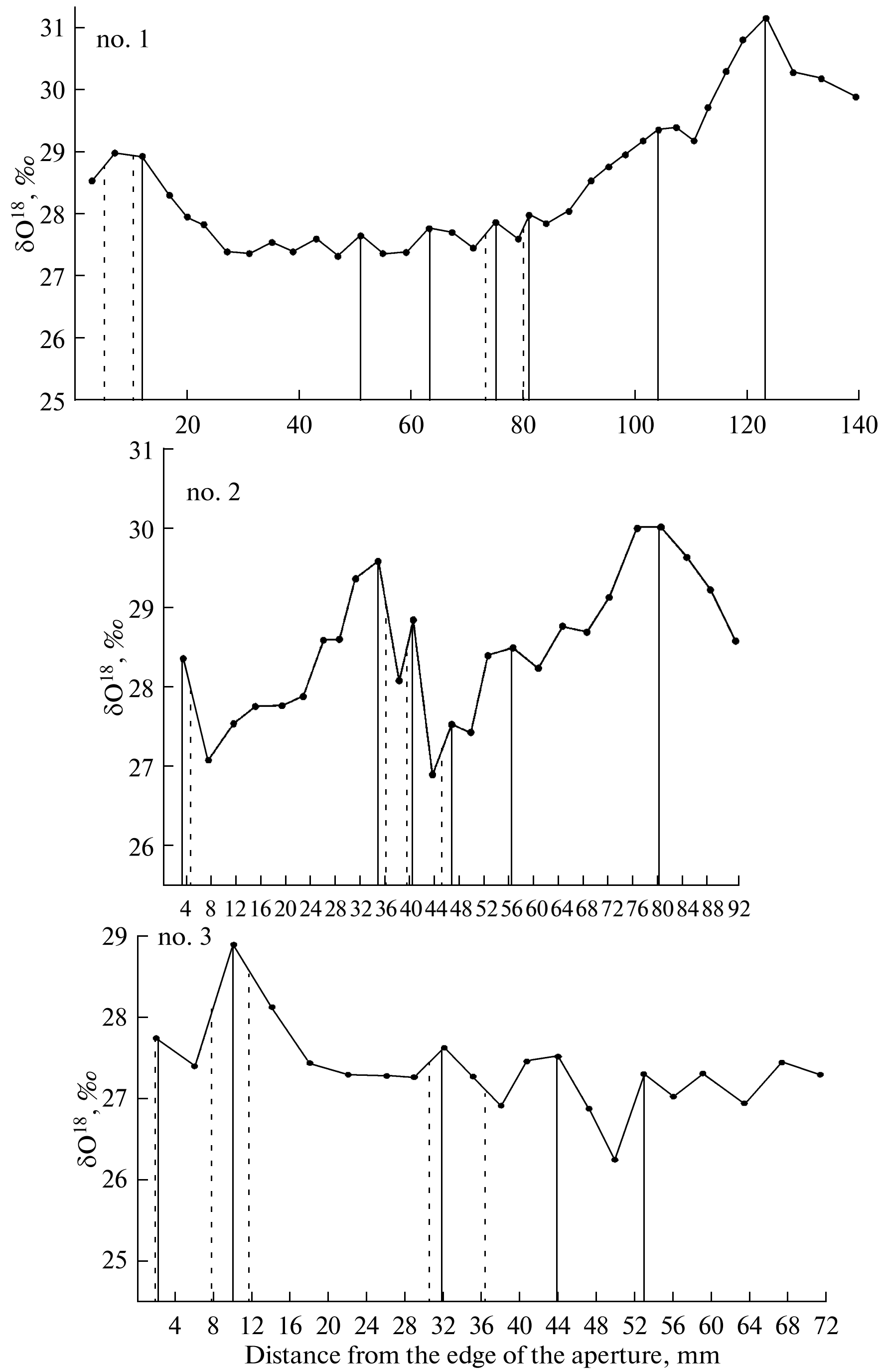

Fig. 2. Seasonal dynamics of $\delta^{18}O$ in rapana shells from Tuzla spit (nos. 1 and 2) and from Orlenok (no. 3). The dashed lines indicate the spawning marks, while the solid lines, the boundaries between the year increments.

mums are poorly expressed. Most probably, the results were affected by the method of the carbonate sampling. For specimen no. 1, the samples were taken at the maximal distance from each other and from a larger part of the shell (Fig. 1f), which might cause mixing of the carbonates from various seasons and the omitting of some seasons.

Orlenok. On specimen no. 3 (table), we found five poorly expressed spawning marks. In the graph of the seasonal $\delta^{18}O$ dynamics, one can see four clearly and two poorly expressed minimums of the $\delta^{18}O$ values (Fig. 2). Obviously, in the two last cases, the sample contains carbonates from various seasons. The age of this individual is six years.

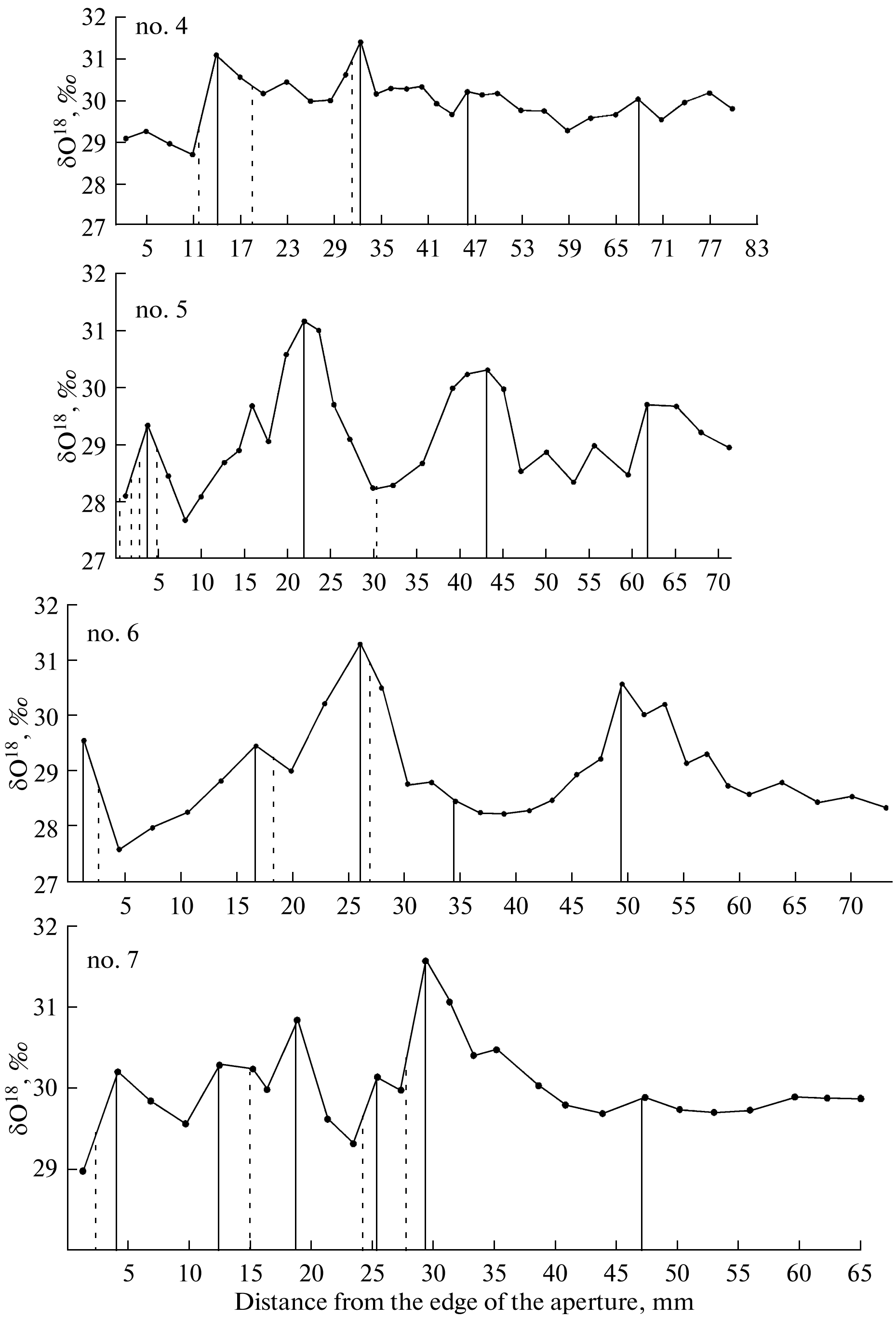

Fig. 3. Seasonal dynamics of $\delta^{18}O$ in rapana shells from Golubaya Bay (nos. 4–7). The dashed lines indicate the spawning marks, while the solid lines, the boundaries between the year increments.

Golubaya Bay. Four individuals taken in Golubaya Bay had shells of nearly equal size (table) with highly eroded surfaces. The number of spawning marks was possible to count only for specimen nos. 4 and 5. In the graph of the seasonal $\delta^{18}O$ dynamics for specimen no. 4, one can see clearly expressed three local minimums corresponding to the age of puberty (Fig. 3). The number of spawning marks in specimen no. 4 (Fig. 1e) is also three. Thus, the individual's age is about five years.

Specimen no. 5 (Fig. 1g) has the last four spawning marks located very close to each other between edge of the aperture and the third sampling point; hence, the several last seasonal increments of the shell were omitted when drilling and were not expressed in the graph (Fig. 3). The rest of the graph contains very explicit

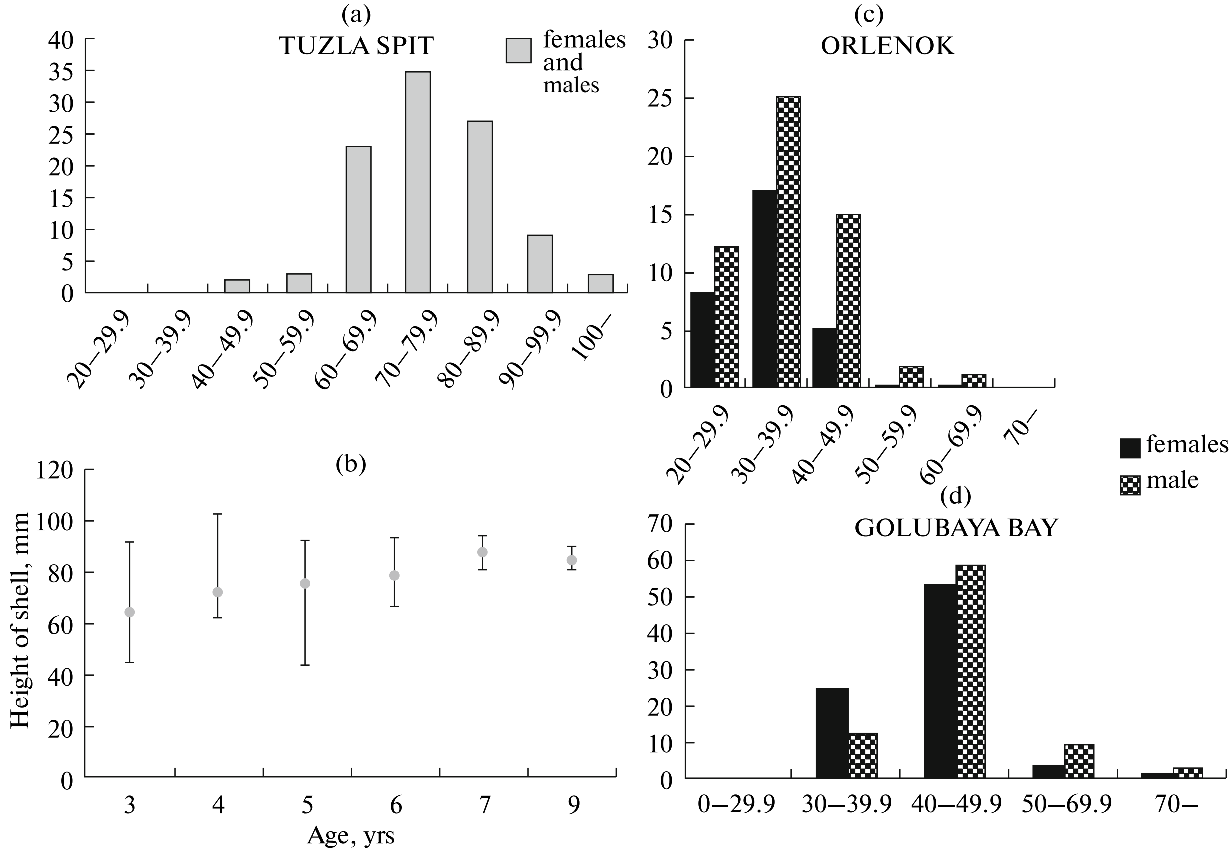

Fig. 4. Population characteristics of rapanas from three sampling sites: (a) size structure of the Tuzla spit population; (b) size–age structure of the Tuzla spit population (the boundaries of the segments are the maximal and minimal heights of the shells in the coeval sampling; the gap in a line marks the average height of the shells from a sampling); (c) size structure of the Orlenok population; (d) size structure of the Golubaya Bay population. The height of the shell (mm) is along the abscissa.

seasonal $\delta^{18}O$ variations. Summarizing all these data, we derived the individual age as seven years.

Specimen no. 6 has more axial dashes similar to spawning marks on its surface than the seasonal $\delta^{18}O$ variations found. In the graph (Fig. 3), we denoted as spawning marks only those which coincide with found seasonal variations. The fourth winter may be absent in the graph since there was a break of the lip there and the shell's fragment corresponding to found winter period was lost. Nine points located between 30 and 47 mm along the abscissa axis correspond to found seasonal minimums of two years. Thus, the age of this shell is supposedly five years.

The spawning marks on specimen no. 7 are virtually indistinguishable. The number of seasonal minimums in the graph (Fig. 3) is five; thus, the individual age of the shell is determined as five years.

We also counted the number of growth rings at found aperture (table). The comparison of the results acquired through different methods showed that the number of rings did not reflect the individual age of the rapanas and the coincidences found for shell no. 6 were most probably random. Distinguishing of the size classes is also ineffective since the growth rates are very different and the corridor of the variations in size for coeval individuals is very broad. For example, Fig. 4 presents the size–age structure of the population from the Tuzla spit.

DISCUSSION

Notably, the sizes of coeval individuals living both in different and in the same biotope may substantially differ; e.g., shell no. 3 from Orlenok and no. 1 from Tuzla spit differ by 2.5 times in terms of their height, whereas the shells from Tuzla spit (table) differ by nearly twice despite being the same age (6 years). One of the probable causes of such a significant difference in the sizes is the living conditions of the rapanas during the first two years after come out of their cocoons (with the leading factor played by the alimentary conditions) since this is the period of the maximal growth; e.g., by the age of puberty, the linear sizes of the shells (in Figs. 2 and 3, this is distance along the abscissa from the apex to the first spawning mark) from the Tuzla spit population reached 60 mm for no. 1 (about 40% of the total length of the whorl) and 45 mm for no. 2 (50%) (Fig. 2). The linear sizes of shell nos. 3–7 by the two-year age were only 37, 38, 40, 38, and 37 mm, respectively; it is about 50% of the total length for the shell from Orlenok and more than 60% for those from Golubaya Bay (Figs. 2 and 3). In further, the growth of the shell decelerated but unevenly: years of fast growth alternated with those of a slow increment.

Augments of the same year period after the age of puberty also vary for individuals from the same biotope; e.g., shell no. 5 from Golubaya Bay has an increment of about 2 mm, whereas no. 4 has the last year augment of 12 mm; nos. 6 and 7 have the penultimate year augments of 16 and 12 mm, respectively (Fig. 3). Two specimens from Tuzla spit, despite their simultaneous living in the same biotope, have unequal augments of the same years as well.

Slowly growing individuals demonstrate an increase in the relative weight of their shell in comparison to rapidly growing ones [13]. It seems that the growth rate and thickness of the shell walls much depend first of all on the alimentary conditions, which are unequal even in one biotope, and on the individual's physiological peculiarities as well. A similar dependence was found in an experiment with litorines [10]: as the available food reduced, the thickness of the shell and the weight share in the total weight of the organism increased.

As was told above, the extereme right parts of the graphs correspond to the first years of the rapana's life, and the ordinate of the point, which is the most distant from the lip of the aperture (and the closest to the apex, at that) can indicate the season when the young rapana came out from its cocoon. The reproduction season of rapana in the Russian sector of the Black Sea lasts from the end May to October (in the Anapa area, one-third of the 160 rapanas taken on September 26, 2010, were spawning and many shells had new-formed cocoons on them). Naturally, the descendants of the individuals who copulated in the early reproduction period would come out from their cocoons in late June–early July, while, for those who copulated in the end of this period, in late October–early November. In July, the water temperature in the coastal zone usually reaches its annual maximum; hence, the $\delta^{18}O$ value in the shells of the young rapanas that appeared in this early period would be minimal. In October–November, the water temperature decreases by 4–6 degrees on average, which leads to a higher $\delta^{18}O$ value in the shells of the rapanas that appeared in this late period. Thus, we can suggest that four of the seven examined rapanas came out from their cocoons in the summer (nos. 1, 4, 5, 7), whereas three of them appeared in the autumn (nos. 2, 3, 6). For specimen nos. 4 and 7, the juvenile part of the graph (Fig. 3) has smoothed seasonal variations of the isotopic signal. This may be related to living at greater depths where the conditions are more stable (in the Black sea, seasonal variations in the temperature become very poorly expressed as early as the 30 m depth).

CONCLUSIONS

It has been found that the method of growth rings counting at operculum does not reflect the individual age of rapana. Distinguishing of modal classes is ineffective as well since the growth rates are very different and the corridor of the variations in size for coeval individuals is very broad. Counting of spawning marks yields a good result, but it is inapplicable when the surface of a shell is corroded or damaged. In this case, supplementary methods are required, for example, the analysis of the content of the stable oxygen isotopes in the shell carbonates.

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