

THE INFLUENCE OF MARINE ANOXIA ON PRECIPITATION OF *MYTILUS GALLOPROVINCIALIS* SHELL CARBONATE IN THE COASTAL ZONE OF THE ROVINJ BAY (NORTHERN ADRIATIC)

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ABSTRACT

The results of this study suggest changes in shell growth structure and in the isotopic, as well as chemical composition of Mytilus galloprovincialis shells due to anoxia in the coastal zone of the Rovinj Bay, Northern Adriatic. The outer aragonitic "stress layer" of Mytilus galloprovincialis shells formed during pronounced summer anoxia due to intensive phytoplankton bloom in the years 1989-1991 is massive, pink coloured and out of oxygen and carbon isotopic equilibria. It also exhibits higher C/O ratios relative to the uncoloured aragonitic layer precipitated by Mytilus galloprovincialis living in normal oxic conditions.

Key words: *Mytilus galloprovincialis*, biomineralization, stable isotopes, anoxia, Rovinj Bay, Northern Adriatic

INFLUENZA DELL'ANOSSIA MARINA SULLA PRECIPITAZIONE CARBONATICA DELLA CONCHIGLIA DI *MYTILUS GALLOPROVINCIALIS* NELLA ZONA COSTIERA DELLA BAIJA DI ROVIGNO (ADRIATICO SETTENTRIONALE)

SINTESI

I risultati di questo studio evidenziano variazioni nella struttura di crescita e nella composizione sia isotopica che chimica delle conchiglie di Mytilus galloprovincialis, dovute ad anossia nella zona costiera della baia di Rovigno, Adriatico settentrionale. Lo strato esterno di aragonite delle conchiglie di Mytilus galloprovincialis, formatosi durante una marcata anossia estiva dovuta ad un'intensiva fioritura fitoplanctonica negli anni 1988-1991, è massiccio, di colore rosa e non rispetta l'equilibrio isotopico di ossigeno e carbonio. Questo strato esibisce, inoltre, frazioni C/O più alte rispetto a quelle dello strato di aragonite incolore, precipitato da Mytilus galloprovincialis in condizioni normali di ossigeno.

Parole chiave: *Mytilus galloprovincialis*, biomineralizzazione, isotopi stabili, anossia, baia di Rovigno, Adriatico settentrionale

INTRODUCTION

Biom mineralization is a complex combination of biochemical and physiological processes, depending on the endogenous activity of the organism and exogenous environmental influences. However, the influence and interaction between environmental factors, such as lack of oxygen and biomineralization, have been insufficiently investigated so far. This paper reports on the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values measured in selected pink coloured shell layers taken from specimens of *Mytilus galloprovincialis*, which were found growing in the coastal zone of the Rovinj Bay, Northern Adriatic, during anoxic conditions caused by intensive phytoplankton bloom in the years 1989-1991. These data were used to make interpretations regarding alterations in biomineralization processes influenced by environmental changes.

MATERIALS AND METHODS

Determination of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ was performed on separate shell layers using a Varian MAT 250 mass spectrometer. Organic matter was eliminated prior to the determination of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ by roasting the molluscan carbonate powder under vacuum for 1 hour at 380°C. Thereupon the carbonate powder was reacted with > 100 % H_3PO_4 at 25°C (McCrea, 1950). CO_2 gas released during acid treatment was cryogenically cleaned and analyzed for O and C isotopic composition. Data quality was maintained in part through frequent comparisons against internationally recognized isotopic standards and frequent processing of secondary laboratory standards. Before acid treatment, the mineralogical composition of the selected layer powder was checked by X-ray diffraction to determine the aragonite/calcite ratio. All results reported here are for samples of > 95 % aragonite or > 95 % calcite. Results are expressed relative to PDB for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. The average difference of duplicate analyses was about 0.1 ‰ for oxygen and 0.09 ‰ for carbon.

For characterization of the microstructure and composition of *Mytilus galloprovincialis* shells, SEM and EDS analyses were applied. A JEOL JSM 5800 SEM equipped with a Link ISIS 300 EDS was used for overall analyses. Quantitative analyses were performed using SEM Quant software and a virtual standard package-library (VSP). Measured peak intensities in the spectra were corrected and quantified using a ZAF matrix correction program. In the case of oxygen, semi-quantitative estimation is possible only by comparison between the peak areas of oxygen peaks and different spectra without exact quantification. The relative random errors of EDS were less than 3% for major and selected minor oxides.

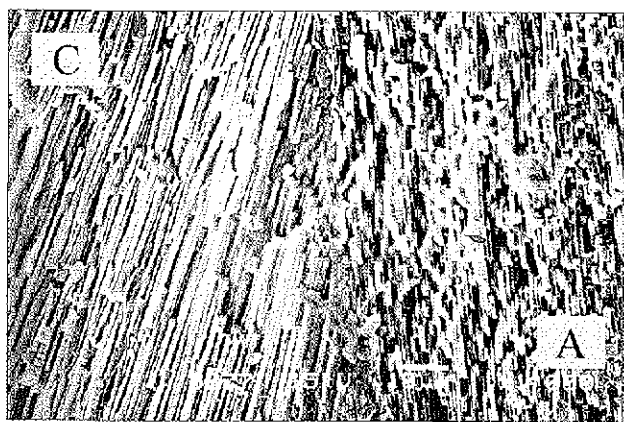


Fig. 1: SEM photo of calcite fibres (C) and prismatic nacreous aragonite (A) layer in *Mytilus galloprovincialis* shell.

Sl. 1: Elektronski (SEM) posnetek apnenčastih vlaken (C) in prizmatičnega biserovinastega aragonitnega sloja (A) pri lupinicah školjke *Mytilus galloprovincialis*.



Fig. 2: SEM photo of the visible difference in morphology between the uncoloured (U) and pink (P) parts of the aragonite layer of a *Mytilus galloprovincialis* shell structure.

Sl. 2: Elektronski (SEM) posnetek vidnih morfoloških razlik med neobarvanim (U) in rožnatim (P) delom aragonitnega sloja v sestavi lupinic školjke *Mytilus galloprovincialis*.

RESULTS AND DISCUSSION

SEM analyses showed that *Mytilus galloprovincialis* has an outer shell layer composed of calcite fibres and an inner nacreous shell layer, which is porcelaneous in appearance. Single fibres of the outer layer are approximately 1.5 μm thick and are inclined towards the shell exterior (Fig. 1). The inner layer is composed of equidimensional, horizontally flattened aragonite prisms about 10 - 20 μm wide, with their long axes oriented perpendicular to the shell surface. In pink coloured

shells, the inner layer is roughly divided into two sublayers. The inner uncoloured white sublayer consists of the same structure of flattened aragonite prisms, while the outer sublayer, up to 900 μm thick, is pink and composed of irregular aragonite grains and shows a massive structure (Fig. 2). These aragonite crystals are supposed to be precipitated during stress conditions and more rapidly than uncoloured ones.

Comparison between peak areas of the oxygen and carbon peaks shows that the pink coloured aragonite contains more carbon than oxygen relative to the uncoloured aragonite or calcite (Figs. 3, 4). The C/O ratio of the pink layer is 1.4 and 2.3 while that of the uncoloured aragonite from uncoloured and coloured shells varies between 0.9 and 1.2. Similar C/O values of 0.98 and 1 were also measured in calcite fibres. We believe that the high C/O ratio of the pink aragonite layer indicates anoxic conditions during its precipitation.

The *Mytilus galloprovincialis* shell carbonates show variation of $\delta^{18}\text{O}$ in the range between + 0.07 and + 2.21 ‰ and $\delta^{13}\text{C}$ between + 0.18 and - 1.31 ‰ (Tab. 1). In uncoloured shells, the calcitic layer is either slightly enriched in the heavy oxygen isotope relative to the inner aragonite layer or contains more or less the same quantity of heavy oxygen isotope as the aragonite one. Biogenic aragonite has been reported as having both ^{18}O depletion (Tarutani et al., 1969; Horibe & Oba, 1972) and enrichment (Sommer & Rye, 1978; Grossman, 1982) compared to biogenic calcite. The

magnitude of the ^{18}O enrichment of aragonite relative to mollusc calcite was not temperature dependent (Grossman & Ku, 1986). In the coloured shell the uncoloured aragonitic sublayer exhibits a higher $\delta^{18}\text{O}$ value than the pink aragonitic sublayer and/or calcitic layer. The 1.22 ‰ enrichment in $\delta^{18}\text{O}$ of the uncoloured aragonite sublayer relative to the pink layer would translate into a change of 4.5°C of seawater temperature using the Grossman & Ku (1986) equation. Such large changes in ambient seawater temperature most probably indicate a temperature-induced seasonal $\delta^{18}\text{O}$ signal as well as isotopic disequilibrium. The pink aragonite layer was formed between July and August during seawater anoxia when the ambient water was warmer.

The $\delta^{13}\text{C}$ of mussel carbonates vary between 0.18 and - 1.26 ‰. In all investigated samples the calcite layer contained more light carbon ^{12}C isotope than the aragonite one. The isotopic difference between the calcite and aragonite layers most probably corresponds to different equilibrium fractionation in the calcite-water and aragonite-water systems. Aragonite was found to be enriched by 1 ‰ in ^{13}C relative to coexisting calcite at the relevant temperatures (Grossman, 1984; Grossman & Ku, 1986). In the coloured shell the pink aragonite sublayer was enriched (up to 0.91 ‰) in light ^{12}C relative to the uncoloured aragonite sublayer. It is interesting to note that there is a negative correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ for the fibrous calcitic layers and a positive one for aragonitic layers (Fig. 5).

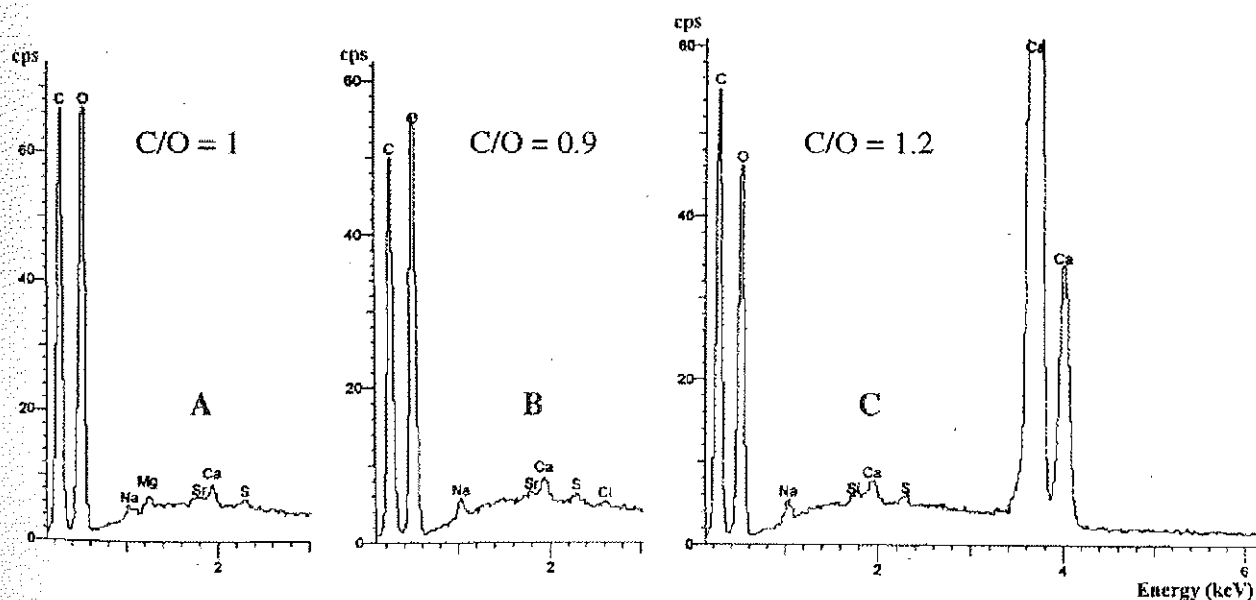


Fig. 3: Energy Dispersive Spectroscopy (EDS) spectra and C/O ratio of an uncoloured *Mytilus galloprovincialis* shell showing only small differences in the maxima of the oxygen and carbon peaks between the carbonate layers. (A) fibrous calcitic layer, (B) outer part of the aragonitic layer, (C) inner part of the aragonitic layer.

Sl. 3: EDS-spektri in C/O-razmerje neobarvane školjke *Mytilus galloprovincialis* kažejo le manjše razlike v največjih vsebnostih kisika in ogljika med dvema karbonatnima slojema. (A) vlaknati apnenčasti sloj, (B) zunanji del aragonitnega sloja, (C) notranji del aragonitnega sloja.

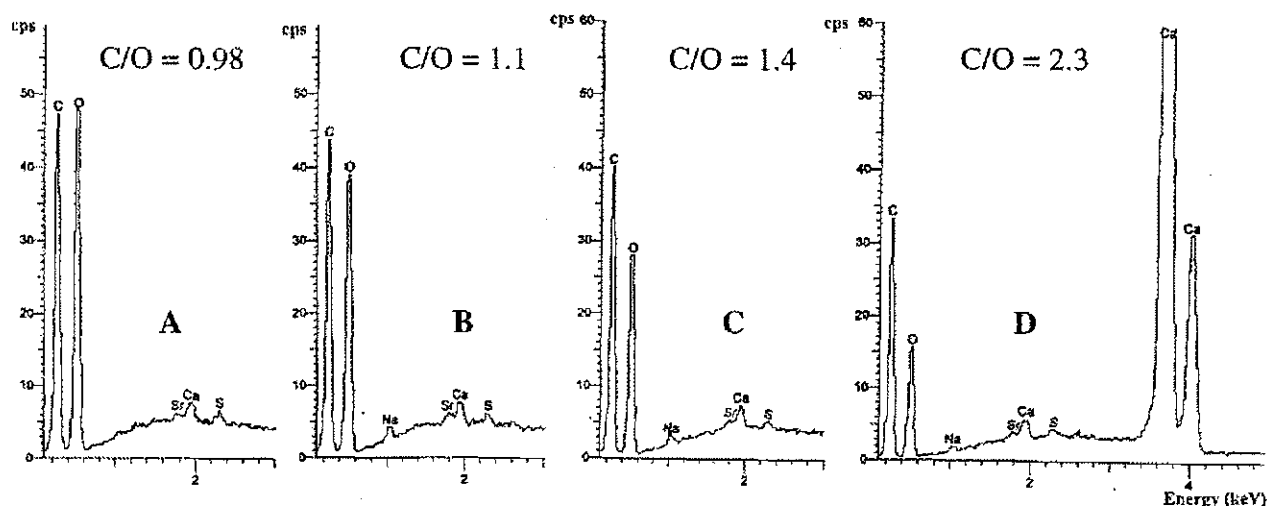


Fig. 4: Energy Dispersive Spectroscopy (EDS) spectra and C/O ratio of pink coloured *Mytilus galloprovincialis* shell showing appreciable differences in the maxima of the oxygen and carbon peaks between different carbonate layers. (A) fibrous calcitic layer, (B) uncoloured aragonitic sublayer, (C) inner part of pink aragonitic sublayer, (D) outer part of pink aragonitic sublayer.

Sl. 4: EDS-spektri in C/O-razmerje rožnato obarvanih lupinic školjke *Mytilus galloprovincialis* kažejo na znatne razlike v največjih vsebnostih kisika in ogljika med dvema karbonatnima slojema. (A) vlaknati apnenčasti sloj, (B) neobarvani aragonitni podsloj, (C) notranji del rožnatega aragonitnega sloja, (D) zunanji del rožnatega aragonitnega sloja.

Tab. 1. Stable oxygen and carbon isotope composition of *Mytilus galloprovincialis* shell carbonates.

Tab. 1: Izotopska sestava kisika in ogljika v karbonatih lupinic školjke *Mytilus galloprovincialis*.

Sample	$\delta^{18}\text{O}$ (PDB ‰)	$\delta^{13}\text{C}$ (PDB ‰)	Remarks
R-202 a	1.65	- 1.04	calcite
R-202 b	1.04	- 0.32	aragonite
O-204 a	1.13	- 0.84	calcite
O-204 b	1.39	0.18	aragonite (u)
O-204 c	0.07	- 0.73	aragonite (p)
M-202 a	1.01	- 1.31	calcite
M-202 b	0.98	- 0.14	aragonite (pk)
S-1 a	2.21	- 1.26	calcite
S-1 b	1.22	- 0.15	aragonite
ST-1 a	0.33	- 0.77	calcite
ST-1 b	0.81	- 0.19	aragonite

R-202, S-1, ST-1 (uncoloured *Mytilus galloprovincialis* shells), O-204, M-202 (pinkly to pink coloured *Mytilus galloprovincialis* shells), (u) - uncoloured, (p) -pink, (pk) -pinkly

R-202, S-1, ST-1 (neobarvane lupinice školjke *Mytilus galloprovincialis*), O-204, M-202 (rahlo rožnato do rožnato obarvane lupinice školjke *Mytilus galloprovincialis*), (u) -neobarvane, (p) - rožnate, (pk) - z rožnatim nadhorn

The water temperature, salinity, and the total dissolved carbon (TDC) content have been recognised as major factors controlling the oxygen and carbon isotope composition in shell carbonate of marine invertebrates (Anderson & Arthur, 1983; Rosenberg, 1980). Molluscs, in general, are believed to exert only a minimal vital effect over their isotopic composition (Jones, 1985), and thus their isotopic values are representative of environmental water conditions. However, calcium carbonate may not always be precipitated in equilibrium with the environment, and in such cases stable isotope analyses of the shell carbonate may be an unreliable technique for environmental reconstruction. Isotopic disequilibrium may be in part due to metabolic effects or kinetic effects that are inherent in fast-growing shells, or areas of shell (Mitchell *et al.*, 1994). Many molluscs use aragonite to build their shell, but calcitic molluscs and those with mixed mineralogy also are abundant. Because aragonite is found to be enriched by 0.7 ‰ in ^{18}O and by 1 ‰ in ^{13}C relative to coexisting calcite at the relevant temperatures (Grossman, 1984; Grossman & Ku, 1986) the calcitic and aragonitic layers in molluscs shells with mixed mineralogy must be sampled and treated separately. In shells with mixed aragonite-calcite composition, low temperatures are favourable for deposition of calcite, while warm waters favour the formation of aragonite (Schifano & Censi, 1986).

The calculated temperature for *Mytilus galloprovincialis* shell calcite according to the equation of Craig (1965):

$$T[^\circ\text{C}] = 16.9 - 4.2(\delta_C - \delta_W) + 0.13(\delta_C - \delta_W)^2$$

(where δ_C is the oxygen isotopic composition of calcite vs. PDB and δ_W is the oxygen isotopic composition of the water from which the calcite was precipitated vs. SMOW), range from 11.8 to 19.8°C, while that of aragonite calculated according to Grossman & Ku (1986):

$$T[^\circ\text{C}] = 21.8 - 4.69(\delta^{18}\text{O}_{\text{aragonite}} - \delta^{18}\text{O}_{\text{water}})$$

is between 20 and 26.2°C. Because no water samples were collected, the $\delta^{18}\text{O}$ of seawater of + 1.0 ‰ had to be taken from the literature (Dolenec *et al.*, 2000). The calculated values are generally regarded as typical of conditions in the investigated areas. The calculated temperature for *Mytilus galloprovincialis* further implies that this mussel most probably calcifies shell carbonate all the year round. During the colder months of the year the mussel precipitated more calcite, while during the summer this organism calcifies more aragonite.

they form (Anderson & Arthur, 1983), and this is probably also the case with the *Mytilus galloprovincialis* from the investigated area. Whereas many organisms display a vital carbon isotope fractionation effect, molluscs are considered to precipitate carbonate in or near isotopic equilibrium with ambient water (Wefer & Berger, 1991). Normally, when calcium carbonate precipitates slowly from solution, equilibrium isotope partitioning is observed. However, faster precipitation may result in isotopic signatures, which are significantly out of equilibrium (Mitchell *et al.*, 1994). In the case of the pink coloured *Mytilus galloprovincialis* shells we feel reasonably confident that the pink aragonite sublayer represents a stress layer formed during summer anoxia. The lower $\delta^{13}\text{C}$ of this layer thus could indicate the incorporation of isotopically light bicarbonate due to the decomposition of organic matter and/or kinetic effects due to the fast growth of the pink biogenic aragonite. This hypothesis is supported by field observations as well as by structural and chemical changes in the minor aragonitic layer, which are coincidental with the higher ^{12}C content. The pink aragonite sublayer is believed to form during pronounced summer anoxia. It contains a signature of stress conditions, which reflects the changes from a slow precipitation of uncoloured flattened aragonite prisms to irregular aragonite grains precipitated at high rates. Faster precipitation may result in isotopic signatures, which are significantly out of equilibrium. Kinetic effect have been described in both fast growing biogenic carbonates (McConnaughey, 1989) and in laboratory precipitated non-biological carbonates (Turner, 1982).

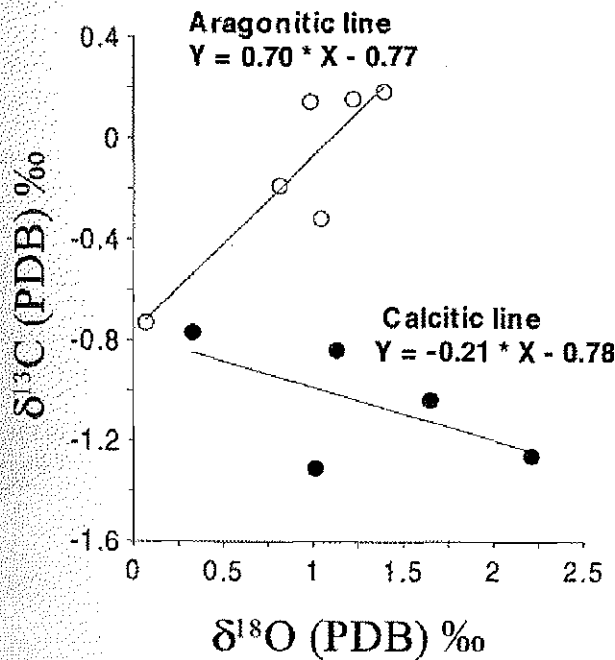


Fig. 5. $\delta^{18}\text{O}$ vs. $\delta^{13}\text{C}$ diagram for *Mytilus galloprovincialis* shell carbonates showing a positive trend for aragonite (○) and a negative one for calcite (●).

Sl. 5: $\delta^{18}\text{O}$ vs. $\delta^{13}\text{C}$ -diagram za karbonate lupinje školjke *Mytilus galloprovincialis* kaže pozitiven trend za aragonit (○) in negativen trend za kalcit (●).

Most marine carbonates reflect the $\delta^{13}\text{C}$ of total dissolved inorganic carbon (TDC) of the water in which

CONCLUSIONS

The impact of environmental factors such as marine anoxia in the coastal zone of the Northern Adriatic on the growth of *Mytilus galloprovincialis* shells is indicated by changes in the growth structure of the outer part of the nacreous aragonitic layer, which is pink in colour and out of isotopic equilibrium with oxygen and carbon from the ambient seawater. Isotopic disequilibrium most probably resulted from faster precipitation of aragonite during the summer period, as well as from lack of oxygen and higher concentrations of isotopically light organic derived CO_2 in the anoxic environment. The influence of higher concentrations of ambient CO_2 is also reflected in a higher C/O ratio of pink coloured aragonite as compared to the uncoloured one.

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VPLIV MORSKE ANOKSIJE NA IZLOČANJE KARBONATA V LUPINICAH ŠKOLJKE *MYTILUS GALLOPROVINCIALIS* IZ PRIOBALNEGA DELA ROVINJSKEGA ZALIVA (SEVERNI JADRAN)

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POVZETEK

Z raziskavo smo ugotovili vpliv morske anoksije v priobalnem delu Rovinjskega zaliva na zgradbo, izotopsko in kemično sestavo lupinic školjke *Mytilus galloprovincialis*. Zunanji aragonitni del "stresne" plasti, ki je nastajal med močno poletno anoksijo zaradi intenzivnega cvetenja fitoplanktona v letih 1989-1991, je masiven in rožnat ter ni v izotopskem ravnotežju s kisikom in ogljikom. Poleg tega kaže tudi večje razmerje C/O v primerjavi z neobarvanim aragonitnim slojem pri školjkah, ki žive v normalnem oksidacijskem okolju.

Ključne besede: *Mytilus galloprovincialis*, biomineralizacija, stabilni izotopi, anoksija, Rovinjski zaliv, severni Jadranski morje

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