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Nitrogen isotope sclerochronology - insights into coastal environmental conditions and *Pinna nobilis* ecology

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Pinna nobilis is a large bivalve endemic to the Mediterranean Sea that lives in shallow coastal areas. Due to its size and relatively fast shell growth rates, it is an interesting taxon for high resolution geochemical and sclerochronological research. Subsequently to previous analyses of $\delta^{18}O$ and $\delta^{13}C$ in *P. nobilis* shells, here, we investigate nitrogen isotopes in the carbonate-bound organic matrix ($\delta^{15}N_{CBOM}$) of this species. Our objectives were to test if *P. nobilis* shells (i) can be used as an indicator of the isotopic baseline of the system, and (ii) is a good candidate for obtaining high-resolution temporal data on environmental $\delta^{15}N$ variability. Due to the multiple mass mortality events of *P. nobilis* spreading throughout the Mediterranean, including the Adriatic Sea, we also tested if (iii) *P. nobilis* geochemistry changes as a response to diseases.

Shells were opportunistically collected by skin diving from 4 shallow coastal localities in the eastern Adriatic, as a part of a project on mortality monitoring. Specimens from Lim channel (October 2019), Kaštela Bay (January 2020) and Mali Ston Bay (November 2019) were collected alive, while in Pag Bay, shells of three recently dead specimens were collected in September 2020. Tissue and epibionts were removed and shells carefully cleaned and air-dried. Shell powder was collected by milling sample swaths by hand using a DREMEL Fortiflex drill equipped with a 300 µm tungsten carbide drill bit. For $\delta^{15}N_{CBOM}$ analysis, three shells from each locality were processed and three replicas were collected from each of these shells by milling shallow lines parallel to the growth axis from the internal shell surface. In addition, high-resolution $\delta^{15}N_{CBOM}$ data were obtained for one shell from Kaštela by milling lines (N=40) perpendicular to the major growth axis from the external shell surface. From this shell we also collected shell powder for $\delta^{18}O_{shell}$ and $\delta^{13}C_{shell}$ analysis to enable placing $\delta^{15}N_{CBOM}$ into temporal context. Isotope samples were analyzed Union College on an elemental analyzer - isotope ratio mass spectrometer.

Results indicate significant differences in $\delta^{15}N_{CBOM}$ between sampling localities, with lowest values recorded for shells from Pag Bay (3.73±0.36‰), and highest for shells sampled in Lim channel (7.04±0.63‰). High-resolution $\delta^{15}N_{CBOM}$ data obtained from the shell collected from Kaštela Bay corresponded to a time interval from spring 2018 to spring 2019. These data showed relatively

small variations (5.02±0.33‰). However, $\delta^{15}N_{CBOM}$ values increased to 8.65±1.61‰ closest to the shell margin, and were coupled with a decrease in $\delta^{13}C_{shell}$ values, indicating that this animal was experiencing stressful conditions several months prior to its death. According to our findings, $\delta^{15}N_{CBOM}$ values serve as an indicator of the isotopic baseline of the ecosystem as well as a potential powerful tool to study bivalve physiology.

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