



Bioerosion features of boring polydorid polychaetes in the North Adriatic Sea

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Abstract Considering the pivotal role played by erosive organisms in the marine habitat and the scanty knowledge of this phenomenon in the Mediterranean Sea, the present study aimed to identify the pioneer excavating organisms occurring in the first stages of bioerosion, providing the first estimation of their rate of erosion along the North Adriatic Sea. Bioerosion activity was investigated by deploying sets of limestone panels for a period of 2 years. Moreover, micro-computed tomography was used for the first time to study polychaete erosion, confirming it is a very useful tool for the description of erosion processes. Blocks were placed in the touristic harbor Marina Dorica (Ancona) in July 2017 and periodically collected, the first time after a 6-month period, then bimonthly. Two species of the polychaete genus *Polydora* were the only boring organisms that

settled on the experimental substrates. Erosion rate was estimated by measuring the holes produced by both worm species, and the eroded volumes obtained by resin casts of the holes. *Polydora* appeared after 6 months of exposure significantly changing its density rates (holes·100 cm⁻²·month⁻¹) during the exposure, being comparable with densities reported from tropical areas. The erosion rate on the contrary is lower, reaching values of 0.053 g·cm⁻²·year⁻¹.

Keywords *Polydora* · Boring worms · Erosion rates · Experimental blocks · Micro-CT · Resin casts

Introduction

Destruction of carbonate substrates due to organism activities, or bioerosion (Neumann, 1966), is a common process in several marine ecosystems from temperate (Cerrano et al., 2001a; Wisshak et al., 2005; Schönberg & Wisshak, 2014), to tropical (Hutchings et al., 1992, 2013; Hutchings & Peyrot-Clausade, 2002; Pari et al., 2002; Tribollet et al., 2002; Hutchings, 2008) and even polar regions (Cerrano et al., 2001b; Meyer et al., 2021). Boring organisms participate to a wide range of regulation and maintenance ecosystem services: in fact, they enhance habitat complexity offering refuge to several taxa and contribute to the carbonate cycle, with the erosion of coastal areas and the production of fine sediments (Naylor et al., 2012; Davidson et al., 2018).

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Moreover, considering that their boring activity can be influenced by a variety of environmental factors (e.g., nutrients, salinity, temperature, pH), in the context of the current climate crisis, the study of the bioerosion processes became a timely topic (Chazottes et al., 2008; Schönberg & Wisshak, 2014; Schönberg et al., 2017).

Schönberg & Wisshak (2014) reported that only the 11% of the research on bioerosion were conducted in the Mediterranean Sea; since then, only 7 papers have been found on SCOPUS (<https://www.scopus.com>) using “bioerosion,” “boring species,” and “Mediterranean” as search terms. In particular, studies about the rates of bioerosion in Mediterranean basin are even scantier (Calcinai et al., 2008, 2011; Färber et al., 2015, 2016) and exclusively about sponges and polychaetes.

Several organisms belonging to different taxa are involved in calcareous-boring activities; they can be microborers as cyanobacteria (Tribollet et al., 2011; Pica et al., 2016) and fungi (Gleason et al., 2017), or macroborers as sponges (Bavestrello et al., 1991; Calcinai et al., 2008, 2011), molluscs (Ćurin et al., 2014; Peharda et al., 2015), echinoderms (Belaústegui et al., 2017), and polychaetes (Martin & Britayev, 1998; Hutchings, 2008). Experimental studies for the evaluation of bioerosion rates are principally conducted using experimental blocks made of dead coral substrate (Tribollet et al., 2002; Hutchings, 2008), limestone panels (Wisshak et al., 2005; Casoli et al., 2019), shells (Calcinai et al., 2007), and wood (Reish et al., 2018). These experimental blocks also provide essential information about successional stages of the boring communities. In the first place, the local pattern of currents influences the larval dispersion (Tribollet et al., 2002) affecting the recruitment of boring organisms. The following successional stages, mainly facilitated by the borers, lead to the achievement of mature boring communities (Hutchings, 2008). Polychaetes have been reported as the pioneer-dominant borers on newly available submerged substrates and considered among major bioeroders along with sponges and molluscs (Hutchings et al., 1992, 2011). Nevertheless, studies about values of bioerosion of polychaetes are not abundant. Numerous researches assessed this topic in coral reefs ecosystems, obtaining a great variation among their estimates: Tribollet and Golubic (2005) ($0.010\text{--}0.130\text{ kg CaCO}_3\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) together with Yeung et al.

(2021) ($0.013\text{--}0.162\text{ kg CaCO}_3\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) presented the lowest values, followed by Kiene and Hutchings (1994) ($0.104\text{ kg}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$), Hutchings (2008) ($0.356\text{ kg}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$), Chazottes et al. (2002) ($0.050\text{--}0.400\text{ kg CaCO}_3\text{ m}^{-2}\cdot\text{year}^{-1}$), and Davies and Hutchings (1983) which presented the highest value in the literature ($1.800\text{ kg}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$). Generally, this group present lower values than sponges, considered as the principal erosive organisms in later succession stages (Pari et al., 2002). Only recently the colonization dynamics of boring polychaetes has been studied in the Mediterranean Sea (Tyrrhenian Sea), using experimental substrates (Casoli et al., 2019; Gravina et al., 2019), but still no data are available regarding their erosion rates.

Several families of polychaetes are involved in bioerosion processes, such as Eunicidae, Cirratulidae, Sabellidae, and Spionidae. These worms exhibit a variety of feeding preferences but are in general considered as filter and surface deposit feeders. Boring polychaetes use both chemical secretions and mechanical excavation for dissolving the rocks and making their holes (Hutchings, 2008); some sabellids, for example, although limited to a few species, have developed glandular areas along the ventral part of the body, while in some polydorids (Spionidae) the erosion of the carbonate substrate is possible thanks to the modified chaetae present on the 5th chaetiger, in addition to the production of chemicals compounds (Zottoli & Carricket, 1974; Sato-Okoshi & Okoshi, 1993; Hutchings, 2011). Polydorid is one of the most studied boring taxa and this is principally due to the serious damages these polychaetes inflict on oysters and abalone shells in aquaculture systems (Sato-Okoshi et al., 2008; Walker, 2011). These polychaetes are easily recognizable on the substrate for their typical 8-shaped holes, due to the two closed openings they produce divided by a thin mucous layer, allowing palps to move in the surrounding ground looking for food particles. The longitudinal section of the hole, where the worm lives in, is U-shaped, and each 8-shaped hole corresponds to a single boring individual. Not all the genera belonging to this taxon have been recognized as boring (Sato-Okoshi, 1999); sometimes, it's not even easy to attribute the species to the correct taxon because of the similar morphological characteristics shared by a group of species, thus making identification a difficult task (see for example Simon & Sato-Okoshi, 2015; Bertasi, 2016; Çinar &

Dagli, 2021), such as the case of the species complex *Polydora ciliata* (Johnston, 1838) (Mustaquim, 1986, 1988; Manchenko & Radashevsky, 1998).

Micro-computed tomography (micro-CT) has been extensively applied for studying internal and external anatomical features of several taxa such as corals (Morales Pinzón et al., 2014; Enochs et al., 2016; Urushihara et al., 2016), sponges (Heim & Nickel, 2010), or polychaetes (Faulwetter et al., 2013; Parapar et al., 2017, 2019, 2021), and in terms of analysis of bioerosion traces produced by micro- and macroborers (Beuck et al., 2007). Regarding annelids, micro-CT has been implemented to visualize, from a three-dimensional perspective, bioturbation in polychaetes (Pennafirme et al., 2019) and the boring pattern produced by the Siboglinid *Osedax* (Higgs et al., 2010). To the best of our knowledge, this is the first time micro-CT is used to visualize the development of boring polychaete cavities.

Considering the role that bioerosion plays in the marine habitat and the scanty knowledge of this phenomenon in the Mediterranean Sea, this study aims to assess on experimental limestone panels: (i) the pioneer, bioerosive organisms occurring in the first stages of bioerosion along the North Adriatic Sea and (ii) the annual rate of bioerosion during this first colonization phase. A diagram summarizing the different techniques implemented along the study was produced and added as Supplementary Material (Online Resource 1).

Methods

Study site, experimental design, and sample treatment

Marina Dorica (43°36.65' N; 13°28.91' E) is a touristic harbor in the northern-west coast of the Ancona Promontory (Adriatic Sea, Italy) (Fig. 1). In this site, five sets of three experimental blocks (15 in total) were placed under a wharf (W3) at a depth of 1 m. These blocks were made of Pietra Leccese (Margiotta, 2006), a common calcareous formation along Italian coasts consisting in a compact biomicrite made of planktonic foraminifera with its origin in the Miocene (Bossio et al., 2002).

Blocks were positioned between the poles using a line and kept separated by plastic tubes 20 cm long.

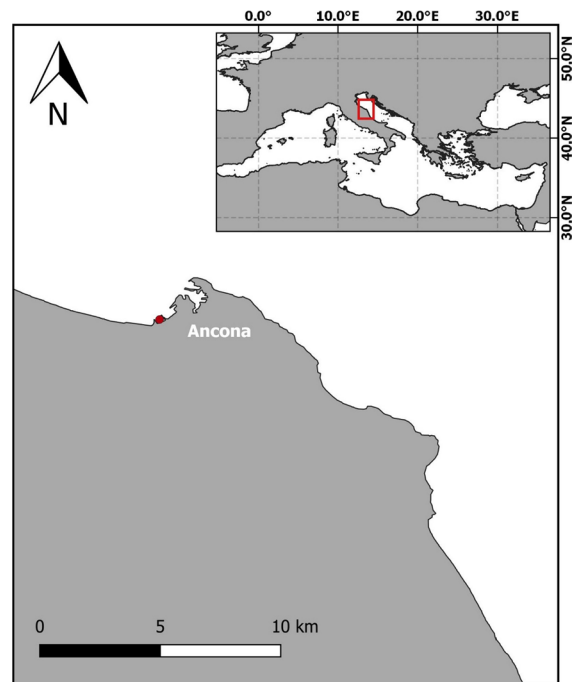


Fig. 1 Map of the study site, located in the touristic harbor of Marina Dorica (Ancona, Italy)

The wharf is in proximity of the canal port where the water exchange, with the open sea, is higher with respect to the inner port. The calcareous blocks were submerged from July 2017 to July 2019, and the first set of three blocks was collected in January '18 after 6 months from the positioning. Later, the other sets were collected bimonthly, up to July '18; the last three blocks were collected after 24 months of immersion (in total), in July 2019. When removed from the structure, the blocks were separately placed in plastic bags with sea water and transferred to the laboratory; at this point, all the encrusting organisms were carefully removed from the blocks and the blocks were kept in aquaria, under a continuous sea-water circulation. The cleaning procedure did not affect the integrity of boring worms and keeping them alive in aquaria allowed to precisely localize their holes. Magnesium chloride (7%) was added to the water to relax polychaetes and to facilitate their extraction from the substrate; right after the worms were extracted, forcing them out of their holes with the help of a Pasteur pipette, fixed in alcohol solution (95%) and identified. For scanning electron microscopy (SEM) analyses, several specimens were primary fixed in ethanol

20%, then dehydrated through a graded ethanol series (50%, 70%, 90%, 100%), and dried with Hexamethyldisilazane 98% until complete evaporation following Shively & Miller (2009). Finally, they were attached on stubs, sputtered with gold-platinum and observed under SEM Philips XL20.

Density and erosion parameters assessment

For each block, the 8-shaped *Polydora*'s holes were counted, calculating their average density (holes·100 cm⁻²). The two openings, creating an 8-shaped sign on the rock surface, approximately have equal diameter (*d*) (Fig. 2a); for this reason, only one diameter (of the single opening) per each hole was measured under stereomicroscope; the surface of each opening (*OS*) (Fig. 2a) was considered circular in shape and calculated with the formula $OS = (d/2)^2 \pi$. The value of the surface erosion produced by a single worm (8-shaped hole) was obtained doubling the *OS* values.

To assess the volume of carbonate material removed by the polychaetes, a polyurethane resin (PU800) (www.antichitabelsito.it) was injected into the holes of the blocks, with a surgical syringe. Then,

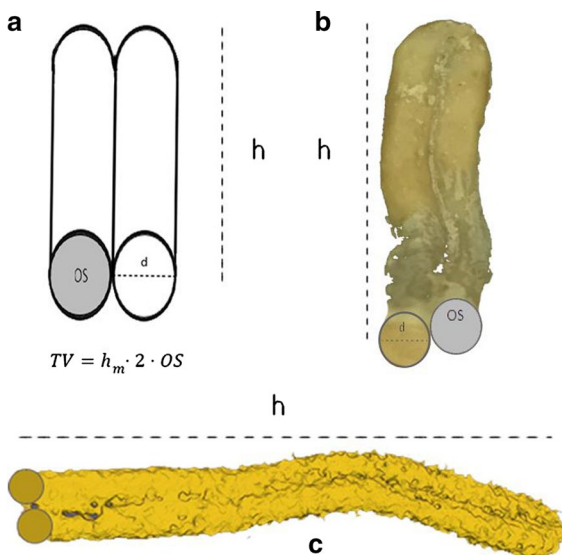


Fig. 2 Three methodologies used to extract polydorida's morphometrics. **a** Model used as a proxy for cavity volume; **b** Resin cast; **c** 3D reconstruction of *Polydora*'s cavities after micro-CT processing. (*d* diameter, *h* height, *OS* surface of a single opening)

the blocks were dissolved in acid chloride (37%) and the hardened resin casts were removed. For this purpose, the blocks of March, May, and July 2018 were used. The resin casts reproduced worm excavations (Fig. 2b), whose regular form allowed us to approximate its shape to two paired cylinders (Fig. 2a) to calculate the total volume (TV) excavated by a single worm; we applied the formula $TV = h_m 2OS$, where 2OS is the average value of the excavated area (i.e., the double openings; see above) calculated for each period of sampling, and *h* stands for the height of the cast; the value *h_m* was obtained by calculating the average height of 15 casts for each period (Fig. 2a, b). The total volume excavated in July 2018 was then used to estimate the erosion rate of polychaetes as mm³ of excavated substrate per mm⁻² of substrate, per year⁻¹. To evaluate the weight (g) of the substrate eroded by polychaetes per surface and year⁻¹, the volumes removed by these after one year of exposure (July 2018), were multiplied by the specific gravity of the "Leccese" rock (2.577 g·cm⁻³) and by the specific gravity of the micritic limestone of the maiolica formation that mainly characterizes the Conero substratum (2.5 g·cm⁻³) (Calcinai et al., 2011).

In order to compare the density of excavating worms (=holes) on the blocks between the different times of exposure, it was standardized by dividing its density values by their time of exposure.

Parallel to the approach made with resin casts, *Polydora* samples from January and July 2018 were micro-CT-scanned at Centro di Ricerca e Servizio di Microscopia delle Nanostrutture (CISMIn) at the Polytechnic University of Marche, using a Skyscan 1174. During scanning, the sample was rotated in 0.20° angular increments reaching a total of 180° rotation, acquiring an X-ray absorption radiograph was digitally recorded at each rotation step. The X-ray source was set at 50 kV and 800 μA and an aluminum filter was placed between the sample and the camera in order to maximize the energy transmission through the sample. Scans were performed at a maximum pixel size of 25.25 μm. Initial processing of the projection images was performed with cone-beam reconstruction software (Nrecon v.1.7.3.1., Skyscan), resulting in a series of axial cross-sections. Post-processing of the cross-sections was conducted using the Amira software edition from the Zuse Institute Berlin, ZIB Amira 5 version 2019.1 (Stalling et al., 2005), where, in the first place, a Gaussian smoothing filter

using a $3 \times 3 \times 3$ voxel kernel was applied to reduce image noise. Then, bioerosive pores were differentiated from the rocky substrate by the following: (i) cross-sections segmentation by an interactive thresholding, defining the solid substrate; (ii) then, ambient occlusion module was applied to the binarized cross-sections in order to allow erosion pores to be visualized separately, as digital ‘casts’ in a greyscale depending on the occlusion of the same; and (iii) a final interactive thresholding was applied again to allow the software to perform volume calculations. Once single cavities were individualized on the 3D reconstructions, dimensions such as diameter, length, and volume values were extracted for each reconstructed erosion channel (Fig. 2c). Two sampling periods were assessed by the tomographic approach in order to test the technique, January and July 2018.

Statistical analysis

Given the proximity among the three blocks extracted at each time period, it was not possible to consider them as independent replicates. This is the reason why a repeated-measures ANOVA test was conducted to compare groups of samples exposed to different periods of immersion. The data were previously checked for homogeneity of variance using the Levene’s test, and whenever data did not meet the assumptions for parametric analyses, they were logarithmically transformed. Moreover, when the assumptions for parametric analyses were not met, even after logarithmic transformation, data were compared by the equivalent non-parametric analysis, the Friedman test.

All statistical analyses were performed using PAST 4 (Hammer et al., 2001) and graphical outputs with R software (R Core Team, 2020).

Results

Boring organism, density, and erosion traces

Boring species recorded into the experimental blocks belonged to two species: *Polydora ciliata* complex (Annelida: Spionidae) (Walker, 2011) and *Polydora* sp.. The *P. ciliata* complex specimens had the typical characters of the species (Fig. 3), but since the first description of the species refers to tubicolous

non-boring forms on mud sediments (Johnston, 1838), for the boring sibling species of this complex, only genetic analyses can distinguish among different taxa (Manchenko & Radashevsky, 1993, 1998). *Polydora* sp. is a form showing some of the characters of the *P. ciliata* complex, but differs in having (i) dark bar pigmentation of the palps, (ii) the pigmentation present on the prostomium and dorsal side of the 3rd–4th segments, and (iii) the stout special chaetae of the 5th segment without a spur or secondary tooth. In fact, the pigmentation on both palps and prostomium, as well as in the anterior segments, closely resembles that of *Polydora haswelli* Blake & Kudenov, (1978) (see figure in Sato-Okoshi & Abe, 2013); however, the shape of the stout modified chaetae of the 5th segment differs from *P. haswelli*, in lacking any clear lateral accessory flange (Radashevsky et al., 2006). The form present in our samples resembles the worms collected by Boscolo and Giovanardi (2002) in the external coloration; these worms were found as borers infesting the bivalve *Ruditapes philippinarum* (A. Adams & Reeve, 1850) (cited as *Tapes philippinarum*) shells in the northern Adriatic and attributed by the Authors to *P. ciliata*. Therefore, considering that *P. haswelli* is actually not reported for the Mediterranean Sea, our specimens deserve further taxonomic analyses, possibly matched with molecular ones, since the 18S gene sequencing is already available for some Japanese specimens of *P. haswelli* (Sato-Okoshi & Abe, 2013).

However, from an ecological and functional point of view, both species found in our samples seem to exert the same role as boring pattern and activity, and therefore, their effects can be considered as they were a single taxonomic entity. Bearing this in mind, signs of bioerosion made by *Polydora* spp. have been present since the first sampling in January 2018, where after six months of immersion, we could already count a total of 80 specimens in all three blocks. Moreover, their density changed along the periods of immersion, reaching the highest value in July 2018, after 1 year of submersion ($n=290$ in three blocks with a density of 32.8 ± 15.8 holes·cm⁻²).

Considering exposure time, the density of excavating worms (=holes) was comprised between 1.51 ± 0.51 worms 100 cm⁻²·month⁻¹ in January 2018 (after 6 months of exposure) and 2.74 ± 1.32 worms 100 cm⁻²·month⁻¹ in July 2018, after 12 months (Fig. 4). Repeated-measures ANOVA test

Fig. 3 SEM pictures of a specimen of *Polydora ciliata* complex, showing the main taxonomic characters. **a–c** Dorsal and lateral views of the prostomium and first few segments showing lack of notochaetae at the 1st segment, and the start of neuropodial uncini and branchiae at the 7th segment. **d** Modified stout chaetae of the 5th segments with a typical spur (sometimes a small tooth). **e–f** posterior part of the body showing the lack of both special chaetae and branchiae in the last segments, and the plate-like pygidium

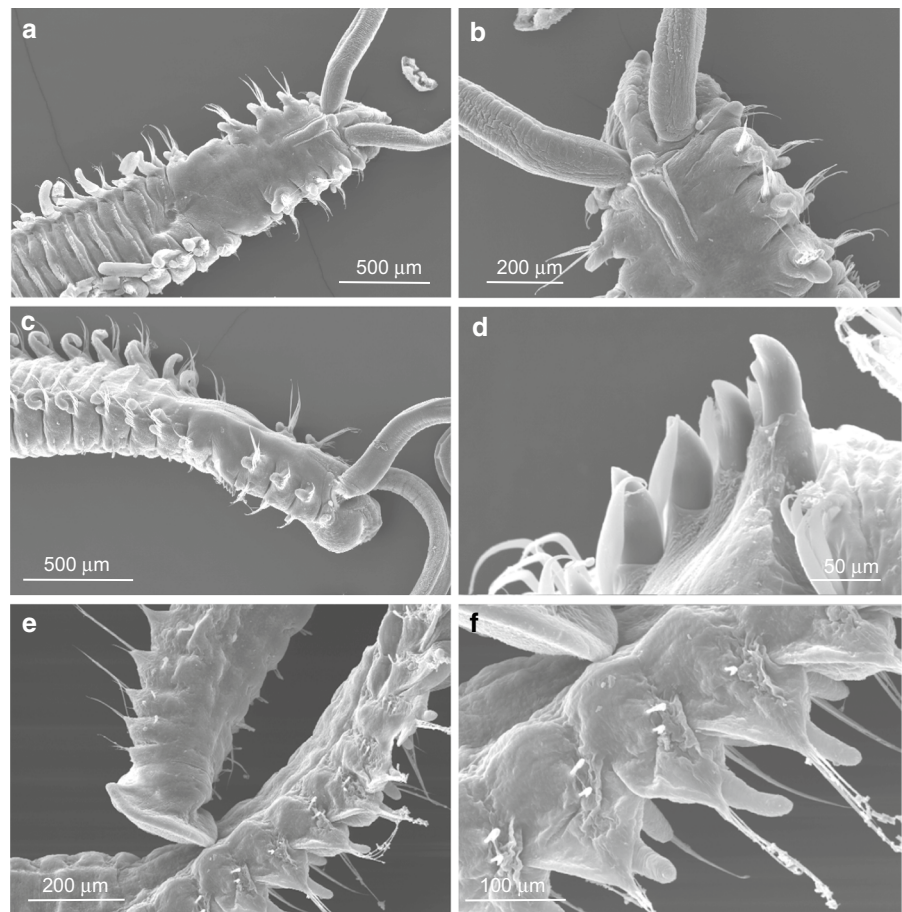
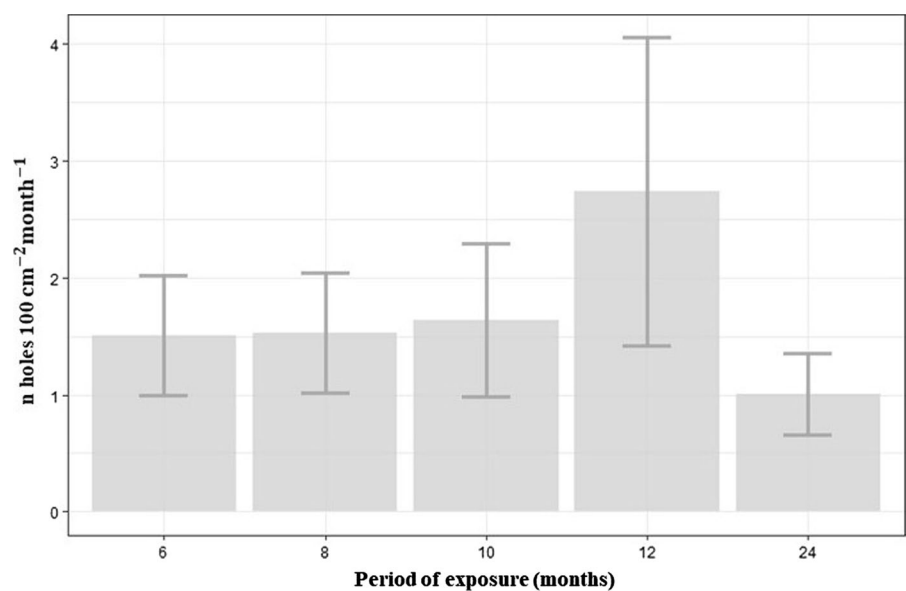


Fig. 4 Density rate of the excavating worms (holes) (average \pm SD) along the period of exposure



revealed significant differences in relation to the periods of immersion ($P < 0.05$), specially between July 2018 and July 2019 (Tukey's test $P = 0.01296$).

The average size of diameters of a single opening is shown in Fig. 5. The smallest value of 0.70 ± 0.19 mm was recorded in May '18 (after 10 months of immersion) while the higher value was 0.81 ± 0.13 mm in July '19, after 24 months of immersion. The Friedman test showed no significant differences ($P > 0.05$) between diameters among periods of immersion.

The heights of the resin casts were evaluated for holes produced in March, May, and July 2018. They are between 18.4 ± 4.5 mm in May and 19.2 ± 4.1 mm in March. Repeated-measures ANOVA test revealed no significant differences ($P > 0.05$) in the heights of the holes, reproduced by the casts.

Erosion rates assessment

The eroded volumes were ranged from $6.4 \text{ mm}^3 \cdot \text{cm}^{-2}$ of substrate (March '18, after 6 months of exposure) to $21 \text{ mm}^3 \cdot \text{cm}^{-2}$ of substrate (July '18, after a year). Assuming that substrate erosion is constant over time and considering the periods of blocks' immersion, the eroded volume rates varied between $9.788 \text{ mm}^3 \cdot \text{cm}^{-2} \cdot \text{year}^{-1}$ (March '18) and $21.086 \text{ mm}^3 \cdot \text{cm}^{-2} \cdot \text{year}^{-1}$ (July '18). The rates obtained using the full-year period eroded volume was considered as a more complete estimate, possibly accounting for

seasonal variations. In this context, erosion rate was calculated by multiplying the yearly eroded volume by the specific gravity of the Leccese rock, obtaining a rate of $0.0543 \text{ g} \cdot \text{cm}^{-2} \cdot \text{year}^{-1}$. If we consider the specific gravity of the Conero rock present along Ancona Promontory, the erosion rates on the Conero area would be around $0.053 \text{ g} \cdot \text{cm}^{-2} \cdot \text{year}^{-1}$.

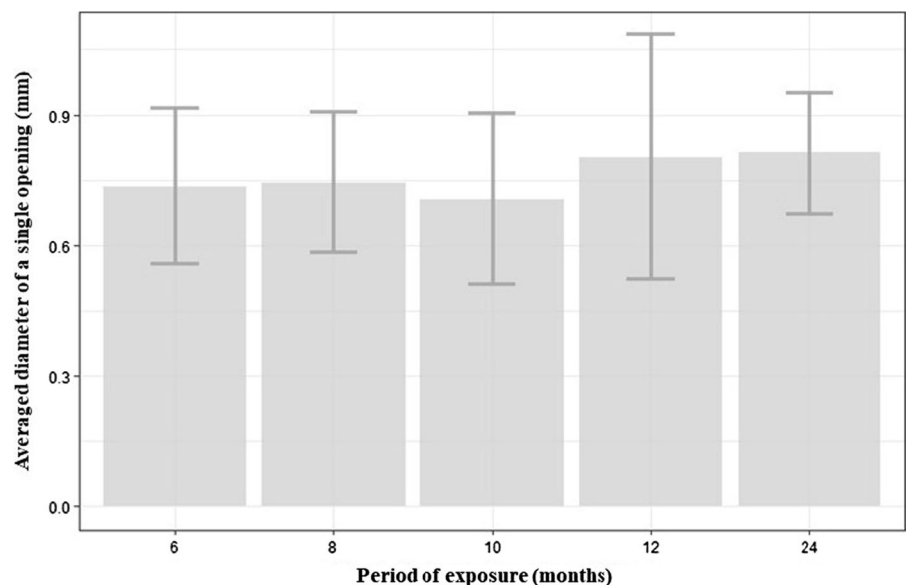
Micro-CT characterization

Micro-CT was applied for two of the experimental time steps: January '18, where two subsamples were scanned resolving three fully reconstructed holes, presenting an averaged diameter of 0.71 ± 0.09 mm and a length of 8.32 ± 3.08 mm; while for July '18, a single subsample resolved three holes, with an averaged diameter of 0.78 ± 0.14 mm and an average length of 9.39 ± 3.29 mm. The 3D reconstructions obtained from the scanning allowed also to calculate the volume for each of the holes, obtaining averaged eroded volumes of $7.52 \pm 4.73 \text{ mm}^3$ for January '18 and $8.51 \pm 5.52 \text{ mm}^3$ for July '18.

Discussion

The aim of this study was to describe the first phases of bioerosion processes along the North Adriatic Sea coast and provide the first estimation of the relative bioerosion rates. The North-Italian Adriatic coast is

Fig. 5 Average diameter (\pm SD) of *Polydora*'s hole (single opening) along the period of exposure



characterized almost entirely by sandy beaches while the Conero Promontory, where Ancona is located, is a massive calcareous promontory colonized by both epilithic and endolithic organisms as sponges and bivalves (Calcinai et al., 2009). Up until today, only sponge bioerosion has been studied on this peculiar ecosystem (Calcinai et al., 2011). At Mediterranean level, knowledge regarding polychaete erosion rates is still absent. For some borers, as sponges (*Cliona adriatica* Calcinai et al., 2011) and bivalves (*Rocellaria dubia* (Pennant, 1777)), it has been possible to estimate the erosion with non-destructive methods, allowing to calculate the volume of the internal cavities by mean of its external openings (Schiaparelli et al., 2005; Calcinai et al., 2011). For polydorids, only destructive methods, such as cracking the substrates, have been used (Simon & Sato-Okoshi, 2015; Radashevsky et al., 2017); considering that heights of the excavations did not vary significantly with the time of exposure, we suggest to estimate the volume eroded by *Polydora* spp. directly by measuring the diameter of the openings, considering the erosion cavity as a double cylinder, and avoiding destructive approach; moreover, we also propose, for the first time, micro-CT three-dimensional reconstructions as a non-destructive method for evaluating polydorids' erosion cavities. The erosion pattern in *Polydora* seems different in respect of other taxa as the bivalve *R. dubia* (see Schiaparelli et al., 2005), the date mussel (*Lithophaga lithophaga*, (Linnaeus, 1758)), or the piddock, (*Pholas dactylus* Linnaeus, 1758) where both the erosion cavities and the superficial openings enlarge following the mollusks' growth.

This study shows that, after 6 months, the polydorid *Polydora* spp. appeared into the blocks, and no other excavating organisms were detected during the following 2 years of experiment. No evidence was recorded for boring sponges, bivalves, or echinoderms, widely considered as the principal taxa involved in boring processes both in tropical and temperate seas (Schönberg & Wisshak, 2014). These organisms are known to appear in late successional stages of the boring community (after 2 years, e.g., Chazottes et al., 1995), after worms that, in the coral reefs, are always reported as pioneers (Hutchings & Peyrot-Clausade, 2002; Hutchings, 2008; Hutchings et al., 2013). In this study, blocks were immersed only for 2 years but, although in the short term, this experiment highlights that also in the Mediterranean Sea,

the first succession phase in the boring community mirrors that of the tropical areas (see, e.g., Hutchings et al., 1992; Tribollet et al., 2002).

The density of *Polydora* spp. significantly changed with the time of exposure, as reported for other bioeroders in studies conducted in the tropical areas (Chazotte et al., 1995; Hutchings et al., 2002; Hutchings et al., 2013). Recently, Casoli et al. (2019) registered the presence of only 10 specimens of *Polydora ciliata* and of another excavating worm (*Dodecaceria concharum* Örsted, 1843), in experimental blocks in the Tyrrhenian Sea. In contrast, our experimental blocks presented a high abundance of *Polydora* spp. specimens, reaching its maximum densities after one year. This sequence coincides with the results of Casoli et al. (2019) and Gravina et al. (2019) that found *Polydora* exclusively present in the first period, in blocks immersed for 1 year, substituted later by *D. concharum*, the only worm present in 2- and 3-year-old blocks. Consequently, species of the genus *Polydora* may be considered as pioneer species characterizing the first period of succession also in the Adriatic Sea.

In terms of eroded volumes, the higher values recorded in July 2018 are probably due to the settlement of a new cohort of *Polydora* spp., that, as reported by Blake (1969), it is characterized by one or two reproductive events in a single season, usually in late spring. In fact, during the observation in vivo, done on the samples of May 2018, several mature specimens were recorded.

Regarding the density, per unit of volume, of the boring polychaetes, our data varied between 10 ± 4.5 and 36.25 ± 23.3 worms·100 cm⁻³. Values comparable to those reported in coral reefs by Hutchings and Peyrot-Clausade (2002) which assessed abundances of some coral-boring polydorids in the French Polynesia, obtaining densities from 0.5 ± 0.8 to 36.3 ± 33.7 ind·100 cm⁻³; higher values as those reported by Hutchings et al. (2013) for *Polydora* spp. (303.0 ± 72.75 ind·100 cm⁻³). On the other hand, bioerosion rates, here assessed, are much lower than those reported in tropical areas (see data in the introduction). This is not surprising, as it is known that the bioerosion rate in the tropics exceeds the ones occurring in temperate and cold waters (Wisshak, 2006). Nevertheless, the comparison between present data and those from literature allows only some very general considerations; in literature, the erosion rates

are often calculated without discriminating among the taxa, i.e., grouping polychaetes and sipunculans as “worms,” and being this group mainly clustered together with bivalves, as macroborers. Moreover, differences observed in terms of density and erosion rates may be due to the different lithologies used during the experiments; in fact, it has been demonstrated that the bioerosion rate is higher in porous substrates (corals) in respect of denser material (Leccese rock) (Calcinai et al., 2007).

Thanks to the micro-CT analysis, a clear three-dimensional view of the erosion patterns produced by *Polydora* spp. was obtained, showing a unified “8-shaped hole” which occasionally gets separated into two single funnels along its development. Measurements taken from the digital casts obtained similar values that the ones extracted from resin casts; yet the tomography did not only enabled us to extract measures, but also to observe the position and orientation of the cavities into the substrate, as well as to explore bioerosion pattern as a whole, otherwise impossible to obtain by more invasive approaches (e.g., Online Resource 2, where two worms appeared to have avoided perforating each other cavities by slightly changing direction during their erosion activity). Nevertheless, even if micro-CT has proved its value on the assessment of erosive patterns, its application on high-density substrates can cause artifacts during the scanning, thus limiting the maximum volume of sample to be analyzed. A posterior subsampling would make the technique to lose its non-invasive character, risking to damage the cavities inside the sample (as observed on Online Resource 2, where the cut on the substrate damaged two cavities). Moreover, operational costs for a high number of samples can be considerable, for which the coupling of more cost-effective techniques, such as resin casts, should be considered.

Conclusions

This is the first study evaluating bioerosion rates produced by polychaetes, organisms which have a dominant role in the bioerosion processes affecting calcareous substrates, both natural and artificial, in the Mediterranean Sea. The study has shown how the spionid polychaetes *Polydora* spp. trigger the earlier stages of bioerosion in the North Adriatic Sea,

and that it is possible to estimate the etched volume with non-destructive methods. In the sea, the bioerosion affects any kind of calcareous substrate, playing a relevant role in several marine ecosystem dynamics. Bio-erosive successions in temperate climates are poorly studied. Moreover, in the near future, due to the increasing of organic pollution in coastal waters together with a general warming, an increment of the bioeroders’ activity is expected. Long-term experiments using substrates having different lithologies, at different depths and considering waters with different contents of organic matter, are needed to provide a wider and more precise picture of the bioerosion trends of the Mediterranean Sea.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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