

Research Article

Antioxidant Efficiency of *Platynereis* spp. (Annelida, Nereididae) under Different pH Conditions at a CO₂ Vent's System

Giulia Valvassori ¹, Maura Benedetti,² Francesco Regoli,² and Maria Cristina Gambi¹

¹Stazione Zoologica Anton Dohrn Napoli, Dept. of Integrative Marine Ecology, Villa Dohrn-Benthic Ecology Center, 80077 Ischia, Napoli, Italy

²Università Politecnica delle Marche, Dept. DiSVA (Dipartimento di Scienze della Vita e dell'Ambiente), Laboratory of Ecotoxicology and Environmental Chemistry, 60131 Ancona, Italy

Correspondence should be addressed to Giulia Valvassori; giulialvalva89@gmail.com

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Marine organisms are exposed to a pH decrease and to alteration of carbonate chemistry due to ocean acidification (OA) that can represent a source of oxidative stress which can significantly affect their antioxidant defence systems efficiency. The polychaetes *Platynereis dumerilii* and *P. massiliensis* (Nereididae) are key species of the benthic community to investigate the effect of OA due to their physiological and ecological characteristics that enable them to persist even in naturally acidified CO₂ vent systems. Previous studies have documented the ability of these species to adapt to OA after short- and long-term translocation experiments, but no one has ever evaluated the basal antioxidant system efficiency comparing populations permanently living in habitat characterized by different pH conditions (acidified vs. control). Here, individuals of both *Platynereis* species, sampled from a natural CO₂ vent system and from a nonventing “control” site in three different periods (April 2016, October 2016, and February 2017), were compared highlighting signals which suggested the ability of both species to acclimatize to high pCO₂-low pH with slight seasonal variations of their antioxidant efficiency and the absence of disturbances of the oxidative status of *Platynereis* spp. tissues.

1. Introduction

World climatic alterations are mainly driven by atmospheric CO₂ partial pressure (pCO₂) increase, as a consequence of the anthropogenic activity, which is predicted to reach 800 ppm by the end of the current century [1–5]. This change at the atmospheric level also affects the ocean surface through the phenomenon of ocean acidification (OA): an increase of the dissolved CO₂, alteration of seawater carbonate chemistry, and the consequent reduction of the ocean pH. Ocean surface pH has fallen by about 0.1 units since the beginning of the industrial era and is expected to further decrease by 0.3–0.5 units by the end of the current century [1, 4, 5]. Marine organisms are continuously exposed to a range of environmental parameters, such as pH, salinity, and temperature, varying over temporal and spatial scales, which may represent a source of oxidative stress that entails Reactive Oxygen Species

(ROS) additional production. ROS, endogenous and highly reactive oxygen-bearing molecules, are commonly produced at low concentrations during several natural cellular pathways of aerobic metabolism, and under basal and stable conditions their adverse effects are prevented by antioxidant defence systems (i.e., low molecular weight scavengers and enzymes). In stressful conditions, this balance may be altered leading to uncontrolled ROS formation that translates into cellular oxidative damage against biological macromolecules including lipids, proteins, and DNA, impairing normal cellular functions. This unbalance in favour of oxidants is termed “oxidative stress”. In the last years, scientific literature has provided evidence that global climate change, especially OA, affects antioxidant systems efficiency of several marine organisms [6–20]. The biological effects of low pH–high pCO₂ have been investigated through not only laboratory/mesocosm experiments, but also studying natural volcanic CO₂ vent

systems which occur in different parts of the world (e.g., [21–28]). Such “natural laboratories” have so far provided environmentally realistic overviews of the conditions with which marine organisms will interface according to the near-future OA predictions [5, 29] and represent an important tool to detect information about putatively tolerant species to OA and their capability to modulate the antioxidant system in response to pH variations. Despite the growing interest of the scientific community in this research field, there is still little knowledge about how the acidification processes affect antioxidant defence systems of the benthic biota.

Within benthic community, polychaetes represent a key group in marine habitat and are often used as bioindicators in monitoring programs for their high sensitivity to metal exposure [30–33] and anthropogenic pressure [34, 35]. Due to their physiological and ecological characteristics, polychaetes are among the most abundant invertebrates under low pH conditions, such as along the naturally acidified CO₂ vent system of the Castello at the Ischia Island [22, 36–39], and various studies investigated the role of antioxidant systems in response to low pH–high pCO₂ [12, 16–18, 24, 40, 41]. The polychaete *Platynereis dumerilii* (Audouin and Milne-Edwards, 1834) (Nereididae) represents a key species: it showed high tolerance to environmental stress [42, 43], including low pH [38], and for this reason it was employed as model organism in two recent *in situ* transplant experiments, carried out to evaluate the effects of OA on the oxidative sensitivity of populations living inside and outside the vent area of Ischia [12, 24]. The short-term translocation (only 5 days) displayed a true local adaptation to low pH conditions, with an exclusive genotype apparently restricted to the acidified areas of Castello Aragonese (Ischia) characterized by higher metabolic rate, measured as oxygen consumption [24]. The genotype of the acidified areas was later identified as the only known sibling species of *Platynereis dumerilii*, *P. massiliensis* (Moquin-Tandon, 1869): morphologically indistinguishable species in its adult (nonreproductive) stage, but characterized by a completely different reproductive biology [44–46]. A recently published study that combined together genetic and reproductive biology analyses revealed that both *Platynereis* species actually represented two different complexes of siblings [47]. Based on preliminary genetic results, the Castello vent site of Ischia appeared dominated by the brooding *P. massiliensis* sibling, while the control site by the broadcasting species *P. dumerilii* [24, 46, 47]. In the long-term translocation (30 days) the antioxidant sensitivity of different polychaete species (*Platynereis dumerilii*, *Polyphthalmus pictus*, and *Syllis prolifera*) and their antioxidant capacity to counteract oxyradicals formation in control and low pH conditions was evaluated, highlighting as the population of *Platynereis* originating from the vent showed higher constitutive antioxidant efficiency [12], which may allow them to cope with short-term and chronic exposure to higher oxidative pressure without further enhancement of antioxidant defences [12]. From these results, a hypothesis of long-term adaptation of the *Platynereis* vent-inhabiting population emerged, suggesting the need of this species for greater antioxidant protection in conditions of chronic oxidative exposure to low pH–high pCO₂ [12]. Recent laboratory

experiments on both species also highlighted a differentiation on the expression of some target genes involved in the oxidative metabolism as a result of the exposure to different pH conditions [48, 49]. Specimens of *Platynereis dumerilii* from a control site near the vents, Sant’Anna, showed significant lower levels of NADH dehydrogenase mRNA expression compared to *P. cfr massiliensis* from Castello acidified sites confirming, as already stated by Calosi et al. [24], that living under acidified conditions entails a higher energetic consumption and metabolic rate [48]. In line with Calosi et al. [24], translocation experiment did not show significant effect for this gene expression in *Platynereis dumerilii* but, in contrast, a significant downregulation of *P. cfr massiliensis* NADH dehydrogenase from low pH to control conditions was observed indicating a reduction in the oxidative metabolism of this species [48]. Differently to what was previously asserted by Lucey et al. [46], recent phylogenetic analysis carried out in the frame of a PhD thesis on *Platynereis* spp. [50], samples collected in the south-acidified areas of Castello Aragonese and the control zone of Sant’Anna rocks highlighted a less evident spatial segregation of the two *Platynereis* species between the two sites. For this reason, it was not possible to consider the Castello vent area as an exclusive domain zone for *P. massiliensis*, as well as the control area of Sant’Anna for *P. dumerilii*.

Considering the preliminary results on antioxidant efficiency of putative *P. dumerilii* and the presence of both species in control and acidified sites, the aim of this study was to provide new insights about the basal levels of the antioxidant system in *Platynereis* spp., comparing populations living at different pH conditions (acidified vs. control). This comparison between populations was carried out in three different periods: April 2016, October 2016, and February 2017, in relation to different temperature conditions. The oxidative effects of different pH levels were evaluated thorough analysis of single antioxidant activities, such as catalase (CAT), glutathione S-transferases (GST), glutathione reductase (GR), and Se-dependent and Se-independent glutathione peroxidases (GPx), that can be very sensitive in revealing a prooxidant condition [51] and thorough their integration with total oxyradical scavenging capacity (TOSC), which quantify the capability of *Platynereis* spp. to neutralize different forms of oxyradicals including peroxy radicals (ROO[•]), hydroxyl radicals (HO[•]), and peroxy nitrite (HOONO) [52].

2. Materials and Methods

2.1. Study Areas, Sample Collection, and Processing. The study was conducted at the Ischia island (Gulf of Naples, Italy), a volcanic island well known for the presence of numerous submarine CO₂ vent systems [29, 53, 54], including the area of Castello Aragonese on the north-eastern side (40° 43.84 N, 13° 57.08 E) as the first vent system studied in the world [29]. Gas bubbles, composed by 90–95% CO₂, 3–6% N₂, 0.6–0.8% O₂, 0.2–0.8% CH₄, with no sulphur, are released at ambient seawater temperature at about 1.4 × 10⁶ l d⁻¹ [21]. The salinity of the water (38) and total alkalinity (2.5 mequiv Kg⁻¹) are relatively uniform [21, 38]. Gas emissions, which occur between 0.5 and 3.0 m depth, create a gradient of pH on both

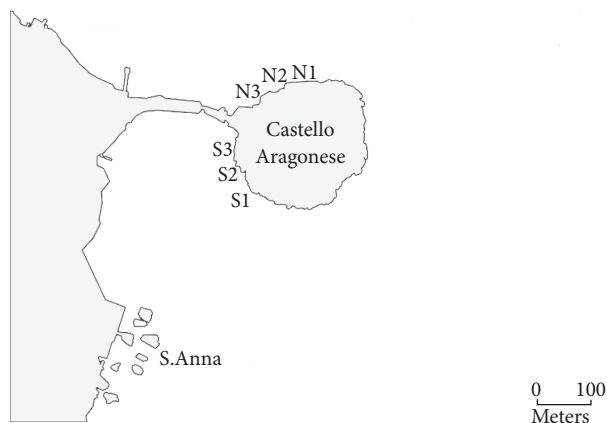


FIGURE 1: Ischia island sampling sites: Castello Aragonese with acidified (S3, S2, N3, N2) and control (S1, N1) stations and the control site at Sant'Anna rocks.

the north and south sides of the Castello islet. According to Kroeker et al. [22], three different pH zones can be identified along a rocky reef of approximately 150 m in length on each side of the islet: a control area with normal pH conditions and no venting activity (N1 and S1), an intermediate area with moderate vent activity and low pH conditions (N2 and S2), and a high venting activity area characterized by extreme low pH conditions (N3 and S3) (Figure 1). The acidified S2–S3 stations where *Platynereis* collection was performed showed a wide range of pH variability as reported by Ricevuto et al. [38, Supplement material]. The control site, called Sant'Anna rocks, characterized by a very stable pH value, which ranged around a mean of 8.01, is located within the Cartaromana Bay, approximately 600 m from the south side of Castello Aragonese [12] (Figure 1).

Platynereis spp. samples were collected in the south-acidified sites of Castello (named as S3 and S2 in previous papers e.g., [36], or as low pH and extreme low pH in [22]) and in the normal/control pH area of Sant'Anna rocks. Samplings were carried out in four different periods: April 2016, October 2016, and February 2017 (mean monthly seawater temperatures 16.5°C, 21.9°C, and 14.6°C, respectively). Worms were collected in each sampling site and period by sampling local macroalgae belonging to the species *Halopteris scoparia*, *Jania rubens*, *Dictyota* spp., and *Cladophora* spp. where these polychaetes live associated. Macroalgal thalli were collected in cotton fabric bags by snorkelling and SCUBA diving at 0.5–2 m depth. After collection, samples were transported to the Villa Dohrn-Benthic Ecology Center (approx. 4 km from the Castello area) inside cool boxes within one hour. Once in the lab, algal thalli were sorted and the *Platynereis* spp. species was identified thanks to the typical sinuous swimming movement, immediately transferred into separated 1.5 ml microcentrifuge tubes (pooling approximately 5–10 individuals per Eppendorf, according to the body mass of the collected samples), frozen, and temporarily stored at -80°C until the transport to the Laboratory of Ecotoxicology and Environmental Chemistry of Ancona (Italy) for the antioxidant analyses.

2.2. Analyses of Antioxidants and Total Oxyradical Scavenging Capacity. For the analysis of the antioxidant enzyme activities, pools were homogenized (1:10 w:v) in 100 mM of potassium phosphate buffer (pH 7.5) containing NaCl (1.5%), 0.1 mg mL⁻¹ phenylmethylsulphonyl fluoride (PMSF), 0.1 mg mL⁻¹ bacitracin and 0.008 TIU mL⁻¹ aprotinin as protease inhibitors. After centrifuging at 100,000 x g for 70 min at 4°C, supernatants were collected and used for the subsequent analyses. Enzymatic activity measurements including catalase (CAT), glutathione S-transferases (GST), glutathione reductase (GR), and glutathione peroxidases (GPx) were carried out using a Varian (Model Cary 3) spectrophotometer at the constant temperature of 18°C according to Bocchetti et al. [30]. CAT activity was determined by the decrease in absorbance at 240 nm ($\epsilon = 0.04 \text{ mM}^{-1} \text{ cm}^{-1}$) due to H₂O₂ consumption (12 mM) in 100 mM K-phosphate buffer (pH 7.0). GST activity was quantified at 340 nm using 1-chloro-2,4 dinitrobenzene (CDNB) as substrate ($\epsilon = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$). The assay conditions were 100 mM potassium phosphate buffer (pH 6.5), 1.5 mM CDNB, and 1.5 mM GSH. GR activity, also known as glutathione-disulphide reductase (GSR), was measured spectrophotometrically at 340 nm following the oxidation of NADPH during the reduction of GSSG (extinction coefficient, $\epsilon = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$). The assay was carried out in 100 mM potassium phosphate buffer (pH 7.0), 1 mM GSSG and 60 μM NADPH. The activity of Se-dependent and Se-independent GPx forms was determined in two enzymatic assays in which GSSG is converted to the reduced form GSH. The consumption of NADPH was quantified as decrease of absorbance at 340 nm ($\epsilon = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) in 100 mM K-phosphate buffer pH 7.5, 1 mM EDTA, 1 mM dithiothreitol (DTT), 2 mM GSH, 1-unit glutathione reductase, 0.24 mM NADPH, and 0.8 mM cumene hydroperoxide as substrate.

For the total oxyradical scavenging capacity (TOSC) analysis, polychaetes were homogenized following the same protocol reported above, with 0.5 $\mu\text{g mL}^{-1}$ pepstatin as additional protease inhibitor and without phenylmethylsulphonyl fluoride (PMSF). This assay was based on the capability of cellular antioxidants to reduce the oxidation of α -keto- γ -methiolbutyric acid (KMBA), and the consequent formation of ethylene gas, in presence of artificially generated oxyradicals. The ethylene formation was monitored at 12 min time intervals by gas-chromatographic analyses and the TOSC values were calculated from the equation: $\text{TOSC} = 100 - (\int \text{SA} / \int \text{CA} \times 100)$, where $\int \text{SA}$ and $\int \text{CA}$ were the integrated areas calculated under the kinetic curve produced during the reaction course for sample (SA) and control (CA) reactions, respectively [52].

In order to obtain the specific antioxidant activity and TOSC values, data were normalized with the relative protein concentration according to Lowry method [55] by using Bovine Serum Albumin (BSA) as standard.

2.3. Statistical Analysis of Data. Permutational multivariate analysis of variance (PERMANOVA) and pairwise non-parametric tests with square root transformation, Euclidean distance, and 9,999 number of permutations were conducted to test the differences ($\alpha = 0.05$). "Population origin" and

TABLE 1: Sampling periods' variations of biomarkers (mean values \pm standard deviations, $n = 5$) in *Platynereis* spp. population from Sant'Anna (SA, normal pH conditions) and Castello Aragonese (CA, acidified pH conditions).

		April		October		February		Period x population
		Mean	s.d.	Mean	s.d.	Mean	s.d.	
CAT	SA	52.91a	15.88	35.52b*	1.85	24.05c*	6.91	
	CA	48.63	20.33	44.82	1.95	45.66	9.78	
GST	SA	58.58a	24.73	29.62b	4.63	30.44b	8.58	
	CA	37.75ab	12.10	34.24a	5.05	26.59b	5.62	
GR	SA	54.53a*	25.45	30.43a	6.94	6.98b*	3.47	$p < 0.001$
	CA	21.68a	2.06	32.11b	5.94	22.67ab	5.15	
GPx	SA	13.91	6.20	26.29	13.67	20.32	5.39	
	CA	16.3a	3.01	23.29b	4.64	18.05ab	3.97	
ROO•	SA	1044.88	359.44	904.99	203.17	774.35	146.88	
	CA	918.83	331.44	988.68	312.71	946.79	270.06	
HO•	SA	654.36	240.36	581.23	157.36	647.05	180.70	
	CA	640.78ab	181.19	748.18a	186.85	446.85b	89.39	
HOONO	SA	500.79a	127.41	786.5b*	101.88	336.42c*	37.99	$p < 0.01$
	CA	451.32	142.57	522.71	134.84	507.98	139.86	

CAT: catalase ($\mu\text{mol}/\text{min}/\text{mg prt}$); GST: glutathione S-transferases ($\text{nmol}/\text{min}/\text{mg prt}$); GR: glutathione reductase ($\text{nmol}/\text{min}/\text{mg prt}$); GPx: glutathione peroxidases ($\text{nmol}/\text{min}/\text{mg prt}$); ROO•, HO•, and HOONO: TOSC toward peroxy, hydroxyl radicals, and peroxyxynitrite (UTosc/ mg prt). The p values are given for the interaction "period x population" when it resulted in statistical significance (PERMANOVA); different letters indicate significant differences among sampling periods ($p < 0.05$), while asterisks indicate significant differences between Sant'Anna and Castello Aragonese *Platynereis* spp. populations ($p < 0.05$) (PERMANOVA pairwise post hoc comparison).

"sampling period" were considered as fixed factors with two and three levels, respectively (sites: Castello and Sant'Anna; periods: April 2016, October 2016, February 2017), to test the response temporal variation between population. Multivariate principal component analysis (PCA) was applied to visualize the relationships among the different populations/sampling periods and all statistical analyses were performed using PRIMER/PERMANOVA v 6 [56].

3. Results

Results are showed in both Table 1 and Figure 2; the activity of GR significantly differed between the two *Platynereis* spp. populations in April and February (pairwise comparison $p < 0.05$, see asterisks Table 1). Sant'Anna population showed significant differences between April–October and April–February (pairwise comparison $p < 0.05$, see letters Table 1). The highest enzyme activity was recorded in April (GR = 54.53 $\text{nmol}/\text{min}/\text{mg prt}$) and the lowest one in February (GR = 6.98 $\text{nmol}/\text{min}/\text{mg prt}$). On the contrary, GR activity was higher in October in organisms sampled from Castello with values comparable to those observed in Sant'Anna population (SA = 30.43 $\text{nmol}/\text{min}/\text{mg prt}$; CA = 32.11 $\text{nmol}/\text{min}/\text{mg prt}$), while constant values were observed for this population in the other months. Concerning the capability to neutralize HOONO, the *Platynereis* spp. populations showed significant differences in October and February between the investigated sites (Figure 2). While the total oxyradical scavenging capacity toward HOONO in organisms from Castello was constant over different sampling periods, conversely, more marked variation was observed for *Platynereis* specimens from Sant'Anna site, with the highest

TOSC efficiency measured in October (HOONO = 786.5 UTosc/ mg prt) and the lowest one in February (336.42 UTosc/ mg prt). Concerning other antioxidant parameters, in worms sampled from Sant'Anna, CAT exhibited the highest activity in April, followed by a significant decrease over the examined periods; on the contrary, Castello specimens showed constant values with some differences ($p < 0.05$) between specimens in October and February (Figure 2). Similar trend, with the highest enzymatic activity in April, was also observed for the GST in both populations. On the contrary, the activity of Se-dependent and Se-independent GPx was lower in April and increased in October and February in organisms sampled from both sites. TOSC values toward ROO• did not show significant differences, while against HO• limited variations were observed in organisms sampled from Castello with a significant decrease in February.

PCA analysis provided a two-dimensional pattern explaining 82.5% of the total variance (56.2% and 26.3% in the first and second axes, respectively) (Figure 3). However, despite some differences occurred mainly for single antioxidants activity between populations and periods, no clear groups' separation occurred (Figure 3).

4. Discussion

This study represents the first attempt of a background analysis of the antioxidant parameters of *Platynereis* spp. (putative different sibling species) living in different pH conditions (normal and acidified conditions). Specimens morphologically identified as *Platynereis dumerilii* were already employed as model organisms for some transplant experiments to investigate the prooxidant effect of ocean

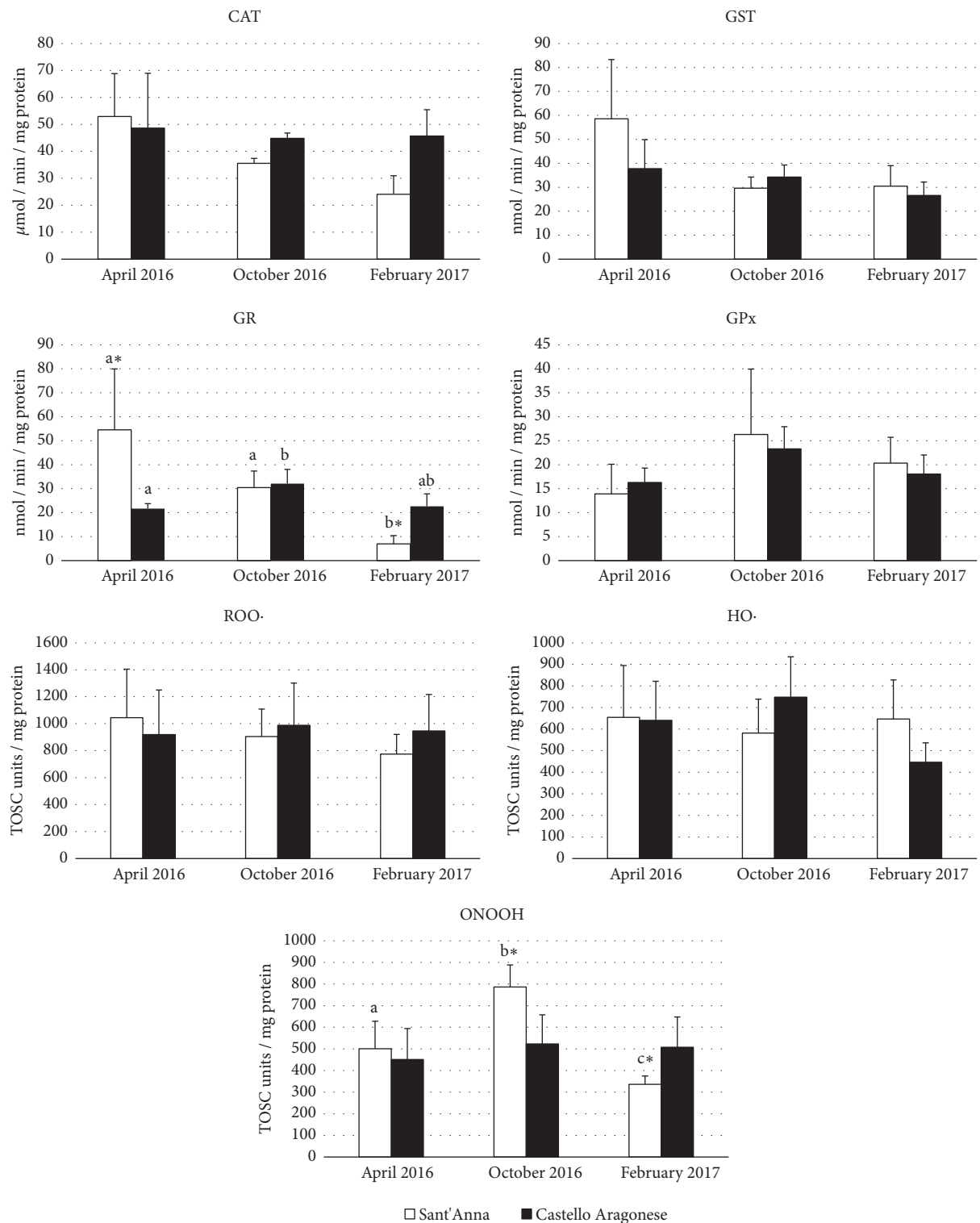


FIGURE 2: Basal antioxidant enzyme activities (CAT, GST, GR, GPx) and total oxyradical scavenging capacity (TOSC) toward peroxyl radicals (ROO^{\bullet}), hydroxyl radicals (HO^{\bullet}), and peroxyxynitrite (ONOOH) in Sant'Anna (white) and Castello Aragonese (black) *Platymereis* spp. populations. Values are expressed as $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein for CAT; $\text{nmol min}^{-1} \text{mg}^{-1}$ protein for GST, GR and GPx; TOSC units/ mg^{-1} protein for ROO^{\bullet} , HO^{\bullet} , and ONOOH . Letters indicate significant differences among sampling periods ($p < 0.05$), while asterisks indicate significant differences between Sant'Anna and Castello Aragonese *Platymereis* spp. populations ($p < 0.05$) (PERMANOVA pairwise post hoc comparison).

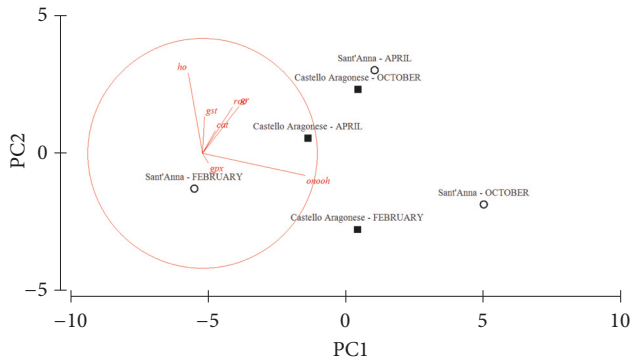


FIGURE 3: Separation of *Platynereis* spp. populations (white circles = Sant'Anna; black squares = Castello Aragonese) and periods of sampling obtained by principal component analysis (PCA) of mean biological responses.

acidification [12, 24, 48, 49], but the basal level efficiency of the antioxidant defence systems in terms of species sensitivities and seasonality of populations submitted to natural acidified and normal pH conditions was never evaluated.

Our overall results indicated that the two populations showed few differences, probably based on seasonal-related conditions in their habitat, in particular for glutathione reductase activity and the total oxyradical capability to counteract HOONO radical. GR catalyses the reduction of glutathione disulphide (GSSG) to the active form of glutathione (GSH) which is a fundamental molecule for preventing oxidative stress and maintaining the reduced environment of cell, using NADPH as cofactor. The GR activity of specimens sampled from control site (Sant'Anna population) showed the highest activity in April and a rapid decrease until February. Conversely, the Castello vent population showed similar trend and values during the whole year, suggesting more stable environmental conditions during the examined periods. Similar considerations were also supposed for TOSC toward HOONO, which showed for specimens collected in Sant'Anna a maximum capability to counteract radical species in October and a minimum one in February, while the vent population displayed constant activity during the whole sampling period.

Based on limited variations, our findings did not show a clear correlation between pH conditions and alteration of the oxidative status in *Platynereis* spp. populations, which was instead already observed in previous ocean acidification experiments with several invertebrate species [6, 8, 10–12, 14–16, 41, 48, 49, 57]. An *in situ* transplant experiment of 30 days into naturally acidified conditions carried out with the fan worm *Sabella spallanzanii* (Sabellidae) highlighted a significant decrease of enzymatic activities of CAT and GPx and the impairment of the overall capability to neutralize hydroxyl radicals (HO[•]) [41]. The effect of pH decrease was also investigated in the polychaete *Diopatra neapolitana*; after 28 days of exposure to low pH levels higher enzymatic activity (CAT, SOD, GSTs) and oxidative alterations were recorded [16]. The results of TOSC assay performed after an *in situ* reciprocal transplant experiment in the Castello vents on

specimens morphologically identified as *Platynereis dumerilii* displayed insights of long-term adaptation of the vent population to greater prooxidant challenge [12]. Conversely to our findings, Ricevuto et al. [12] observed that *Platynereis* vent specimens showed more elevated basal antioxidant efficiency toward ROO[•] and HOONO when compared to the population collected in control pH conditions, suggesting the need of a greater antioxidant protection in conditions of chronic oxidative exposure [12]. A different response to pH conditions was also highlighted by Wäge et al. [48] in whose study *P. cfr massiliensis* from acidified sites showed a marked upregulation of gene expression involved in the energy metabolism compared to *P. dumerilii* from control site. This inconsistency with our results might be due to a mixing of the two species (or complexes) in the studied areas, Castello Aragonese and Sant'Anna rocks, only 600 meters away from each other. The two species would therefore be separated exclusively by a chemical barrier (pH) for which nonsignificant effects were detected at least in *P. dumerilii* [48].

The PCA analysis suggested that the antioxidant response of Sant'Anna population was more differentiated when compared with specimens collected in the acidified areas of Castello, without a clear trend in the different periods (Figure 3). Both statistical analyses supported that the two populations seemed to have different temporal-related trends of the antioxidant defence systems, even if the lowest mean values of antioxidant capacity were mainly recorded in the period characterized by the lowest water temperature (February). This phenomenon was most evident in the Sant'Anna population, which showed a marked decrease of antioxidant activities of CAT, GST, and GR and a lower scavenging capacity toward ROO[•] and HOONO, in February. The antioxidant defence systems' efficiency of marine organisms can be influenced by several environmental factors, such as annual fluctuations in solar irradiance, changes in water temperature, fluctuating oxygen concentration, and exposure to chemical pollutants [58–63]. The lowest temperature of the winter season could entail lower prooxidant pressure and, as a consequence, the need of a decreased antioxidant efficiency, as reported for the European eel, *Anguilla anguilla*, [64], in order to counteract the increase of environmental ROS formation during the summer. High temperature increased ROS production and the consequent enhancement of antioxidant enzymes' activity also in the mussel species *Mytilus coruscus* [57].

In this study, *Platynereis* spp. population from control pH conditions displayed a higher temporal variability, showing the need to modulate the redox response to keep the oxidative stress level of the tissues under control during different periods of the year. Conversely, the overall ability of the vent population to maintain stable levels of antioxidant defences, regardless the period of the year and seasonal-related trends, suggested that natural enhancement of environmental prooxidant conditions was balanced with slight changes of individual antioxidants. The different native pH conditions, over three sampling periods, were not translated into significant differences between populations in the other antioxidant biomarkers analysed, and the similar antioxidant responses

highlighted between the two populations confirmed the high tolerance of these species (or complex of sibling species, [47]). The long-term exposure to moderately elevated $p\text{CO}_2$ conditions, such as those expected in global climate change scenarios at the end of this century [1], could minimally affect the cellular redox status, as already observed in two marine bivalve species, *Crassostrea virginica* and *Mercenaria mercenaria* [9].

5. Conclusions

In conclusion, this study provided the first baseline for a direct characterization of antioxidant responses in *Platynereis* spp. from naturally acidified and control pH conditions. The different $p\text{CO}_2$ and pH levels of the studied habitats did not seem to strongly affect *Platynereis* spp. defence systems' efficiency and no one of the two populations stood out for a stronger or lower antioxidant capacity. *Platynereis* spp. specimens appeared able to acclimatize to low pH conditions with slight seasonal variations of the antioxidant defence systems, with enzymatic activity and TOSC kept constant throughout the year. The inconsistency with previous studies suggested the need to further investigate the seasonal variation of the basal antioxidant systems efficiency of genetically characterized *Platynereis* specimens collected in vent and nonvent sites.

Data Availability

The individual raw data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

- [1] K. Caldeira and M. E. Wickett, "Oceanography: anthropogenic carbon and ocean pH," *Nature*, vol. 425, no. 6956, p. 365, 2003.
- [2] K. Caldeira and M. E. Wickett, "Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean," *Journal of Geophysical Research: Oceans*, vol. 110, no. 9, pp. 1–12, 2005.
- [3] J. C. Orr, V. J. Fabry, O. Aumont et al., "Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms," *Nature*, vol. 437, no. 7059, pp. 681–686, 2005.
- [4] J. Raven, K. Caldeira, H. Elderfield et al., "Ocean acidification due to increasing atmospheric carbon dioxide," The Royal Society Policy Document 12.05, Clyvedon Press, Cardiff, UK, 2005.
- [5] IPCC, *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Core Writing Team, R. K. Pachauri, and L. A. Meyer, Eds., IPCC, Geneva, Switzerland, 2014.
- [6] L. Tomanek, M. J. Zuzow, A. V. Ivanina, E. Beniash, and I. M. Sokolova, "Proteomic response to elevated $p\text{CO}_2$ level in eastern oysters, *Crassostrea virginica*: Evidence for oxidative stress," *Journal of Experimental Biology*, vol. 214, no. 11, pp. 1836–1844, 2011.
- [7] D. Zhang, S. Li, G. Wang, D. Guo, K. Xing, and S. Zhang, "Biochemical responses of the copepod *Centropages tenuiremis* to CO_2 -driven acidified seawater," *Water Science and Technology*, vol. 65, no. 1, pp. 30–37, 2012.
- [8] V. Matozzo, A. Chinellato, M. Munari, M. Bressan, and M. G. Marin, "Can the combination of decreased pH and increased temperature values induce oxidative stress in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*?" *Marine Pollution Bulletin*, vol. 72, no. 1, pp. 34–40, 2013.
- [9] O. B. Matoo, A. V. Ivanina, C. Ullstad, E. Beniash, and I. Sokolova, "Interactive effects of elevated temperature and CO_2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*)," *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, vol. 164, no. 4, pp. 545–553, 2013.
- [10] A. Vehmaa, H. Hogfors, E. Gorokhova, A. Brutemark, T. Holmborn, and J. Engström-Öst, "Projected marine climate change: Effects on copepod oxidative status and reproduction," *Ecology and Evolution*, vol. 3, no. 13, pp. 4548–4557, 2013.
- [11] M. S. Pimentel, F. Faleiro, M. Diniz et al., "Oxidative stress and digestive enzyme activity of flatfish larvae in a changing ocean," *PLoS ONE*, vol. 10, no. 7, Article ID e0134082, 2015.
- [12] E. Ricevuto, M. Benedetti, F. Regoli, J. I. Spicer, and M. C. Gambi, "Antioxidant capacity of polychaetes occurring at a natural CO_2 vent system: Results of an *in situ* reciprocal transplant experiment," *Marine Environmental Research*, vol. 112, pp. 44–51, 2015.
- [13] S. Uthicke, T. Ebert, M. Liddy, C. Johansson, K. E. Fabricius, and M. Lamare, "Echinometra sea urchins acclimatized to elevated $p\text{CO}_2$ at volcanic vents outperform those under present-day $p\text{CO}_2$ conditions," *GCB Bioenergy*, vol. 22, no. 7, pp. 2451–2461, 2016.
- [14] Q. Wang, R. Cao, X. Ning et al., "Effects of ocean acidification on immune responses of the Pacific oyster *Crassostrea gigas*," *Fish and Shellfish Immunology*, vol. 49, pp. 24–33, 2016.
- [15] R. Freitas, Á. Almeida, V. Calisto et al., "The impacts of pharmaceutical drugs under ocean acidification: New data on single and combined long-term effects of carbamazepine on *Scrobicularia plana*," *Science of the Total Environment*, vol. 541, pp. 977–985, 2016.

- [16] R. Freitas, A. Pires, A. Moreira, F. J. Wrona, E. Figueira, and A. M. V. M. Soares, "Biochemical alterations induced in *Hediste diversicolor* under seawater acidification conditions," *Marine Environmental Research*, vol. 117, pp. 75–84, 2016.
- [17] R. Freitas, A. Pires, C. Velez et al., "Effects of seawater acidification on *Diopatra neapolitana* (Polychaete, Onuphidae): Biochemical and regenerative capacity responses," *Ecological Indicators*, vol. 60, pp. 152–161, 2016.
- [18] R. Freitas, L. de Marchi, A. Moreira et al., "Physiological and biochemical impacts induced by mercury pollution and seawater acidification in *Hediste diversicolor*," *Science of the Total Environment*, vol. 595, pp. 691–701, 2017.
- [19] A. Nardi, L. F. Mincarelli, M. Benedetti, D. Fattorini, G. d'Errico, and F. Regoli, "Indirect effects of climate changes on cadmium bioavailability and biological effects in the Mediterranean mussel *Mytilus galloprovincialis*," *Chemosphere*, vol. 169, pp. 493–502, 2017.
- [20] A. Nardi, M. Benedetti, D. Fattorini, and F. Regoli, "Oxidative and interactive challenge of cadmium and ocean acidification on the smooth scallop *Flexopecten glaber*," *Aquatic Toxicology*, vol. 196, pp. 53–60, 2018.
- [21] J. M. Hall-Spencer, R. Rodolfo-Metalpa, S. Martin et al., "Volcanic carbon dioxide vents show ecosystem effects of ocean acidification," *Nature*, vol. 454, no. 7200, pp. 96–99, 2008.
- [22] K. J. Kroeker, F. Micheli, M. C. Gambi, and T. R. Martz, "Divergent ecosystem responses within a benthic marine community to ocean acidification," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 35, pp. 14515–14520, 2011.
- [23] V. R. Johnson, B. D. Russell, K. E. Fabricius, C. Brownlee, and J. M. Hall-Spencer, "Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO₂ gradients," *GCB Bioenergy*, vol. 18, no. 9, pp. 2792–2803, 2012.
- [24] P. Calosi, S. P. S. Rastrick, C. Lombardi et al., "Adaptation and acclimatization to ocean acidification in marine ectotherms: An *in situ* transplant experiment with polychaetes at a shallow CO₂ vent system," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 368, no. 1627, Article ID 20120444, 2013.
- [25] S. Vizzini, R. Di Leonardo, V. Costa, C. D. Tramati, F. Luzzu, and A. Mazzola, "Trace element bias in the use of CO₂ vents as analogues for low pH environments: Implications for contamination levels in acidified oceans," *Estuarine, Coastal and Shelf Science*, vol. 134, pp. 19–30, 2013.
- [26] K. E. Fabricius, G. De'ath, S. Noonan, and S. Uthicke, "Ecological effects of ocean acidification and habitat complexity on reef-associated macroinvertebrate communities," *Proceedings of the Royal Society B Biological Science*, vol. 281, no. 1775, Article ID 20132479, 2013.
- [27] S. Goffredo, F. Prada, E. Caroselli et al., "Biomineralization control related to population density under ocean acidification," *Nature Climate Change*, vol. 4, no. 7, pp. 593–597, 2014.
- [28] M. Milazzo, R. Rodolfo-Metalpa, V. B. S. Chan et al., "Ocean acidification impairs vermetid reef recruitment," *Scientific Reports*, vol. 4, article no. 4189, 2014.
- [29] S. A. Foo, M. Byrne, E. Ricevuto et al., "The carbon dioxide vents of Ischia, Italy, a natural laboratory to assess impacts of ocean acidification on marine ecosystems: an overview of research and comparisons with other vent systems," *Oceanography and Marine Biology: an Annual Review*, vol. 56, pp. 233–306, 2018.
- [30] R. Bocchetti, D. Fattorini, M. C. Gambi, and F. Regoli, "Trace Metal Concentrations and Susceptibility to Oxidative Stress in the Polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae): Potential Role of Antioxidants in Revealing Stressful Environmental Conditions in the Mediterranean," *Archives of Environmental Contamination and Toxicology*, vol. 46, no. 3, pp. 353–361, 2004.
- [31] L. A. Geracitano, R. Bocchetti, J. M. Monserrat, F. Regoli, and A. Bianchini, "Oxidative stress responses in two populations of *Laeonereis acuta* (Polychaeta, Nereididae) after acute and chronic exposure to copper," *Marine Environmental Research*, vol. 58, no. 1, pp. 1–17, 2004.
- [32] E.-J. Won, J.-S. Rhee, R.-O. Kim et al., "Susceptibility to oxidative stress and modulated expression of antioxidant genes in the copper-exposed polychaete *Perinereis nuntia*," *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, vol. 155, no. 2, pp. 344–351, 2012.
- [33] L. A. Maranhão, R. M. Baena-Nogueras, P. A. Lara-Martín, T. A. DelValls, and M. L. Martín-Díaz, "Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete *Hediste diversicolor* as bioindicator species," *Environmental Research*, vol. 134, pp. 353–365, 2014.
- [34] L. Musco, A. Terlizzi, M. Licciano, and A. Giangrande, "Taxonomic structure and the effectiveness of surrogates in environmental monitoring: a lesson from polychaetes," *Marine Ecology Progress Series*, vol. 383, pp. 199–210, 2009.
- [35] M. Díaz-Jaramillo, J. L. Ferreira, L. L. Amado et al., "Biomonitoring of antioxidant and oxidative stress responses in *Perinereis gualpensis* (Polychaeta: Nereididae) in Chilean estuarine regions under different anthropogenic pressure," *Ecotoxicology and Environmental Safety*, vol. 73, no. 4, pp. 515–523, 2010.
- [36] M. Cigliano, M. C. Gambi, R. Rodolfo-Metalpa, F. P. Patti, and J. M. Hall-Spencer, "Effects of ocean acidification on invertebrate settlement at volcanic CO₂ vents," *Marine Biology*, vol. 157, no. 11, pp. 2489–2502, 2010.
- [37] E. Ricevuto, M. Lorenti, F. P. Patti, M. B. Scipione, and M. C. Gambi, "Temporal trends of benthic invertebrate settlement along a gradient of ocean acidification at natural CO₂ vents (Tyrrhenian Sea)," *Biologia Marina Mediterranea*, vol. 19, no. 1, pp. 49–52, 2012.
- [38] E. Ricevuto, K. J. Kroeker, F. Ferrigno, F. Micheli, and M. C. Gambi, "Spatio-temporal variability of polychaete colonization at volcanic CO₂ vents indicates high tolerance to ocean acidification," *Marine Biology*, vol. 161, no. 12, pp. 2909–2919, 2014.
- [39] M. C. Gambi, L. Musco, A. Giangrande, F. Badalamenti, F. Micheli, and K. J. Kroeker, "Distribution and functional traits of polychaetes in a CO₂ vent system: Winners and losers among closely related species," *Marine Ecology Progress Series*, vol. 550, pp. 121–134, 2016.
- [40] S. D. Batten and R. N. Bamber, "The effects of acidified seawater on the polychaete *Nereis virens* sars, 1835," *Marine Pollution Bulletin*, vol. 32, no. 3, pp. 283–287, 1996.
- [41] E. Ricevuto, I. Lanzoni, D. Fattorini, F. Regoli, and M. C. Gambi, "Arsenic speciation and susceptibility to oxidative stress in the fanworm *Sabella spallanzanii* (Gmelin) (Annelida, Sabellidae) under naturally acidified conditions: An *in situ* transplant experiment in a Mediterranean CO₂ vent system," *Science of the Total Environment*, vol. 544, pp. 765–773, 2016.
- [42] G. Bellan, "Annélides polychètes des substrats solides de trois milieux pollués sur les côtes de Provence (France): Cortiou, Golfe de Fos, Vieux Port de Marseille," *Tethys*, vol. 9, no. 3, pp. 260–278, 1980.

- [43] G. Bellan, G. Desrosiers, and A. Willsie, "Use of an Annelid Pollution Index for monitoring a moderately polluted littoral zone," *Marine Pollution Bulletin*, vol. 19, no. 12, pp. 662–665, 1988.
- [44] C. Hauenschild, "Nachweis der sogenannten atoken Geschlechtsform des Polychaeten *Platynereis dumerilii* Aud. et M. Edw. als eigene Art auf Grund von Zuchtversuche," *Zoologische Jahrbücher*, vol. 63, pp. 107–128, 1951.
- [45] S. Schneider, A. Fischer, and A. W. C. Dorresteijn, "A morphometric comparison of dissimilar early development in sibling species of *Platynereis* (Annelida, Polychaeta)," *Roux's Archives of Developmental Biology*, vol. 201, no. 4, pp. 243–256, 1992.
- [46] N. M. Lucey, C. Lombardi, L. De Marchi, A. Schulze, M. C. Gambi, and P. Calosi, "To brood or not to brood: Are marine invertebrates that protect their offspring more resilient to ocean acidification?" *Scientific Reports*, vol. 5, pp. 12009–12009, 2015.
- [47] J. Wäge, G. Valvassori, J. D. Hardege, A. Schulze, and M. C. Gambi, "The sibling polychaetes *Platynereis dumerilii* and *Platynereis massiliensis* in the Mediterranean Sea: are phylogeographic patterns related to exposure to ocean acidification?" *Marine Biology*, vol. 164, no. 10, p. 199, 2017.
- [48] J. Wäge, J. M. Rotchell, M.-C. Gambi, and J. D. Hardege, "Target gene expression studies on *Platynereis dumerilii* and *Platynereis* cfr *massiliensis* at the shallow CO₂ vents off Ischia, Italy," *Estuarine, Coastal and Shelf Science*, vol. 207, pp. 351–358, 2017.
- [49] J. Wäge, A. Lerebours, J. D. Hardege, and J. M. Rotchell, "Exposure to low pH induces molecular level changes in the marine worm, *Platynereis dumerilii*," *Ecotoxicology and Environmental Safety*, vol. 124, pp. 105–110, 2016.
- [50] G. Valvassori, *Genomic and Phenotypic Analyses of Polychaete Sibling Species *Platynereis dumerilii* and *Platynereis massiliensis* in relation to Ocean Acidification [Doctoral thesis]*, The Open University, 2018.
- [51] F. Regoli, S. Gorbi, G. Frenzilli et al., "Oxidative stress in ecotoxicology: From the analysis of individual antioxidants to a more integrated approach," *Marine Environmental Research*, vol. 54, no. 3-5, pp. 419–423, 2002.
- [52] S. Gorbi and F. Regoli, "Total oxyradical scavenging capacity as an index of susceptibility to oxidative stress in marine organisms," *Comments on Toxicology*, vol. 9, no. 5-6, pp. 303–322, 2003.
- [53] D. Tedesco, "Chemical and isotopic investigations of fumarolic gases from Ischia island (southern Italy): Evidences of magmatic and crustal contribution," *Journal of Volcanology and Geothermal Research*, vol. 74, no. 3-4, pp. 233–242, 1996.
- [54] M. C. Gambi, "Emissioni sommerse di CO₂ lungo le coste dell'isola d'Ischia. Rilievi su altre aree come possibili laboratori naturali per lo studio dell'acidificazione e cambiamento climatico a mare," *Notiziario S.I.B.M.*, vol. 66, pp. 67–79, 2014, <http://www.sibm.it/>.
- [55] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.
- [56] K. R. Clarke and R. N. Gorley, "PRIMER/PERMANOVA v6: User Manual/Tutorial," *PRIMER-E*, p. 192, 2006.
- [57] M. Hu, L. Li, Y. Sui et al., "Effect of pH and temperature on antioxidant responses of the thick shell mussel *Mytilus coruscus*," *Fish and Shellfish Immunology*, vol. 46, no. 2, pp. 573–583, 2015.
- [58] D. Abeleoeschger, R. Oeschger, and H. Theede, "Biochemical adaptations of *Nereis diversicolor* (Polychaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats," *Marine Ecology Progress Series*, vol. 106, no. 1-2, pp. 101–110, 1994.
- [59] D. Abele, B. Burlando, A. Viarengo, and H.-O. Pörtner, "Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna*," *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, vol. 120, no. 2, pp. 425–435, 1998.
- [60] D. Abele-Oeschger and R. Oeschger, "Enzymatic antioxidant protection in spawn, larvae and adult worms of *Phyllodoce mucosa* (Polychaeta)," *Ophelia*, vol. 43, no. 2, pp. 101–110, 1995.
- [61] T. Buchner, D. Abele-Oeschger, and H. Theede, "Aspects of antioxidant status in the polychaete *Arenicola marina*: Tissue and subcellular distribution, and reaction to environmental hydrogen peroxide and elevated temperatures," *Marine Ecology Progress Series*, vol. 143, no. 1-3, pp. 141–150, 1996.
- [62] O. Nusetti, M. Esclapés, G. Salazar, S. Nusetti, and S. Pulido, "Biomarkers of oxidative stress in the polychaete *Eurythoe complanata* (Amphinomidae) under short term copper exposure," *Bulletin of Environmental Contamination and Toxicology*, vol. 66, no. 5, pp. 576–581, 2001.
- [63] R. Bocchetti, C. V. Lamberti, B. Pisanelli et al., "Seasonal variations of exposure biomarkers, oxidative stress responses and cell damage in the clams, *Tapes philippinarum*, and mussels, *Mytilus galloprovincialis*, from Adriatic sea," *Marine Environmental Research*, vol. 66, no. 1, pp. 24–26, 2008.
- [64] S. C. Gorbi, C. Baldini, and F. Regoli, "Seasonal variability of metallothioneins, cytochrome P450, bile metabolites and oxyradical metabolism in the European eel *Anguilla anguilla* L. (Anguillidae) and striped mullet *Mugil cephalus* L. (Mugilidae)," *Archives of Environmental Contamination and Toxicology*, vol. 49, no. 1, pp. 62–70, 2005.



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