# Condition of pteropod shells near a volcanic CO₂ vent region

Clara Manno[a,*], Paola Rumolo[b], Marco Barra[b], Sergio d'Albero[b], Gualtiero Basilone[c], Simona Genovese[c], Salvatore Mazzola[c], Angelo Bonanno[c]

[a] British Antarctic Survey, Natural Environmental Research Council, Cambridge, UK
[b] Istituto di Scienze Marine, Consiglio Nazionale delle Ricerche, Naples, Italy
[c] Istituto per lo studio degli impatti Antropici e Sostenibilita' in ambiente Marino, Consiglio Nazionale delle Ricerche, Campobello di Mazara, Italy

ABSTRACT

Natural gradients of pH in the ocean are useful analogues for studying the projected impacts of Ocean Acidification (OA) on marine ecosystems. Here we document the *in situ* impact of submarine CO₂ volcanic emissions (CO₂ vents) on live shelled-pteropods (planktonic gastropods) species *Creseis conica* in the Gulf of Naples (Tyrrhenian Sea, Mediterranean). Since the currents inside the Gulf will likely drive those pelagic calcifying organisms into and out of the CO₂ vent zones, we assume that pteropods will be occasionally exposed to the vents during their life cycle. Shell degradation and biomass were investigated in the stations located within and nearby the CO₂ vent emission in relation to the variability of sea water carbonate chemistry. A relative decrease in shell biomass (22%), increase in incidence of shell fractures (38%) and extent of dissolution were observed in *Creseis conica* collected in the Gulf of Naples compared to those from the Northern Tyrrhenian Sea (control stations). These results suggest that discontinuous but recurrent exposure to highly variable carbonate chemistry could consistently affect the characteristic of the pteropod shells.

## 1. Introduction

Marine ecosystems are increasingly influenced by decreasing seawater pH and carbonate chemistry changes resulting from oceanic absorption of anthropogenic CO₂, a process now well known as Ocean Acidification (OA) (Feely et al., 2004). Calcifying organisms are particularly susceptible to OA because perturbations in the seawater carbonate system, including changes in H⁺ and CO₂ (aq), can reduce their ability to synthesize and/or maintain calcium carbonate skeletons and shells. In efforts to understand the implications of these changes on marine organisms, shallow submarine volcanic CO₂ vents have been identified as useful analogues for studying the prospective impacts of Ocean Acidification on marine ecosystems (Hall-Spencer et al., 2008) since the water surrounding the CO₂ vent naturally lowers the pH of the water column (Williams et al., 1992).

Identifying the natural response of marine organisms to OA is a difficult task in laboratory conditions since the behaviour of the organism is constrained and the feeding environment is poorly simulated (Howes et al., 2014). However, the combination of laboratory experiments with the assessment of naturally acidified environmental gradients (such as CO₂ vent environments), can provide further insights into the threshold pH affecting the performance of vulnerable marine species (Basso et al., 2015). Volcanic CO₂ vents have been widely used as a proxy for future OA conditions by numerous authors showing the negative response of the higher pCO₂ conditions to which benthic organisms have commonly been exposed for their entire life span (i.e. Ricevuto et al., 2012; Milazzo et al., 2014; Langer et al., 2014).

Marine volcanic CO₂ vents are abundant in the Mediterranean Sea, especially around Italy (Dando et al., 1999). Recent studies in the Gulf of Naples (Tyrrhenian Sea, Italy), on the impact of CO₂ vents on marine benthic organisms inhabiting shallow coastal waters, showed a shift from benthic calcareous communities to communities lacking scleractinian coral (Hall-Spencer et al., 2008). Furthermore, settlement and colonization by mollusks and microfauna decreased at the acidified stations (Ricevuto et al., 2012; Milazzo et al., 2014). In the same region, the natural pH gradient negatively affected the growth and survival in bivalves *Pinna nobilis* (Ricevuto et al., 2012) while the patellogastropod limpet *Patella caerulea* was able to counteract the low pH induced shell corrosion by the addition of aragonitic shell layers (Langer et al., 2014).

With specific reference to the hydrological features, the Gulf of Naples is characterised by the presence of two main water masses typical of the southern Tyrrhenian Sea: the Modified Atlantic Water (MAW) and the Levantine Intermediate Water (LIW) (Uttieri et al., 2011). Even if in the study area the water masses are essentially the

---

* Corresponding author.
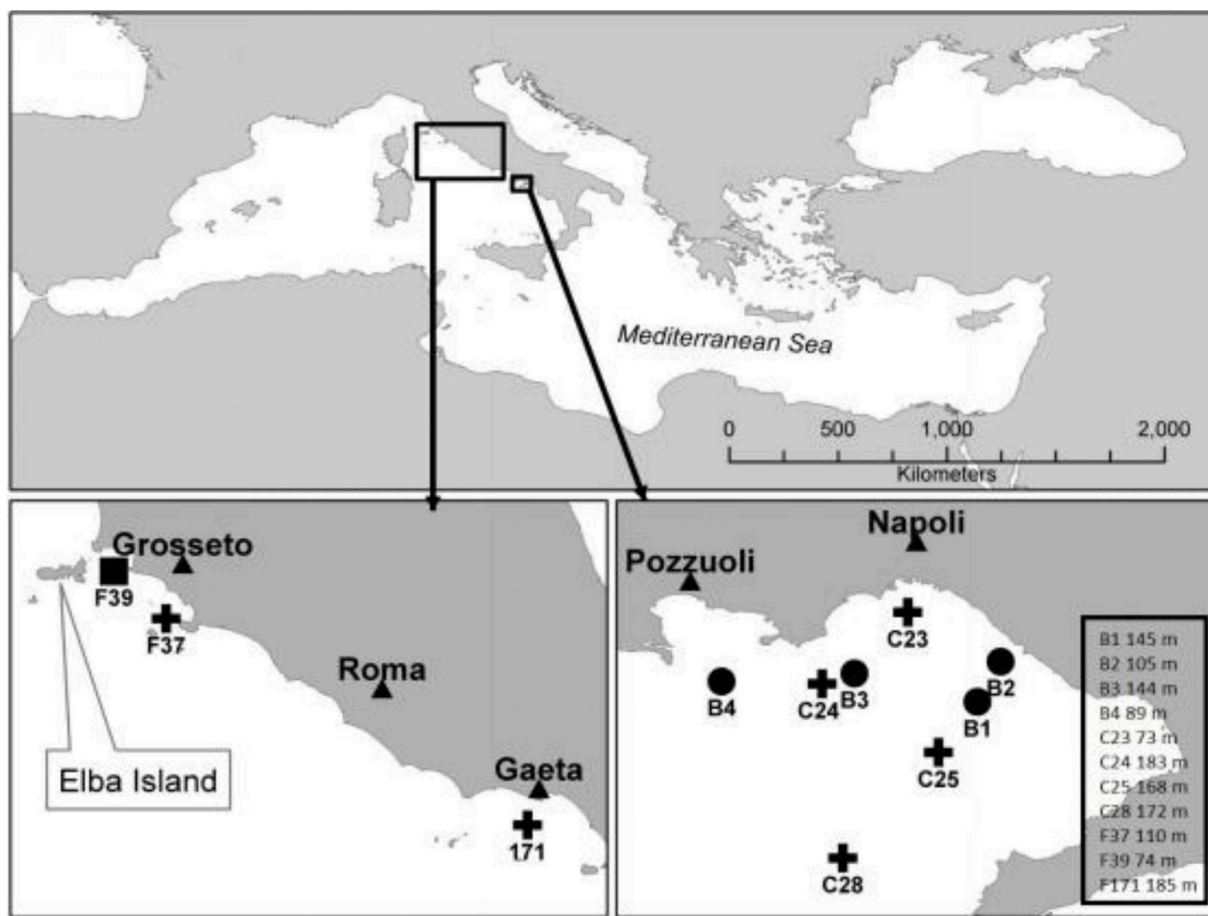  E-mail address: clanno@bas.ac.uk (C. Manno).

**Fig. 1. Sampling station positions in the Northern Tyrrhenian Sea and in the Gulf of Naples (Mediterranean Sea)**. Stations B1-B2-B3-B4 are characterized by the presence of natural submarine volcanic $CO_2$ emissions. Each station depth is indicated. See Passaro et al., 2014, 2016 for a detailed map of $CO_2$ vents emission points.

same as for the southern Tyrrhenian Sea, the presence of $CO_2$ submarine emissions alters the carbonate chemistry nature of the water masses. The presence of natural submarine gas emissions was suggested by (Sacchi et al., 2005). More recently (Passaro et al., 2014, 2016), detected and mapped the gas discharge (dominated by $CO_2$) at the seafloor of the Gulf of Naples and suggested that the occurrence of $CO_2$ vents in this area could be linked to the interaction between volcanic related seafloor morphologies and the main, North East striking faults present in the area, (i.e., Vesuvian fault).

However, all the $CO_2$ vent related studies have been mainly focused on the response of the coastal benthic ecosystem, while the impact of these natural pH gradients on the planktonic calcifying population has not been explored. Unlike sessile benthic organisms, pelagic species can move in and out of waters surrounding the $CO_2$ vents and experience a pronounced variability of $pCO_2$ conditions over time. This mobility makes it difficult to quantify the exposure of pelagic organisms to high $pCO_2$ levels. However, a recent study on corals found that repetitive exposure to high $pCO_2$ conditions may cause greater responses within certain organisms than exposure to static OA (Roleda et al., 2015).

Euthecosome pteropods (planktonic shelled gastropods) have been identified as indicator for OA (OSPAR/ICES advisor group, 2015); as their thin shells are made of aragonite, a metastable form of biogenic $CaCO_3$ (Mucci, 1983), shelled pteropods are extremely sensitive to changes in marine carbonate chemistry. These organisms have been widely studied for OA effects, both in simulated OA conditions in the lab and in the field where high $pCO_2$ levels already occur. Short-term lab experiments (up to a month), examining the impact of exposure to high $pCO_2$, document pteropod shell dissolution, lowered shell

calcification, altered metabolism, behavior, gene expression and decreased survivorship (i.e. Manno et al., 2007; Comeau et al., 2010; Lischka and Riebesell, 2012; Moya et al., 2016). In the field, changes in pteropod species community composition, geographical distribution and presence of shell dissolution have been observed as a result of co-variation of natural high $CO_2$ and low dissolved oxygen across a frontal system in the Southern California Current (Bednaršek et al., 2014, Bednaršek and Ohman, 2015) and within an upwelling region in the Scotia Sea (Bednaršek et al., 2012). Maas et al. (2016) suggested that natural environmental exposure to low pH and oxygen influences pteropod metabolic sensitivity in the Oxygen Minimum Zone in the North Atlantic.

Here we present our observations of pteropods collected around the $CO_2$ vent region in the Gulf of Naples (Tyrrhenian Sea). We aim to assess the condition of pteropod shells (in terms of biomass and dissolution) to episodic exposure to high $pCO_2$ in the presence of volcanic $CO_2$ vents. We focus on the species *Creseis conica* (*C. conica*) which are common and distributed in tropical and subtropical water masses worldwide.

This study documents, for the first time, the impact of natural $CO_2$ volcanic emissions on live pteropods extracted directly from the natural environment. In particular the present work adds new insight to the *in situ* response of pteropods *C. conica* to recurrent exposure to critical carbonate chemistry environments. This study also highlights the importance of including $CO_2$ vent regions within a long term monitoring program to investigate the potential ability of pteropods to persist in a high $CO_2$ ocean.

## 2. Methods

### 2.1. Study region

This study was performed within the framework of the Medias (Mediterranean International Acoustic Survey) project in the Tyrrhenian and Ligurian seas. All the samples and data were collected on August 2015 during an oceanographic cruise in the Tyrrhenian Sea on board of the R/V "G. Dallaporta". A total of 8 stations were sampled in the Gulf of Naples characterized by on site (4 stations, group "B") and nearby (4 stations, group "C") presence of natural submarine volcanic $CO_2$ emissions. Since currents inside the gulf will likely drive the pteropods in (B stations) and out (C stations) the $CO_2$ vent zones, we assume that those organisms will be periodically exposed to the vents during their life. In addition, more stations (3 stations, group "F") were sampled outside of the Gulf of Naples, in the northern Tyrrhenian Sea (Fig. 1), where no $CO_2$ vents have been identified, to provide a control suite of samples (Fig. 1).

Stations characterized by natural gas emissions were identified during a previous oceanographic survey in the same area (Passaro et al., 2014) by means of the Simrad EK60 Scientific Echosounder. Such instrumentation is typically used for estimating biomass and distributions of small pelagic fish species in many areas of the Mediterranean Sea (Bonanno et al., 2014) but also readily identify plumes of bubbles derived from $CO_2$ vents at the seafloor.

### 2.2. Hydrology and carbonate chemistry measurements

Full depth hydrological casts were acquired across all the stations using an SBE 9/11 Plus CTD, with temperature, oxygen, conductivity and fluorometer sensors. The probes were calibrated before the cruise at Sea-Bird Electronics in Kempten, Germany. The collected downcast data were quality-checked and processed using the Seasoft-Win32 software. The overall accuracies are within 0.001 °C for temperature, 0.001 sm$^{-1}$ for conductivity, and 0.015% of full scale for pressure. Raw fluorescence values were converted to Chl a biomass (μg*l$^{-1}$) using the factory calibration.

Discrete Total Alkalinity (TA) and Dissolved Inorganic Carbon (DIC) samples were collected at different depths of the water column using a carousel equipped with Niskin bottles and then poisoned with HgCl$_2$ (2% saturated solution) to prevent biological alteration.

Seawater TA and DIC were measured by potentiometer titration, employing the open-cell procedure. The precision for TA was ± 2.0 mmol kg$^{-1}$ and 4 mmol kg$^{1}$ for DIC. Data accuracy was confirmed by regular analyses of Certified Reference Materials (Scripps Institution of Oceanography). Carbonate saturation states of aragonite ($\Omega_{ar}$) were indirectly calculated from TA and DIC data using the CO2SYS software (Lewis and Wallace, 1998), with carbonate dissociation constants by (Mehrbach et al., 1973) refitted by (Dickson and Millero, 1997) and sulfate dissociation constants by (Dickson, 1990). Note that for logistical reasons no chemistry samples, were collected at station F39.

### 2.3. Pteropod collection and investigation

On board, living pteropods were collected from near bottom depth (ranging between 65 m and 170 m) to the surface by a Bongo-40 zooplankton net (200 μm mesh size). Sampling took place over one time at each station during the day time. The volume of sea water sampled was measured by General Oceanics mechanical flow-meters attached to the ring net. Samples were stored for 3 weeks within buffered formalin solution and kept at 4 °C. pH was measure in all the samples, at the beginning and the end of the storing period to ensure that the state of the shells were not affected by the preservation technique. After three weeks from the collection, pteropod species were identified and counted using a light microscope Olympus SZX16. Pteropod abundance within the water column was calculated as individuals per cubic meter

(Ind*m$^{-3}$).

Investigation of shell morphology and shell biomass was determined only for the dominant pteropod species *C. conica*. Shell morphology was performed using a Scanning Electron Microscope (SEM). The number of individuals analysed for SEM ranged between 10 or 20 for each station (except for station B2 where we analysed only 5 organisms) depending on the availability of specimens. Only individuals with similar shell size (juveniles ranging between 280 μm and 320 μm) were selected to facilitate comparison between different groups assuming same life stage has similar susceptibility to high pCO$_2$ level. Before SEM imaging, individuals were carefully washed with DI water to remove salt on the shell and then air-dried for 24h. The shells outer organic layer (periostracum) was not removed. We acknowledge that the exclusion (Bednaršek et al., 2016) or inclusion (Peck et al., 2016b) of periostracum for evaluate shell dissolution is still in debate. However our rationale for not removing the periostracum prior to imaging shells follows previous studies (Peck et al., 2015) suggesting that the removal of the organic outer layer, which also has an intra-crystalline matrix (Marin et al., 1996), can expose crystals in a way which could be misinterpreted as shell dissolution.

Shell degradation was evaluated by applying a semi-quantitative index of dissolution (Gerhardt and Henrich, 2001; Lischka et al., 2011; Manno et al., 2012). This Dissolution Index is represented by six preservation stages (from 0 = best preservation to 5 = highest degree of dissolution), determined by: shell surface lustre (whether lustrous or dull); shell damage (surface with shell corrosion and/or perforation of at least one layer of aragonite). For each station, we calculated the % of shell falling in four dissolution levels: no corrosion (transparency, preservation stage 0); low corrosion (opacity with small sign of dissolution, preservation stage 1–2); high corrosion (periostracum and the first aragonite prismatic are partially missing, preservation stage 3–4); damage (presence of perforation, preservation stage 5).

Shell surface was inspected for the presence or absence of fracture zone (i.e., resulting from *in situ* mechanical damage) and represented as % shells presenting fractures to the total shells. To discriminate between "natural fractures" and fractures due to mechanical damage from the net and collection processing, we only considered the "historical fractures" where it appears that the animal has built up shell material to weld the shell back together (Peck et al., 2018).

For the measurement of shell biomass (carbonate content expressed as μg CaCO$_3$), individuals were heated to 550 °C for 5 h to eliminate organic matter content and the ashes (representing the remains of the shells) weighed using a Toledo microbalance. The ash weight can be considered an indirect estimate of CaCO$_3$ content. As for SEM investigation, to allow us to estimate shell biomass difference between groups, we only used individuals with similar shell diameter (juveniles, 302 μm ± 11, for a total of 76 specimens) and presenting the best shell condition within each group (31, F; 30 C; 15, B specimens).

### 2.4. Data analysis

For each station, the average values of carbonate chemistry parameters (pH, TA, $\Omega_{ar}$, DIC, pCO$_2$) were computed together with total abundance, shell biomass, shell dissolution level, and percentage of fractured shells. Temperature, salinity, Chl a and oxygen values recorded at the same depth of carbonate chemistry measurements were extracted from CTD profiles to obtain the average hydrological conditions at each station. Obtained data matrix was then used in the statistical analysis. The pairwise correlation between all above-mentioned factors was computed by using Spearman correlation coefficient. PCA was used to investigate the presence of pattern of variables (that could be interpreted as "processes") as well as to best explain the variation observed among stations. The differences among the identified groups of stations were assessed with parametric statistical tests (namely ANOVA and *t*-test according to the nubers of groups). If serious violations in the assumption required by parametric tests were identified, the
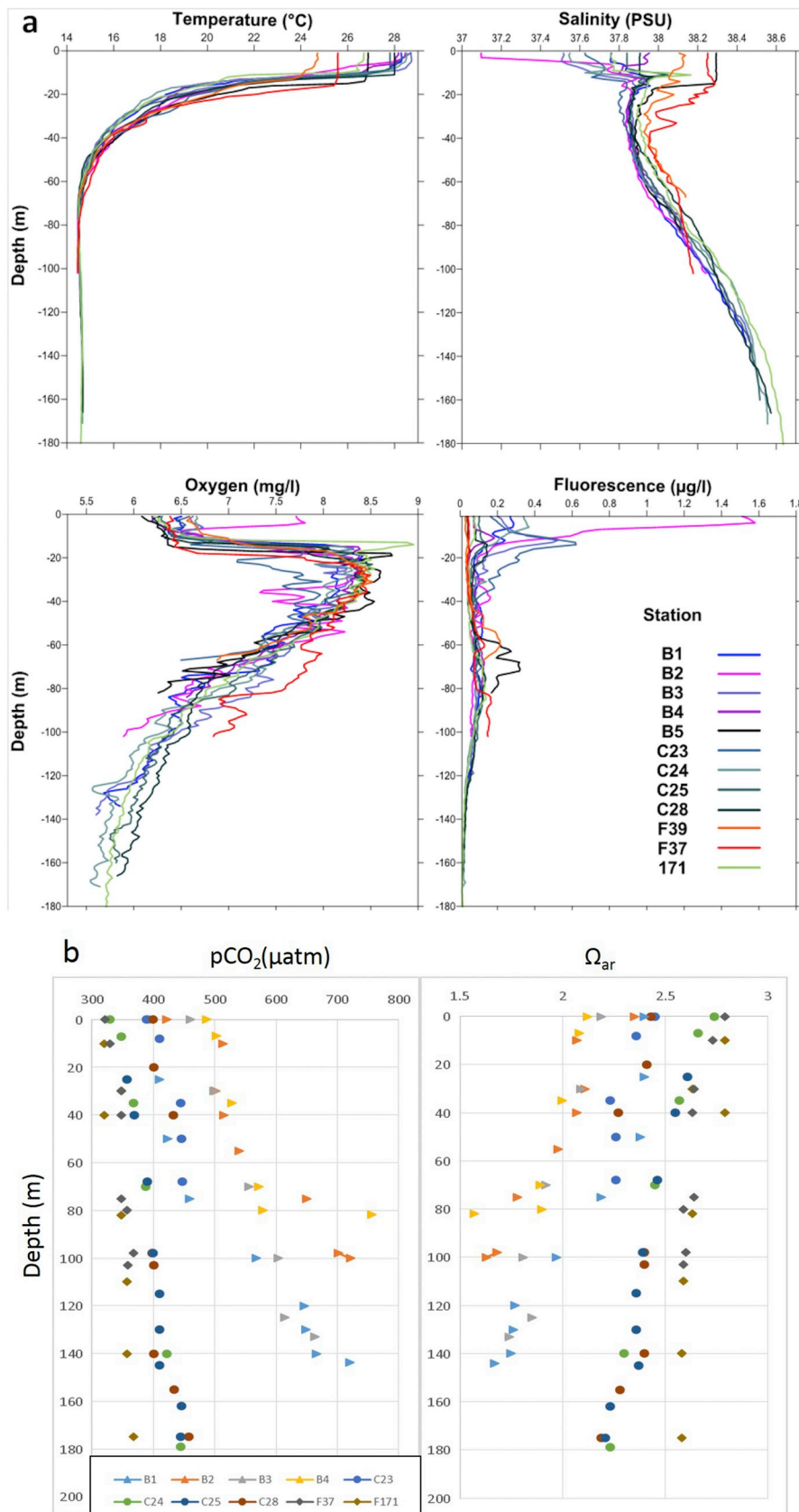
Fig. 2. Vertical hydrological and carbonate chemistry profiles. a) Temperature, salinity, oxygen (mg*l$^{-1}$), Chl a (µg*l-1) and b) pCO$_2$ (µatm) and Ωar (aragonite saturation state) at the sampling stations (F control, C nearby vents and B vents station).

non-parametric alternative were used (Kruskal-Wallis ANOVA and Wilcoxon rank sum test).

Shannon diversity index was used to characterize the pteropods biodiversity in the stations. All statistical analysis were carried out in R statistical environment (R Core Team, 2018).

## 3. Results

### 3.1. Hydrology profiles

At all stations mean surface and bottom temperature ranged between 25.6 °C and 28.2 °C and between 14.45 °C and 14.67 °C respectively (Fig. 2a). Surface salinity was strongly influenced by the river outflow with values ranging from 37.09 to 38.25. The salinity minimum due to the presence of the Modified Atlantic Water (MAW) was typically positioned between 30 and 45 m (Fig. 2a). Oxygen concentration exhibited a similar profile at all stations except B2 (Fig. 2a) where higher surface oxygen values were mainly influenced by the Sarno river outflow. Fluorescence values ranged between 0.01 and 1.58 µg*$1^{-1}$. The higher values were recorded in the Gulf of Naples and in particular in the B2, B3 and C23 stations (Fig. 2a). Dataset of the hydrological parameters is available in Table 1 in S1_Table.

### 3.2. Carbonate chemistry

Significant differences were recorded among the three groups of stations for both $\Omega_{ar}$ (ANOVA, F(2,7) = 101.4, p < 0.001) and $pCO_2$ (ANOVA, $F_{(2,7)}$ = 240.7, p < 0.05). In particular, although seawater was not under-saturated with respect to the aragonite at any of the stations (i.e., $\Omega_{ar}$ > 1 at all stations), $\Omega_{ar}$ and $pCO_2$ values in the Gulf of Naples (stations B and C) were respectively significantly lower and higher than at control site (stations F). Differences in carbonate chemistry were also evaluated by grouping the B and C stations (Gulf of Naples) and comparing such group with the stations outside the gulf (control). Obtained results showed that stations outside the gulf were significantly different from the B + C group ($\Omega$ar;$t_{(7)}$ = 5.78; p < 0.05 and $pCO_2$; $t_{(7)}$ = 4.7, p < 0.05).

Dataset of carbonate chemistry is available in Table 2 in S1_Table.

### 3.3. Difference in pteropod abundance and "shell fitness" between stations

Pteropod abundance was significantly different between the three groups of stations (K-W ANOVA, H $_{(2)}$ = 7.13, p < 0.05). In particular, pteropod abundance was significantly lower at group C (40%) and B (82%) stations than the control stations, group F.

Pteropod diversity was significantly different (Shannon diversity index, (K-W ANOVA, H (2) = 6.76, p < 0.05) between the three groups of stations also. In particular, diversity was significantly higher at the control stations outside the Gulf of Naples (group F, 100% of identified species) than in the stations of the groups B and C. Dataset of pteropod relative abundance is available in Table 3 in S1_Table.

The state of *C. conica* shell condition is presented in (Fig. 3a). ANOVA test showed that the percentage of shells presenting no signs of corrosion, low corrosion and high corrosion were significant different among the considered groups (No Corr: F(2,8) = 9284, p < 0.05; Low Corr: K-w: H(2) = 9.07, p < 0.05; High Corr: F(2,8) = 102.6, p < 0.05). Pteropods collected from the $CO_2$ vent stations (group B) presented a significantly higher degree of dissolution than pteropods collected from stations C and F. In particular all pteropods collected in group F had a well preserved and transparent shells (stage 0). Conversely, within group B, 60% of shells showed stage 4 levels of dissolution. Shell dissolution (even if moderate) was also observed in the group C, with 70% of shells exhibiting opacity and dullness (stage 3). SEM pictures in Fig. 3b are representative of the different *C. conica* shell dissolution stages observed. We did not observe evidence of shell perforation (stage 5) in any specimens.

Significant differences among the three groups were evidenced also in terms of incidence of shell fractures (F (2, 8) = 51, p < 0.001) and biomass. In particular, at stations within group B the highest incidence of shell fractures and the lowest biomass was recorded. Significant differences were also recorded between C and F stations, the latter presenting the lowest incidence of shell fractures and the highest biomass. Dataset of pteropod shell biomass, fractures and dissolution are available in Table 4, 5 and 6 in S1_Table.

Comparing the B + C stations against the F group, the presence of significant differences between the Gulf of Naples (B + C) and the Control station (F) were confirmed (No Corr: $t_{(7)}$ = 103.97, p < 0.05; Low Corr: $t_{(7)}$ = 9.26, p < 0.05; High Corr: $t_{(7)}$ = 6.32, p < 0.05; incidence of shell fractures: $t_{(7)}$ = 7.8, p < 0.05; biomass: $t_{(7)}$ = 7.35, p < 0.05).

### 3.4. Relationships with carbonate chemistry and hydrology

The pairwise correlation analysis (Fig. 4a) showed the presence of strong correlations between carbonate chemistry and some parameters related to the condition of pteropods (such as the lowest and the highest dissolution levels, the biomass and the percentage of shell fractures). In particular, the absence of dissolution was positively related to high $\Omega_{ar}$ values. Conversely, the highest level of corrosion was negatively related to $\Omega_{ar}$. The abundance and the shell biomass were found positively correlated with $\Omega_{ar}$ while the opposite was true for the percentage of shell presenting fractures. No significant differences were found among the three groups of stations in terms of temperature, salinity and oxygen, evidencing the presence of comparable hydrological conditions.

PCA analysis further confirm the relationships observed in correlation analysis, providing a more clear picture of the factors driving differences among the three groups of stations (Fig. 4a and b). The first two PCA axis explained 82% of the total variance. In particular, the 1st PC axis was significantly (see Table 7 in S1_Table) related to $\Omega_{ar,}$ and $pCO_2$, absence of corrosion (No Corr) and higher corrosion, as well as to abundance, percentage of shell with fractures and biomass. Such patterns evidenced that stations having lower values on the first PCA axis were characterized by higher abundance, biomass, $\Omega_{ar}$ and lower shell dissolution (No Corr.) as well as by lower $pCO_2$, percentage of shell with fractures and lower proportion of pteropods shell characterized by higher degree of corrosion. As the 1st axis accounts for 62.76% of the total variance it is clear that most of the variability among stations is related to pteropods and carbonate chemistry parameters. In this context all the B stations were clustered on the right side of the 1st PC axis, while the F stations showed the lowest values with respect to such axis. C stations were mainly found in intermediate position along the 1st PC axis evidencing the presence of intermediate conditions between B and F stations in terms of the parameters related to the 1st PC. Regarding the 2nd PC axis, it was found strongly related only to the hydrological parameters. Also, as it accounts for a much lower proportion of the total variance, the weak effect of hydrological condition in driving the differences among the stations was confirmed. Dispersion of stations along the 2nd PC axis is much lower than the one along the 1st PC, and the observed differences along the 2nd PC are mainly due to local factors, such as distance from the coast (leading to higher Chl a) or the presence of freshwaters input (it is the case of C23 and F37 stations).

## 4. Discussion

### 4.1. Pteropod shell fitness around $CO_2$ vents

This study documents, for the first time, the impact of natural $CO_2$ volcanic emissions on live pteropods extracted directly from the natural environment. We illustrated that the decrease of $\Omega_{ar}$, associated with the presence of $CO_2$ vents, can alter the chemical environment for planktonic calcifying organisms in the vicinity. In particular, *in situ*
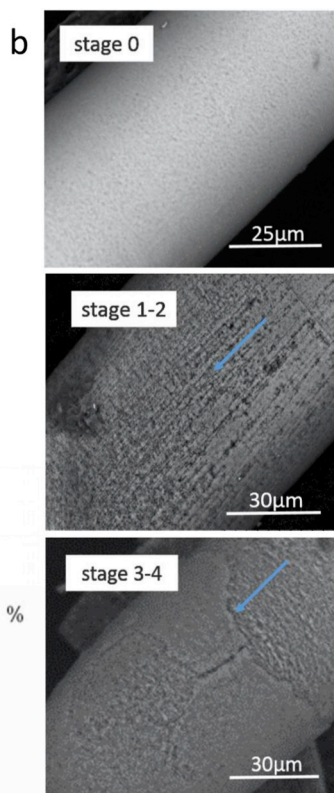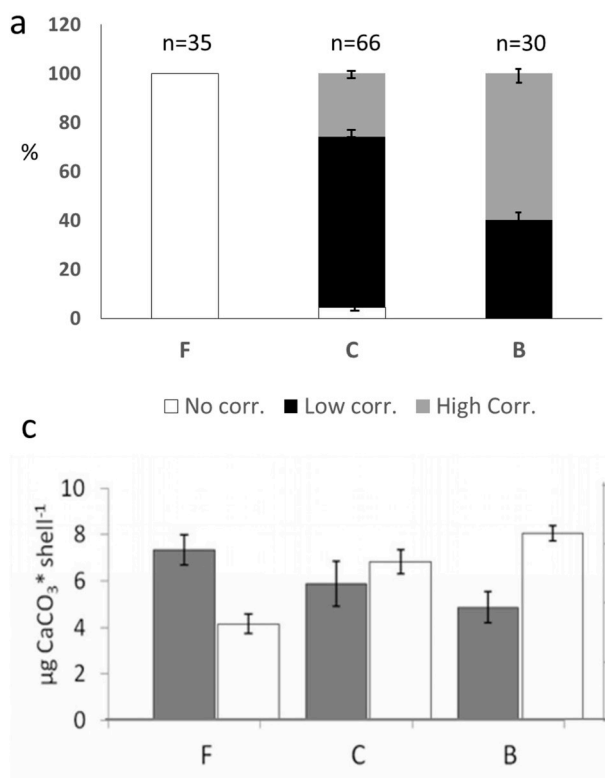
Fig. 3. Difference in pteropod abundance and "shell fitness" between stations a) Shell dissolution level (%) of *C. conica* collected at the group B (vent stations), C and station F; b) SEM images showing different levels of dissolution for *C. conica* shells. The top image shows a detail of *C. conica* shell in perfect condition (stage 0, mainly found in the group F); in the middle *C. conica* shell lustreless with sign of dissolution (stage 2–3, mainly found in group C and B); on the botttom *C. conica* shell with high dissolution where the periostracum and the first aragonite prismatic are partially missing (stage 4, mainly found in the group B); c) shell biomass (grey histogram, µg CaCO3* shell$^{-1}$) and shell presenting fractures (white histogram, %) of *C. conica*.
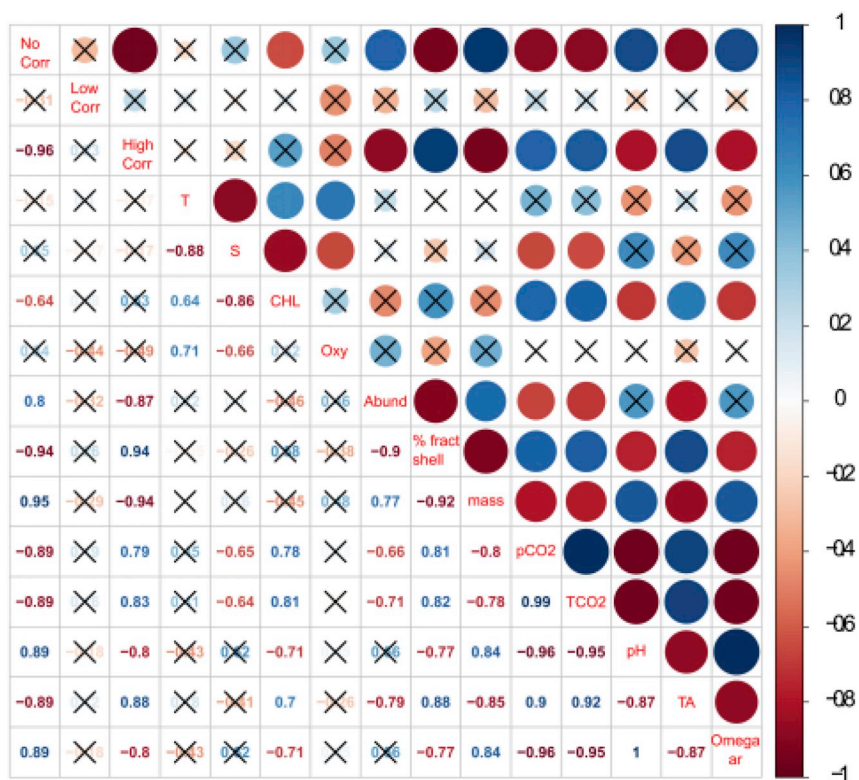


Fig. 4a. Pairwise Spearman correlation plot among the considered variables. Correlation values are reported in the lower triangular matrix. In the upper triangular matrix a graphical representation of correlation values is reported (higher the correlation, bigger the circle; blue and red colours indicate positive and negative correlations respectively). The "X" symbol is used to mark the non-significant ($p > 0.05$) correlations. Note: no corrosion = % shell presenting preservation stage 0; low corrosion = % shell presenting preservation stage 1–2; high corrosion = % shell presenting preservation stage 3–4; DIC = $TCO_2$ (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

shell dissolution and change in shell biomass were the predominant features observed in the live pteropods collected in the Gulf of Naples (in the station located within and nearby the $CO_2$ vent discharge) compare to pteropods collected in the control stations. Unfortunately, so far there are no studies on seasonal variability of the carbonate chemistry in this region, however, the pH difference between the $CO_2$

vent stations and the controls is higher than the natural seasonal variability of the Liguria coastal site (Howes et al., 2015), located on the border with the Tyrrhenian Sea.

The difficulty in investigating pelagic organisms along a "natural gradient" is determining the residence-times of populations within the $CO_2$ vent stations, so as to parameterise the duration of their exposure
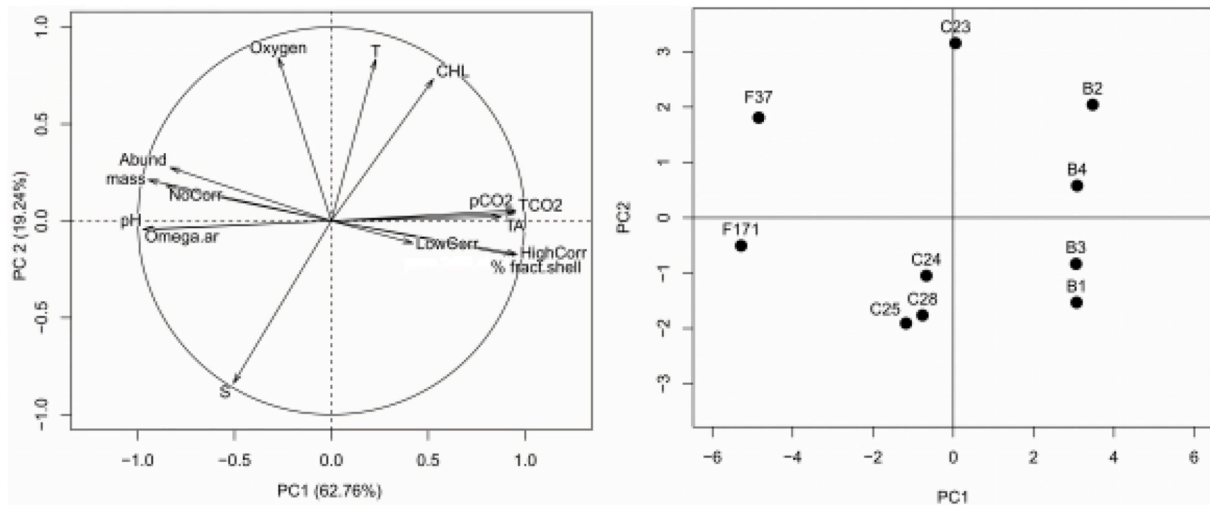
**Fig. 4b. PCA of the considered variables.** Visual representation of the correlation among environmental, chemistry, biological factors variables and PCs (left panel) and distribution of the stations along the 1$^{th}$ and 2nd PCs space (right panel). Note corr = corrosion; $TCO_2$ = DIC.

to the stressor. However, pteropods may perform diel and/or seasonal vertical migration, spending part of their time under low $\Omega_{ar}$ (nearby the $CO_2$ vent source at the bottom) and part in the more saturated waters at the surface (Bednaršek et al., 2012; Manno et al., 2016). In particular, Creseidae (such as *C. conica*) seems to perform diel vertical migration (Be and Gilmer, 1977; Hsueh, 1995) with a vertical distribution > 100 m (Van der Spoel, 1967; Be and Gilmer, 1977). Consequently, despite we do not have information on the vertical distribution of pteropods at the time of collection, we can assume that the organisms collected at the $CO_2$ vent stations (group B, emission depth ranging from 89m to 145m) will have been daily exposed to $pCO_2$ fluctuation.

Another challenge is that pteropods are also not static spatially and will likely move around and outside the Gulf of Naples transported by currents. Two different water inflow and outflow regimes are present in the Gulf of Naples, with a tendency towards stagnation inside the basin during spring and summer and a more effective water renewal mechanism in fall and winter under NE winds (Cianelli et al., 2015). In particular, Mazzocchi et al. (2012) outlined that the only few species representative of the coastal area dominate the zooplankton assemblage in summer owing to coastal retention (Cianelli et al., 2015). Thus, given a growth rate of about 0.33 mm per month (Bednaršek et al., 2012; Wells, 1976) and a mean diameter of pteropod shell investigated in this study of 302 μm, it is likely that specimens were retained in the Gulf of Naples from the very beginning of their life cycle and within the same water mass condition. As Bednaršek et al., 2012 and Wells, 1976 reported similar values of shell growth rates on pteropods collected from very different regions (i.e. Scotia Sea and West India respectively), we assume that the used growth rate can be representative for pteropods collected in the present study.

Uttieri et al. (2011) using a model simulations of particle transport (in the summer period) demonstrate the presence of a scarce renewal of coastal waters, both over short (i.e., 48 h) and long (i.e., 1 month) periods. The authors found that the residence times was very high, with particles remaining in the deployment area on average for more than 15 days. This simulation confirms that pteropods will spend a relevant amount of time in the station of collection before to be moved back and forward around the Gulf. Thus, although we do not have information about the resident time of pteropods in the Gulf of Naples, we can assume that pteropods collected in August (this study) have likely been trapped in the Gulf and have experienced intermittent $CO_2$ vent impact for months. The presence of impacted pteropod shell in the stations (group C) not directly located on the $CO_2$ vent discharge, confirm the role of currents within the Gulf of Naples, driving the pelagic calcifiers

inside and outside the $CO_2$ vent emissions. Conversely, sessile benthic calcifiers (as for result of their sedentary behaviour) experience shell degradation only when directly located around the $CO_2$ vents (i.e., Basso et al., 2015; Milazzo et al., 2014).

The variability nature of the $CO_2$ vent system over the time is a key factor in the interpretation of the observed negative impact on pteropods shell and in part explains the high level of shell dissolution despite the presence of oversaturated seawater ($\Omega_{ar} > 1$). We are aware that our data are not representative of the carbonate chemistry condition over time and a detailed survey throughout the year will be an important next step. However, Passaro et al. (2016) found that in the Gulf of Naples bubble plumes generated at the $CO_2$ vent are highly variable: from a continuous, dense bubble-flux to short-lived phenomena. In particular, the authors found the pH values above a shallow $CO_2$ vent emission (75 m depth) decrease from 8.4 (at 70 m depth) to 7.8 (at the bottom). Unfortunately, the authors did not provide $\Omega_{ar}$ values but the pH values at the bottom are lower than the pH we observed near the $CO_2$ vent emissions and it could likely correspond to lower $\Omega_{ar}$ than the values observed in our study. Therefore, periodical exposition to critical low $\Omega_{ar}$ values may drive the dissolution state of pteropod shells.

Overall, we suggest that pteropods around the $CO_2$ vents in the Gulf of Naples, are negatively impacted when periodically exposed to high spatial and temporal variability in $\Omega_{ar}$. Evidence of impact on pteropod shell dissolution has already been reported in the field. Within an upwelling system, Bednaršek et al. (2014), observed higher levels of shell dissolution (up to preservation stage 5) than the present study. This can reflect either the higher magnitude as well as time of exposure of pteropods in this region compared to the Gulf of Naples. Further, the absence of additive environmental stressors in the Gulf of Naples such as variability in oxygen and nutrient concentration could also partially explain the lower impact on the shell compared to the upwelling system.

In the future targeted research, focused on the investigation of vertical distribution and migration of pteropods in the $CO_2$ vent regions, will be crucial to improve our understanding on the potential ability of these organisms to avoid water depths with critical carbonate saturation state. It will be important to use Lagrangian modelling studies (to track pteropods across temporal and spatial scales) since in addition to intensity and duration of exposure (Manno et al., 2012, 2016), the impact of $CO_2$ vents on pteropods is likely to be also a function of the recovery time between the exposures itself. Lagrangian particle tracking models coupled with hydrodynamic models are particularly efficient tools to examine the role played by various physical

processes and to study transport processes over an entire basin to simulate zooplankton dispersion and distribution at different scales (e.g. Speirs et al., 2006; Lett et al., 2007).

### 4.2. Impact on the pteropod shell biomass

We found that shell biomass was significantly lower in pteropods living within the Gulf of Naples compared to those in the Northern Tyrrhenian Sea. Only individuals of the similar length (juveniles, $302 \mu m \pm 11$) were used to measure shell biomass in order to compare the different groups. The decrease in shell biomass suggests that calcification was lower than dissolution and in turn the shell biomass decrease. This was more the case of the individuals presenting high level of shell dissolution (group B) where likely the dissolution exceed the calcification. However, shell biomass of individuals in the group C, which present manly shells with opacity and/or low level of dissolution was still significantly lower, suggesting that the lower shell biomass was a common features of pteropods in the Gulf of Naples (compared to the shell biomass values of the control stations outside the Gulf). We are aware that other environmental factors can play a role in pteropods shell growth (e.g. temperature and salinity) (Lalli and Gilmer, 1989), however differences in salinity and temperature between the stations during the summer were within the natural seasonal variability of the Tyrrhenian Sea and well within the pteropods' tolerance window (Lalli and Gilmer, 1989). Further, incubation experiment of pteropods under a range of salinity (Manno et al., 2012) and temperature (Lischka and Riebesell, 2012) show that those parameters have to change quite considerably before a negative effect is detectable (i.e., shell growth, behaviour, survival). Similarly, the potential role of temperature on shell dissolution was excluded because previous works found that under manipulate water condition increasing in temperature not leads to dissolution on pteropods (Lischka et al., 2011; Gardner et al., 2018). Food availability may also play a critical role in determining the shell growth because food supply is required to support the metabolic processes facilitating bio-calcification as well as the resistance of calcifiers to adverse condition such as OA (Ramajo et al., 2016). Particulate food availability to pteropods, as inferred indirectly from average Chl a fluorescence in each station was not significantly different between the three groups of stations, suggesting pteropods were not limited by food availability in the region around the $CO_2$ vents.

Evidence of change in shell morphology in response to change in carbonate chemistry associated with shallow-water $CO_2$ vents has already been observed in benthic molluscs (i.e., Langer et al., 2014; Garilli et al., 2015). Garilli et al. (2015) show that benthic gastropod species (*Cyclope neritea* and *Nassarius corniculus*) adapted to acidified seawater ($\Omega_{ar} = 0.68$) were smaller than those found in normal pH conditions (8.1) while Langer et al. (2014) found that the patellogastropod limpet *Patella caerulea* counteracted the induced shell dissolution in the $CO_2$ vent waters ($\Omega_{ar} = 3.01$) by enhanced production of internal aragonite shell layers. Incubation experiments on the Mediterranean pteropod, *Creseis acicula*, reported a 30% decrease in calcification with a decrease in $\Omega_{ar}$ from 3.3 to 2.0 (Comeau et al., 2012). Moya et al. (2016) show that pteropod *Heliconoides inflatus* exhibited a 50% decrease in gross calcification when exposed to waters of $\Omega_{ar} = 2$ (compare to control condition $\Omega_{ar} = 2.9$).

Our results provide *in situ* evidence that shifts away from an organisms optimum $\Omega_{ar}$ values can significantly affect calcification despite waters remaining oversaturated. In support our observation, pteropod shells collected within sediment traps became significantly lighter over recent decades as $\Omega_{ar}$ decreased (Roberts et al., 2011). A decrease in the shell thickness of modern (2000+, $\Omega_{ar} = 4.0$) tropical pteropod *D. longirostris* compared to 1960s ($\Omega_{ar} = 3.5$) samples has been observed (Roger et al., 2012). Further, Howes et al. (2017) compared the difference in shell thickness of pteropod samples (*Cavolinia inflexa* and *Styliola subula*) collected in the Tyrrhenian Sea with archived samples from 1910's. The authors observed that shell thickness

from modern pteropods ($\Omega_{ar} = 3.4$) was significantly less than from individuals collected on 1910's ($\Omega_{ar} = 3.88$) (despite they state those decrease in shell thickness should be treated with caution). Comparison with the present study and Howes et al. (2017), both made in the Tyrrhenian Sea, highlights the relevance of using natural environmental gradients to forecast the impact of high $pCO_2$ on marine organisms as spatial change (natural variability of the carbonate chemistry, associated to $CO_2$ vents) can be a substitute for time (100's older vs. modern samples, Howes et al., 2017). Further short time experimental studies (up 29 days), where pteropods were incubated at undersaturated $\Omega_{ar}$ levels, found a decrease of calcium carbonate precipitation and shell diameter, respectively up 28% (Comeau et al., 2010) and 12% (Lischka and Riebsell, 2012) confirming the relevance of short episodic exposure in natural environments.

We observe an inverse relationship between shell biomass and the incidence of shells presenting fractures, indicating that fractures are most commonly found in shells with low biomass i.e., thinner/low density shells. Assuming predation pressure is comparable across all sites, we consider that thin shells found at station B are more fragile and therefore more prone to fracture than the more robust, high biomass shells. Although the effectiveness of the periostracum for pteropods is a matter of debate (Peck et al., 2016b; Bednaršek et al., 2016). Peck et al. (2016a) indicated that the shells of healthy, living pteropods are only susceptible to dissolution of the shell where the periostracum has been breached and the aragonite beneath is exposed to undersaturated waters. The susceptibility of the thin, fragile shells of pteropods at the $CO_2$ vent stations to fracturing increases the incidence of aragonite being exposed beneath the damaged periostracum. The consequence of increased incidence of mechanical damage to the shell and exposure to undersaturated waters is consistent with our observation of heightened incidence of shell dissolution.

In this study, *C. conica* (as well as the total pteropod assemblage) in the Gulf of Naples were lower in abundance compared to those collected in the control stations (Northern Tyrrhenian Sea). Due to the highly patchy distribution of pteropod abundance and sampling collection being limited to one time, the impact of $CO_2$ vents on pteropod survivorship can only be speculative and any interpretation have to be evaluated with extremely caution. However, it is likely that the observed increasing in shell degradation and decreasing in shell biomass could contribute to increasing in pteropod mortality (because affecting shell buoyancy, defence against predator etc.). Bednaršek et al. (2014) also observed a relationship between shell dissolution and decrease in pteropod abundance within the upwelling system and suggested that increased dissolution combined with increased shell fragility could potentially induce pteropod population decline.

Marine organisms have the potential to adapt to changes in ocean pH and adaptation potential can be inferred from existing genetic diversity related to patterns of local adaptation across present gradients in environmental pH (Stilmann and Paganini, 2015). Even if not explored in this study, the decrease in shell biomass of pteropods as potential local adaptation to natural low saturation state of $CaCO_3$, is an interesting matter for future investigation. At the high latitudes, for example, due to the natural lower saturation state of $CaCO_3$, shell-building materials are more difficult to extract from seawater and calcifying organisms present thinner shells than individuals living at medium and low latitudes (Graus, 1974). Understanding the persistence of populations of marine organisms in future altered environments requires first an understanding of extant phenotypic plasticity under realistic environmental conditions and the potential for adaptation (Stilmann and Paganini, 2015). $CO_2$ vent regions might help to improve our understanding to predict if pteropod populations possess adequate genetic variation to adapt to forecasted environmental change. Future long term monitoring of the *in situ* population dynamics as well as study on phenotypic plasticity and genetic variation across natural small scale gradients (such as $CO_2$ vent) will be crucial to understanding the plasticity-adaptive-defence of this organism to persist in a more

acidified ocean over short (< 10 year) to medium (10–100 year) temporal scales.

## Acknowledgements

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2018.11.003.

## References

Basso, L., Hendriksa, I., Steckbauera, A., Duarte, C., 2015. Resistance of juveniles of the Mediterranean pen shell, (*Pinna nobilis*) to hypoxia and interaction with warming. Estuar. Coast Shelf Sci. 165, 199–203.

Be, A.W.H., Gilmer, R.W., 1977. A zoogeographic and taxonomic review of euthecosomatous Pteropoda. In: Ramsay, A.T.S. (Ed.), Oceanic Micropalaeontology. Academic Press, London, pp. 733–808.

Bednaršek, N., Ohman, M., 2015. Changes in pteropod distributions and shell dissolution across a frontal system in the California current system. Mar. Ecol. Prog. Ser. 523, 93–103.

Bednaršek, N., Tarling, G.A., Fielding, S., Bakker, D.C.E., 2012. Population dynamics and biogeochemical significance of *Limacina helicina antarctica* in the Scotia Sea (southern ocean). Deep-Sea Res. II 59, 105–116.

Bednaršek, N., Feely, R.A., Reum, J.C.P., Peterson, B., Menkel, J., Alin, S.R., Alin, B.H., 2014. *Limacina helicina* shell dissolution as an indicator of declining habitat suitability owing to ocean acidification in the California Current Ecosystem. Proc. R. Soc. Ser. B 281, 20140123.

Bednaršek, N., Johnson, J., Feely, R.A., 2016. Comment on Peck et al: vulnerability of pteropod (Limacina helicina) to ocean acidification: shell dissolution occurs despite an intact organic layer. Deep-Sea Res. II 127, 53–56.

Bonanno, A., Giannoulaki, M., Barra, M., Basilone, G., Machias, A., Genovese, S., Goncharov, S., Popov, S., Rumolo, P., Di Bitetto, M., Aronica, S., Patti, B., Fontana, I., Giacalone, G., Ferreri, R., Buscaino, G., Somarakis, S., Pyrounaki, M.M., Tsoukali, S., Mazzola, S., 2014. Habitat selection response of small pelagic fish in different environments. Two examples from the oligotrophic Mediterranean Sea. PloS One 9 (7), e101498.

Cianelli, D., Falco, P., Iermano, I., Mozzillo, P., Uttieri, M., Buonocore, B., Zambardino, G., Zambianchi, E., 2015. Inshore/offshore water exchange in the Gulf of Naples. J. Mar. Syst. 145, 37–52.

Comeau, S., Jeffree, R., Teyssié, J.L., Gattuso, J.P., 2010. Response of the Arctic pteropod *Limacina helicina* to projected future environmental conditions. PloS One 5, e11362.

Comeau, S., Gattuso, J.P., Jeffree, R., Gazeau, F., 2012. Effect of carbonate chemistry manipulations on calcification, respiration, and excretion of a Mediterranean pteropod. Biogeosciences 9, 6169–6189.

R Core Team, 2018. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Dando, P.R., Stüben, D., Varnavas, S.P., 1999. Hydrothermalism in the Mediterranean Sea. Prog. Oceanogr. 44, 333–367.

Dickson, A.G., 1990. Standard potential of the reaction: AgCl(s) + 1/2H2 (g)=Ag(s) + HCl(aq), and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. J. Chem. Thermodyn. 22, 113–127.

Dickson, A.G., Millero, F.J., 1997. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. 34, 1733–1743.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J., Millero, F.J., 2004. Impact of anthropogenic CO2 on the CaCO3 system in the oceans. Science 305, 362–366.

Gardner, J., Manno, C., Bakker, D.C.E., Peck, V.L., Tarling, G.A., 2018. Southern Ocean pteropods at risk from ocean warming and acidification. Mar. Biol. 165, 1–12.

Garilli, V., Rodolfo-Metalpa, R., Scuderi, D., Brusca, L., Parrinello, D., Rastrick, S.P.S., Foggo, A., Twitchett, R.J., Jason, Hall-Spencer, J.M., Milazzo, M., 2015. Physiological advantages of dwarfing in surviving extinctions in high-CO2 oceans. Nat. Clim. Change 5, 668–672.

Gerhardt, S., Henrich, R., 2001. Shell preservation of *Limacina inflata* (Pteropoda) in surface sediments from the Central and South Atlantic Ocean: a new proxy to determine the aragonite saturation state of water masses. Deep-Sea Res. I 48, 2051–2071.

Graus, R.R., 1974. Latitudinal trends in the shell characteristics of marine gastropods. Lethaia 7, 303–314.

Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, S., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D., Buia, M.C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454, 96–99.

Howes, E.L., Bednaršek, N., Büdenbender, J., Comeau, S., Doubleday, A., Gallager, S.M., Hopcroft, R.R., Lischka, S., Maas, A.E., Bijma, J., Gattuso, J.P., 2014. Sink and swim: a status review of thecosome pteropod culture techniques. J. Plankton Res. 36, 299–315.

Howes, E.L., Stemmann, L., Assailly, C., Irisson, J.O., Dima, M., Bijma, J., Gattuso, J.P.,
2015. Pteropod time series from the North Western Mediterranean (1967-2003): impacts of pH and climate variability. Mar. Ecol. Prog. Ser. 531, 193–206.

Howes, E.L., Eagle, R.A., Gattuso, J.P., Bijma, J., 2017. Comparison of mediterranean pteropod shell biometrics and ultrastructure from historical (1910 and 1921) and present day (2012) samples provides baseline for monitoring effects of global change. PloS One 12 (1), e0167891.

Hsueh, P.W., 1995. Abundance of larval *Creseis virgula conica* (Mollusca: Thecosomata) in daytime and nighttime high tides. Bulletin Natl. Mus. Nat. Sci. 6, 131–134.

Lalli, C.M., Gilmer, R.W., 1989. Pelagic Snails: the Biology of Holoplanktonic Gastropod Mollusks. Stanford University Press, Stanford, CA.

Langer, G., Nehrke, G., Baggini, C., Rodolfo-Metalpa, R., Hall-Spencer, J.M., Bijma, J., 2014. Limpets counteract ocean acidification induced shell corrosion by thickening of aragonitic shell layers. Biogeosciences 11, 7363–7368.

Lett, C., Veitch, J., van der Lingen, C.D., Hutchings, L., 2007. Assessment of an environmental barrier to transport of ichthyoplankton from the southern to the northern Benguela ecosystems. Mar. Ecol.: Prog. Ser. 347, 247–259.

Lewis, E., Wallace, D.W.R., 1998. CO2sys - Program Developed for CO2 System Calculations Report ORNL/CDIAC-105 (Carbon Dioxide Information and Analysis Centre, vol 1998 Oak Ridge Natl. Lab., US Dep. of Energy.

Lischka, S., Riebesell, U., 2012. Synergistic effects of ocean acidification and warming on overwintering pteropods in the Arctic. Global Change Biol. 18, 3517–3528.

Lischka, S., Büdenbender, J., Boxhammer, T., Riebesell, U., 2011. Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation and shell growth. Biogeosciences 8, 919–932.

Maas, A.E., Lawson, G.L., Wang, Z.A., 2016. The metabolic response of thecosome pteropods from the North Atlantic and North Pacific Oceans to high CO2 and low O2. Biogeosciences 13, 6191–6210.

Manno, C., Sandrini, S., Tositti, L., Accornero, A., 2007. First stages of degradation of *Limacina helicina* shells observed above the aragonite chemical lysocline in Terra Nova Bay (Antarctica). J. Mar. Sci. 68, 91–102.

Manno, C., Morata, N., Primicerio, R., 2012. *Limacina retroversa's* response to combined effects of ocean acidification and sea water freshening. Estuar. Coast Shelf Sci. 113, 163–171.

Manno, C., Peck, V., Tarlig, A.G., 2016. Pteropod eggs released at high pCO2 lack resilience to ocean acidification. Sci. Rep. 6, 25752.

Marin, F., Smith, M., Isa, Y., Muyzer, G., Westbroek, P., 1996. Skeletal matrices, muci, and the origin of invertebrate calcification. Proc. Natl. Acad. Sci. 93, 1554–1559.

Mazzocchi, M.G., Dubroca, L., García Comas, C., Di Capua, I., Ribera d'Alcalà, M., 2012. Stability and resilience in coastal copepod assemblages: the case of the Mediterranean long-term ecological research at Station MC (LTER-MC). Prog. Oceanogr. 97–100, 135–151.

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.

Milazzo, M., Rodolfo-Metalpa, R., Chan, V.B.S., Fine, M., Alessi, C., Thiyagarajan, V., Hall-Spencer, J.M., Chemello, R., 2014. Ocean acidification impairs vermetid reef recruitment. Sci. Rep. 4, 4189.

Moya, A., Howes, E.L., Lacoue-Labarthe, T., Foret, S., Hanna, B., Medina, M., Munday, P.L., Ong, J.S., Teyssié, J.L., Torda, G., Watson, S.A., Miller, D.J., Bijma, J., Gattuso, J.P., 2016. Near future pH conditions severely impact calcification, metabolism and the nervous system in the pteropod Heliconoides inflatus. Global Change Biol. 22, 3888–3900.

Mucci, A., 1983. The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. Am. J. Sci. 283, 780–799.

Passaro, S., Genovese, S., Sacchi, M., Barra, M., Rumolo, P., Tamburrino, S., Mazzola, S., Basilone, G., Placenti, F., Aronica, S., Bonanno, A., 2014. First hydroacoustic evidence of marine, active fluid vents in the Naples Bay continental shelf (Southern Italy). J. Volcanol. Geoth. Res. 285, 29–35.

Passaro, S., Tamburrino, S., Vallefuoco, M., Tassi, F., Vaselli, O., Giannini, L., Chiodini, G., Caliro, S., Sacchi, M., Rizzo, A.L., Ventura, G., 2016. Seafloor doming driven by degassing processes unveils sprouting volcanism in coastal areas. Sci. Rep. 6, 22448.

Peck, L.S., Clark, M.S., Power, D., Reis, J., Batista, F.M., Harper, E.M., 2015. Acidification effects on biofouling communities: winners and losers. Global Change Biol. 21, 1907–1913.

Peck, V., Tarling, L., Geraint, A., Manno, C., Harper, E.M., 2016b. Response to comment "Vulnerability of pteropod (*Limacina helicina*) to ocean acidification: shell dissolution occurs despite an intact organic layer" by Bednaršek et al. Deep-Sea Res. II 2016. https://doi.org/10.1016/j.dsr2.2016.03.007.

Peck, V.L., Tarling, G.A., Manno, C., Harper, E.M., 2016a. Outer organic layer and internal repair mechanism protects pteropod *Limacina helicina* from ocean acidification. Deep-Sea Res. II 127, 57–59.

Peck, V.L., Oakes, R.L., Harper, E.M., Manno, C., Tarling, G.A., 2018. Pteropods counter mechanical damage and dissolution through extensive shell repair. Nat. Commun. 9, 1–7.

Ramajo, L., Pérez-León, E., Hendriks, I.E., Marbà, N., Krause-Jensen, D., Sejr, M.K., Blicher, M.E., Lagos, N.A., Olsen, Y.S., Duarte, C.M., 2016. Food supply confers calcifiers resistance to ocean acidification. Sci. Rep. 6, 19374.

Ricevuto, E., Lorenti, M., Patti, F.P., Scipione, M.B., Gambi, M.C., 2012. Temporal trends of benthic invertebrate settlement along a gradient of ocean acidification at natural CO2 vents (Tyrrhenian Sea). Biol. Mar. Mediterr. 19, 49–52.

Roberts, D., Howard, W.R., Moy, A.D., Roberts, J.L., Trull, T.W., Bray, S.G., Hopcroft, R.R., 2011. Interannual pteropod variability in sediment traps deployed above and below the aragonite saturation horizon in the Sub-Antarctic Southern Ocean. Polar Biol. 34, 1739–1750.

Roger, L.M., Richardson, A.J., McKinnon, A.D., Knott, B., Matear, R., Scadding, C., 2012.

Comparison of the shell structure of two tropical Thecosomata (*Creseis acicula* and *Diacavolinia longirostris*) from 1963 to 2009: potential implications of declining aragonite saturation. J. Mar. Sci. 69, 465–474.

Roleda, M.Y., Cornwall, C.E., Feng, Y., McGraw, C.M., Smith, A.M., Hurd, C.L., 2015. Effect of ocean acidification and pH fluctuations on the growth and development of coralline algal recruits, and an associated benthic algal assemblage. PloS One 10 (10), e0140394.

Sacchi, M., Insinga, D., Milia, A., Molisso, F., Raspini, A., Torrente, M., Conforti, A., 2005. Stratigraphic signature of the vesuvius 79 AD event off the Sarno prodelta system, Naples bay. Mar. Geol. 222, 443–469.

Speirs, D.C., Gurney, W.S.C., Heath, M.R., Horbelt, W., Wood, S.N., Cuevas, B.A., 2006. Ocean-scale modelling of the distribution, abundance, and seasonal dynamics of the copepod *Calanus finmarchicus*. Mar. Ecol. Prog. Ser. 313, 173–192.

Stillman, J.H., Paganini, A.W., 2015. Biochemical adaptation to ocean acidification. J. Exp. Biol. 218, 1946–1955.

Uttieri, M., Cianelli, D., Nardelli, B.B., Buonocore, B., Falco, P., Colella, S., Zambianchi, E., 2011. Multiplatform observation of the surface circulation in the gulf of Naples (southern Tyrrhenian Sea). Ocean Dynam. 61, 779–796.

Van der Spoel, S., 1967. In: Bohn, Scheltema, Holkema (Eds.), Euthecosomata, a Group with Remarkable Developmental Stages (Gastropoda. Pteropoda). Utrecht, pp. 375.

Wells, F.E., 1976. A preliminary examination of euthecosomatous pteropod of the central coast of western Austarlia. Record West Aust. Mus. 4 (4), 327–334.

Williams, N.S., Schaefer, S.J., Calvache, V.M.L., Lopez, D., 1992. Global carbon dioxide emission to the atmosphere by volcanoes. Geochem. Cosmochim. Acta 56, 1765–1770.