



RESEARCH ARTICLE

WILEY

Effects of low pH and high temperature on two *Palythoa* spp. and predator–prey interactions in the subtropical eastern Atlantic

Cataixa López¹ | María Bas-Silvestre¹ | Adriana Rodríguez^{1,2} | Alberto Brito¹ | Sabrina Clemente¹

¹Departamento de Biología Animal, Edafología y Geología, Facultad de Ciencias, Universidad de La Laguna, San Cristóbal de La Laguna, Spain

²Grupo de investigación Biodiversidad Marina y Conservación, IU-ECOQUA, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

Correspondence

Cataixa López, Departamento de Biología Animal, Edafología y Geología, Facultad de Ciencias, Universidad de La Laguna, San Cristóbal de La Laguna, Spain.
Email: clopezba@ull.edu.es

Funding information

Fundación Caja-Canarias, Grant/Award Number: INDICATROP; European Social Fund; Canarian Agency for Research, Innovation and Information Society of the Ministry of Economy, Industry, Trade and Knowledge

Abstract

1. In the current context of climate change, benthic cnidarians of the genus *Palythoa* have been suggested to be resistant owing to their intrinsic biological characteristics. In tropical regions, some species are currently proliferating in areas where environmental conditions are less suitable for other organisms, even replacing hard coral ecosystems.
2. Considering their tropical affinities, phase-shifts towards *Palythoa*-dominated areas could become more frequent in future climate change scenarios, leading to changes in ecosystem organization. The aim of this study was to evaluate the effect of climate change stressors in two common *Palythoa* spp. with different habitat affinities within a subtropical region, and the effect upon their predator–prey interactions.
3. The results of this experimental study demonstrated that colonies of *P. aff. clavata* and *P. caribaeorum* were significantly affected by exposure to temperature and pH conditions predicted for 2100 in the Canary Islands, during 62 days.
4. Despite zoantharians' lack of carbonate in their body wall, *Palythoa* spp. were most affected in their growth rates by lowered pH, and colonies significantly decreased in weight and size. Although all colonies exhibited symptoms of bleaching at high temperature, a reduction in chlorophyll content was also observed at low pH.
5. Predation by *Platypodiella picta* crabs decreased on *P. aff. clavata* exposed to acidic conditions, which may compensate for the lowered ecological performance of the species in these climate change conditions. In contrast, *P. picta* was able to actively feed on *P. caribaeorum* colonies regardless of the experimental conditions.
6. Despite being suggested as winner species in a climate change scenario, our study demonstrated that low pH negatively impacted *Palythoa* spp. survival. If the species are not able to acclimatize to the new conditions, changes in their populations may be expected, although their magnitude could be ameliorated by means of a decrease in predation rates.

KEYWORDS

acidification, benthos, indicator species, invertebrates, ocean warming, predation experiments

1 | INTRODUCTION

Rising concentrations of atmospheric carbon dioxide (CO₂) is one of the most critical problems of climate change because its effects are globally pervasive and irreversible on ecological timescales (Doney et al., 2009). The ocean is able to mitigate the effects of climate change through absorption of ~30% of the total CO₂ emissions from human activities (Feely et al., 2004; Sabine et al., 2004) but with consequences for marine ecosystems. Such accumulation of CO₂ is causing ocean acidification (Gattuso & Buddemeier, 2000) that leads to a decrease in calcification rates of many marine organisms with calcareous structures such as algae (Feely et al., 2004), molluscs (Michaelidis et al., 2005), echinoderms (Shirayama & Thornton, 2005) and corals (Langdon et al., 2003). However, not only calcareous organisms are affected by lower pH conditions. For instance, some species of non-calcareous macroalgae are favoured by elevated CO₂ that promote photosynthesis and growth (Gao et al., 1993; Kübler et al., 1999; Riebesell et al., 2007), while others are disadvantaged (Israel et al., 1999; Rodríguez et al., 2018) or suffer no observable effects (Israel & Hophy, 2002; Jokiel et al., 2008). Recent models estimate a reduction in surface ocean pH ranging from 0.3 to 0.5 units over the next 100 years, depending on the CO₂ emission scenario used (Caldeira & Wickett, 2005; Lüthi et al., 2008). Under these conditions, it is expected that morphological, physiological and ecological modifications in marine organisms will become more frequent, compromising the stability of the whole ecosystems.

Another consequence of the increase of CO₂ concentrations is that global air and sea surface temperatures (SST) have risen considerably (Bindoff et al., 2007). This trend is expected to accelerate in the current century (IPCC, 2013), causing modifications in ecosystems worldwide. Most evident effects of ocean warming are changes in patterns of species distributions, which are leading to global tropicalization (Perry et al., 2005; Sunday et al., 2012; Horta e Costa & Gonçalves, 2013; Vergés et al., 2014; López et al., 2015) and meridionalization (Yapici et al., 2016) of the marine biota. Furthermore, an increase in ocean temperatures would have a negative impact on the survival of marine species that already live near their limit of distribution (Somero, 2002; Hughes et al., 2003; Harley et al., 2006).

Organisms most affected by rising ocean temperatures are those that live in association with zooxanthellae belonging to the family Symbiodiniaceae. In hermatypic corals, up to 143% of the host's daily energy requirements are provided by symbiotic dinoflagellates through photosynthesis (Muscatine et al., 1984; Davies, 1991). It is known that symbioses between Symbiodiniaceae and hermatypic corals are very sensitive to changes in temperature. As sea water temperatures increase, this association breaks down and corals expel their dinoflagellates (coral bleaching) (Winter et al., 1998; Michalek-Wagner & Willis, 2001), thus losing their main source of

energy (Baker, Glynn & Riegl, 2008). Such coral bleaching events have already led to extensive mortality in both hard (Glynn et al., 2001; Loya et al., 2001; Depczynski et al., 2013) and soft corals around the world (Harvell et al., 2001; Prada, Weil & Yoshioka, 2010; Dias & Gondim, 2015). The occurrence and harshness of mass coral bleaching events have increased in the last decades, triggering a situation in which coral reefs and their entire associated flora and fauna communities are under threat (Hoegh-Guldberg, 1999; Raymundo et al., 2019).

There are many effects of both ocean warming and acidification at the individual level, e.g. changes in morphology, physiology and behaviour (Pörtner & Knust, 2007; García et al., 2015; Nagelkerken & Munday, 2016). Additionally, indirect effects of such alterations can be detected at community levels, e.g. modifications of the outcomes of species interactions such as competition for space or resources, or predatory interactions (Rodríguez et al., 2017; Ullah et al., 2018). Most experimental studies have only explored the effects of these factors in isolation (Dupont et al., 2012; Luz et al., 2018), but the number of studies combining both factors is starting to increase (e.g. Holcomb et al., 2012; Kroeker et al., 2013; Heuer & Grosell, 2014; Cole et al., 2018; Ullah et al., 2018). Moreover, there is also a need for experiments focusing on assessing not only direct effects of climate change conditions on single species but also indirect effects such as predator-prey interactions (Dixon, Munday & Jones, 2010; Ferrari et al., 2011; Ghedini, Russell & Connell, 2015).

Zoantharian species of the genus *Palythoa* (Cnidaria: Anthozoa) are zooxanthellate benthic cnidarians widely distributed in tropical and subtropical regions around the world (Acosta et al., 2005; Ong, Reimer & Todd, 2013; Santos et al., 2016). Contrary to scleractinian corals, *Palythoa* spp. lack carbonate in their body wall and incorporate sand and detritus instead (Mueller & Haywick, 1995). For this reason, they have been suggested to be more robust to changes in ocean pH than hard corals (Reimer et al., 2008). On the other hand, owing to their sensitivity to thermal stress, *Palythoa* spp. have been considered as early indicators of bleaching events (Williams & Bunkley-Williams, 1990; Goldberg & Wilkinson, 2004; Parkinson et al., 2016), although they rarely die owing to their mixotrophic nature (Reimer, 1971; Jiménez, 2001; Tanner, 2002; Santana et al., 2015). These intrinsic features combined with their fast growth rates (Karlson, 1988; Rabelo, Soares & Matthews-Cascos, 2013), efficient sexual and asexual reproduction strategies (Acosta, 2001; Acosta et al., 2005; Hirose et al., 2011; Polak et al., 2011; Wee et al., 2017), and their anti-predator mechanisms through synthesis of toxic chemicals (Sebens, 1982; Tubaro et al., 2011), make zoantharians especially resilient to different sources of environmental stress. Ecological implications of any population outbreak of zoantharians still need to be disentangled, but available information and biological characteristics of the species support the idea of a significant alteration of marine communities. Such processes have been mainly described in abrupt

decreases in reef-building coral abundances, following the proliferation and dominance of zoantharians (Cruz et al., 2015; Cruz, Kikuchi & Creed, 2016). However, the replacement of macroalgae communities by colonies of *Palythoa* spp. also leads to modification of the composition of associated mesofauna (González-Delgado et al., 2018) and even the whole intertidal and subtidal landscapes (López et al., 2020). In a climate change context, zoantharians may become ecologically dominant species to the detriment of other sessile organisms (Reimer et al., 2008; Durante, Cruz & Lotufo, 2018), compromising native ecosystems' biodiversity and function.

The Canary Islands constitute the northern limit of the distribution ranges of *Palythoa caribaeorum* and *P. aff. clavata* in the East Atlantic, except for some isolated colonies of *P. aff. clavata* found on Madeira (Araújo & Freitas, 2003; López et al., 2019). Populations of both species occur widely in the Canaries with some evidence of recent expansion in their distribution (López et al., 2020), which might be related to the high dispersal abilities of its larvae (Ryland et al., 2000) combined with current increases in ocean temperatures (Vélez et al., 2015; López et al., 2020). In fact, their actual patterns of distribution are related to the temperature gradient over the regional geographic range of the Canary Islands (López et al., 2020), located between the cool and nutrient-rich waters from the Saharan upwelling and the warmer and nutrient-poor open ocean waters (Barton et al., 1998; López et al., 2020). In this sense, *P. caribaeorum* is extensively distributed in subtidal habitats of the western warmest islands within the archipelago, where it can cover large expanses of rocky platforms. Conversely, *P. aff. clavata* is more abundant in intertidal habitats but also more frequently found in the western islands (López et al., 2020).

The aim of this study was to evaluate the combined effects of ocean warming and acidification levels expected for a future scenario of climate change, on these two zooxanthellate zoantharian species with their different habitat affinities (subtidal and intertidal). Because the direct responses of marine organisms to climate change greatly determine many other biotic interactions, we also evaluated the effects of high temperature and low pH regimes on the susceptibility of both *Palythoa* species to predation by the crab *Platypodiella picta*, one of their few known predators (Den Hartog & Holthuis, 1984; Den Hartog & Türkay, 1991; López & Clemente, 2019). We hypothesize that, in a climate change context, these species may be favoured against macroalgae-dominated systems of the Canary Islands, potentially compromising local ecosystem function.

2 | MATERIAL AND METHODS

2.1 | Specimens' collection and acclimatization to laboratory conditions

Collection of specimens of *Palythoa aff. clavata* and *P. caribaeorum* from the field was undertaken in November 2016, when SST is highest at Tenerife Island (22°C) (Canary Islands, Spain), in order to minimize any potential temperature shock experienced by the

colonies during laboratory experiments. Approximately 70 fragments of different colonies of *P. aff. clavata* were collected from the intertidal zone of La Barranquera, north of Tenerife (28°32'17.98" N, 16°23'50.87" W). Likewise, 70 fragments of *P. caribaeorum* belonging to different colonies were collected at 3–6 m depth in the subtidal zone of Tajao, on the south-eastern coast of Tenerife (28°6'46.95" N, 16°27'48.04" W). Specimens were immediately transported to the laboratory in dark and wet conditions.

Fragments of *P. aff. clavata* (9–16 polyps) and *P. caribaeorum* (6–9 cm²) were glued to artificial substrates and placed in tanks with running filtered sea water and 12 h light/12 h dark cycle under LED lighting (Leddy Tube, 6 W). Specimens were maintained during 53 days for acclimatization to laboratory conditions at the natural values of sea water temperature and pH in the Canary Islands (22.132°C ± 0.36 and pH 8.1 ± 0.08). Only colonies with open polyps and without any external sign of damage were selected to perform further climate change experiments. Colonies were acclimated progressively from 22°C and pH 8.1 to the experimental conditions during one week (see the next section).

2.2 | Experimental design and sea water chemistry

Sixty colonies of each species, *Palythoa aff. clavata* and *P. caribaeorum*, were placed into 1 L beakers and distributed in four 300 L tanks, in which sea water temperature was controlled using thermostat coolers (ECHEIM Aquatic, 50 W ± 0.5°C) and heaters (Tetra HT200) and pH using computerized pH systems (AquaMedic). The experiment consisted of four treatments that combined contrasting levels of SST and pH (NBS scale) corresponding to the natural sea water conditions at the Canary Islands, and SST and pH predicted for the region for the year 2100 (IPCC, 2013): the first tank was a control treatment at 22°C (control SST in early autumn in the Canary Islands) and pH 8.1 units (average current sea water pH), the second tank at 26°C (SST predicted for the warmest season for the year 2100) and pH 8.1 as a temperature condition treatment, the third tank at 22°C and pH 7.5 (predicted pH for the year 2100 following Caldeira & Wickett, 2005) as a pH condition treatment, and the fourth tank combining both high temperature and low pH (26°C and pH 7.5), which represented the climate change scenario for the year 2100 in the sampling region. Low pH treatments were maintained with a computerized system (AquaMedic) that regulated pH by injecting pure CO₂ directly into the sea water, to a resolution of ±0.01 pH units. Both temperature and pH conditions were manipulated in an adjacent tank through a hosepipe circuit provided with 50, 10 and 1 µm Unicel polyamide paper filters. Sea water was continuously renewed in each beaker while experimental conditions for each colony were kept isolated for each beaker.

In both acclimatization and experimental periods, salinity and temperature (WTW Cond 3,110), and pH (Hanna HI 98919) parameters were monitored twice a day. Given their mixotrophic conditions, colonies were fed weekly with a mix of phyto and zooplankton (AF Phyto Mix, Aquaforest). After 4 h of colony feeding, the sea water

with excess nutrients was eliminated and replaced with fresh sea water to avoid eutrophication (approximately one-third of each tank). In addition, sea water in each tank was completely replaced once a week.

Water quality parameters for marine aquariums (NO_3 , NO_2 , NH_3/NH_4 and PO_4) were checked weekly using the Marine Care Test Kits (Red Sea). Total alkalinity for each treatment was also measured weekly by titration (Methrom, 665 Dosimar, Switzerland), using an open cell titration system and following the Standard Operation Procedure 3b (Dickson et al., 2007). Other sea water carbonate chemistry parameters (pCO_2 , calcite saturation state, Ω_c) and aragonite saturation state (Ω_a) were calculated from total alkalinity and pH results, using CO_2sys software (Lewis & Wallace, 1998). Calculations for pCO_2 , Ω_c and Ω_a were based on a set of constants K1 and K2 from Mehrbach et al. (1973), refitted by Dickson & Millero (1987).

2.3 | Survival, growth and health status of coral species

Fifteen replicates per species per combination of temperature and pH treatments were maintained during 62 days of experiment. Coral growth rates were measured by differences in weight between the beginning and end of the experiment using a laboratory scale with a precision ± 0.001 g. In the case of *P. caribaeorum*, growth in terms of colony surface area was also measured by means of analysing photographs with ImageJ 1.50 software. Because zoantharian survival also depends on their symbiotic dinoflagellates, their health status was evaluated by means of measuring the content of chlorophyll *a* (Chl-*a*), which can give information about the colony's health condition (Iglesias-Prieto et al., 1992). Concentration of Chl-*a* was obtained through fluorometry following the procedure by Costa, Sassi & Gorch-Lira, (2008) and the results were normalized using the weight of each colony sample. To assess any potential effect of colony manipulation and laboratory conditions on the status of the symbiont, extraction of dinoflagellates was also performed with colonies at the moment of collection from the field and compared with colonies kept in control treatment after 115 days (53 days of acclimatization plus 62 days of experiment). In order to evaluate whether experimental treatments affected zooxanthellae status, and considering that the Chl-*a* concentration method was destructive, only two colony replicates per species and per treatment were used to extract dinoflagellates in the middle of the experiment (i.e. after 31 days of exposure) and three at the end of the experiment (i.e. after 62 days). Additionally, in order to detect any potential bleaching, changes in the colour of experimental colonies were estimated every two weeks using a 'CoralWatch Coral Health Chart' (Siebeck et al., 2006), which has been proved to be a useful complementary technique in assessing *Palythoa* zooxanthellae condition (Hibino et al., 2013; Parkinson et al., 2016). This non-invasive method allowed the indirect evaluation of the symbiosis quality of all colony replicates throughout the experiment, as a measure of

bleaching experienced by colonies under contrasting treatments of pH and temperature.

2.4 | Predation experiments

After 62 days under the experimental conditions, six colonies of *Palythoa* aff. *clavata* and six of *P. caribaeorum* of each combination of temperature and pH treatments were used in a predation experiment. Potential indirect effects of such sea water conditions on colonies' susceptibility to the predatory crab *P. picta* were evaluated. Specimens of *P. picta* were collected from Tajao ($28^\circ 6' 46.95''$ N, $16^\circ 27' 48.04''$ W), in Tenerife Island, two days prior to the starting of the experiment. During these two days the crabs were acclimatized to experimental conditions in each tank without food, to standardize their feeding status (Scheibling & Robinson, 2008). Then, one individual of *P. picta* was placed into each replicate beaker containing an experimental colony of *P. aff. clavata* or *P. caribaeorum*. Respective controls without predatory crabs were also established using another set of six colonies per species and combination of temperature and pH conditions. All experimental and control colonies were weighed just before the commencement of predatory assays, and then daily for up to 6 days (when some experimental colonies showed $\sim 90\%$ of their biomass predated) to assess changes in colony weight owing to crab feeding activity.

2.5 | Statistical analyses

Colony survival of *Palythoa* aff. *clavata* and *P. caribaeorum* was evaluated throughout the course of the experiment by means of distance-based permutational analyses of variance (ANOVAs) (Anderson, 2001). Two-way designs were performed with two fixed factors, 'pH' (two levels: 8.1 and 7.5 units) and 'Temperature' (two levels: 22 and 26°C). For each species, growth rates of experimental colonies, in terms of both weight and surface cover increments, were also analysed by means of distance-based permutational ANOVAs (Anderson, 2001) using the previous design.

To evaluate whether symbiotic Symbiodiniaceae were affected by laboratory conditions, distance-based permutational ANOVAs (Anderson, 2001) of the concentration of Chl-*a* of samples of the two zoantharian species under control conditions (pH 8.1 and 22°C) was performed. A one-way design was carried out with 'Time' treated as a fixed factor with two levels (1, time of specimens' collection; and 2, end of the experiment, after 115 days under laboratory conditions). A second set of analyses was performed in order to check for any changes in zooxanthellae status owing to experimental treatments. In this model, Chl-*a* concentration was compared using a three-way design in which factors 'pH' (two levels: 8.1 and 7.5 units), 'Temperature' (two levels: 22 and 26°C) and 'Time' (two levels: at 31 days of the experiment and at the end of the experiment, 62 days) were treated as fixed factors. The colour score of experimental colonies with the 'CoralWatch

Coral Health Chart' and ranging from 1 (completely bleached) to 6 (healthy colonies) as described by Siebeck et al. (2006), was examined by means of frequency analyses (χ^2) using the SPSS 15.0 statistical software.

Susceptibility of zoantharians to the predatory crab *P. picta* was assessed by means of calculating predation rates upon experimental specimens as the difference in colony weight between the commencement and end of the experiment (six days). A distance-based permutational ANOVA (Anderson, 2001) for each *Palythoa* species was used to evaluate differences in predation rates owing to experimental conditions. A three-way design was carried out with factors 'Experimental vs. control' (two levels: with and without predatory crab, respectively), 'pH' (two levels: 8.1 and 7.5 units) and 'Temperature' (two levels: 22 and 26°C) treated as fixed factors.

In all ANOVA analyses, Euclidean distances of raw data and 9,999 permutations of the appropriate exchangeable units were used (Anderson, 2001). Whenever enough permutations were not obtained for a valid test, the *p*-values were corrected with Monte Carlo method (Anderson & Robinson, 2003). Significant terms in the full models were examined using *a posteriori* pairwise comparisons by permutations (Anderson, 2001). All statistical analyses were carried out using PRIMER 7 and PERMANOVA+ v. 1.0.1 software.

3 | RESULTS

3.1 | Sea water chemistry

The physico-chemical parameters of sea water during the course of the experiment are given in Table 1. Target temperatures and pH were achieved in each replicated treatment (Table 1). The partial pressure of CO₂ (pCO₂) increased at low pH levels in all temperature treatments and saturation levels of calcite (Ω_C) and aragonite (Ω_A) decreased in low pH conditions (Table 1). Sea water only approached subsaturation values with respect to aragonite ($\Omega_A < 1$) under conditions of acidic pH (Table 1).

TABLE 1 Mean values (\pm SD) of the physicochemical parameters of sea water measured in each experimental treatment during 62 days of the experiment

	Control treatment	Temperature treatment	pH treatment	Climate change treatment
Temperature (°C)	22.060 \pm 0.170	25.660 \pm 0.160	22.100 \pm 0.370	25.720 \pm 0.470
pH	8.090 \pm 0.085	8.094 \pm 0.077	7.529 \pm 0.069	7.504 \pm 0.072
AT (mmol kg ⁻¹)	2.547 \pm 0.062	2.620 \pm 0.056	2.768 \pm 0.138	2.918 \pm 0.164
pCO ₂ (μ atm)	364.160 \pm 9.110	367.420 \pm 8.230	1,925.330 \pm 95.620	2,046.880 \pm 115.760
H ₂ CO ₃ (mmol kg ⁻¹)	1.932 \pm 0.048	1.911 \pm 0.043	2.565 \pm 0.129	2.674 \pm 0.152
CO ₃ ⁻² (mmol kg ⁻¹)	0.253 \pm 0.007	0.293 \pm 0.007	0.085 \pm 0.005	0.103 \pm 0.006
Ω_C	5.971 \pm 0.154	6.869 \pm 0.154	1.994 \pm 0.107	2.416 \pm 0.139
Ω_A	3.912 \pm 0.101	4.554 \pm 0.102	1.306 \pm 0.070	1.602 \pm 0.093

Note: AT, Total alkalinity; pCO₂, partial pressure of CO₂; H₂CO₃, carbonic acid; CO₃⁻², carbonate; and Ω_C , Ω_A , saturation levels of calcite and aragonite, calculated by means of CO2SYS (Lewis & Wallace, 1998). Control treatment, 22°C and pH 8.1; temperature treatment, 26°C and pH 8.1, pH treatment, 22°C and pH 7.5; and climate change treatment, 26°C and pH 7.5.

3.2 | Survival, growth and health status of zoantharian species

All colonies of *P. aff. clavata* survived to the 62 days of experiment, regardless of the experimental conditions. However, survival of *P. caribaeorum* showed a marginally significant two-way interaction of factors 'pH \times temperature' ($F = 3.62$; $p = 0.059$). *A posteriori* pairwise analyses only showed significant differences between temperatures (22 vs. 26°C) at pH 7.5 units ($t = 3.54$; p (MC) = 0.001), with all colonies surviving at control temperature and low pH conditions in contrast to the 66.6% of colonies that survived at the combination of 26°C and 7.5 units of pH.

The results of the permutational ANOVA, analysing the growth rate of *Palythoa* spp. under experimental conditions measured as variation in colony weight, showed significant differences at low pH for both species, whereas no differences were found between temperature treatments or within the interaction of factors (Table 2). In general, *P. aff. clavata* showed a decrease in colony weight in all

TABLE 2 Results of the two-way permutational ANOVAs analysing differences in colony weight of *Palythoa aff. clavata* (a) and *Palythoa caribaeorum* (b) during the laboratory experiment at two different pH levels (7.5 and 8.1) and temperatures (22 and 26°C)

PERMANOVA					
Source	d.f.	SS	MS	Pseudo-F	<i>p</i> (perm)
(a) <i>P. aff. clavata</i>					
Temperature (Te)	1	2.61	2.61	1.94	0.174
pH	1	70.08	70.08	52.09	0.001
Te \times pH	1	3.98	3.98	2.96	0.100
Total	51	141.25			
(b) <i>P. caribaeorum</i>					
Temperature (Te)	1	17.14	17.14	1.33	0.279
pH	1	87.14	87.14	6.76	0.005
Te \times pH	1	2.55	2.55	0.20	0.719
Total	40	571.12			

MS, mean sum of squares; SS, sum of squares

experimental treatments (Figure 1) but it was more evident at pH 7.5 (-3.30 ± 0.71 g, Figure 1). *Palythoa caribaeorum* specimens only experienced a decrease in colony weight at low pH that was more severe combined with high temperature (-1.042 ± 0.29 g, Figure 1). Despite the lack of significance of factor temperature, it can be noticed that both *Palythoa* species experienced the greatest decrease in colony weight under the combined effects of low pH and high temperature (Figure 1).

Although measurements of *P. caribaeorum* colony area also showed a noticeable decrease at low pH (-4.49 ± 2.38 mm², Figure 2), specimens were affected also by high temperature as only the colonies that grew under control conditions showed a slight increment in area (0.29 ± 1.78 mm², Figure 2). However, the ANOVA assessing differences in *P. caribaeorum* colony area only showed a

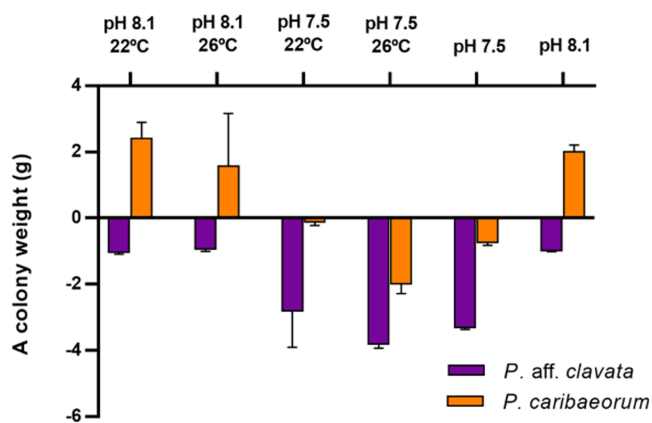


FIGURE 1 Mean difference in colony weight (\pm SD) at each combined treatment of temperature (22 and 26°C) and pH (7.5 and 8.1 units) for *P. aff. clavata* and *P. caribaeorum* in laboratory experiments. Statistically different ($p < 0.01$) overall differences in colony weight at pH 7.5 and 8.1 for each species are also included

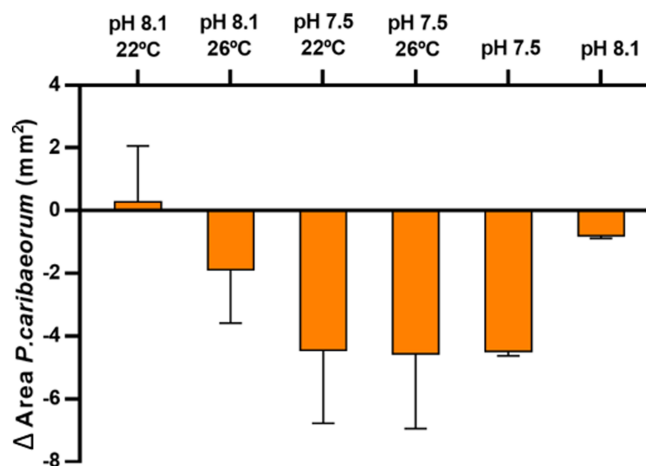


FIGURE 2 Mean difference in colony coverage area (\pm SD) of *P. caribaeorum* at each combined treatment of temperature (22 and 26°C) and pH (7.5 and 8.1 units) in laboratory experiments. Statistically different ($p < 0.001$) overall differences in colony area at pH 7.5 and 8.1 are also included

significant effect of pH, while no effect of temperature or of the interaction of both factors was observed (Table 3).

Values of Chl-*a* concentration in experimental colonies of *P. aff. clavata* ranged from 99.99 ± 26.80 mg/m³ at specimen collection vs. 71.80 ± 48.93 mg/m³ at the end of the experiment, showing not significant differences after 115 days under laboratory conditions ($F = 3.12$; $p = 0.107$). In the same way, the results of the Chl-*a* content in *P. caribaeorum* were not significant ($F = 3.12$; $p = 0.107$), with 2.50 ± 3.76 mg/m³ at specimen collection vs. 6.92 ± 4.89 mg/m³ at the end of the experiment.

Results of the ANOVA analysing the differences in Chl-*a* concentrations under experimental conditions showed a significant three-way interaction of factors 'pH \times temperature \times time' for *P. aff. clavata* (Table 4). This result indicates that the effects of pH and temperature in the Chl-*a* content varied significantly across the course of the experiment. *A posteriori* pairwise analyses showed that after 31 days, significant differences in Chl-*a* concentration between temperature treatments were found at pH 7.5, while no differences among temperature regimes were obtained at pH 8.1 (Table 5). There was an increase in Chl-*a* at pH 7.5 when colonies were kept at 26°C compared with 22°C (Figure 3). Despite marginally non-significant differences, a trend towards higher Chl-*a* content was detected with increasing temperature at pH 8.1 at the end of the experiment (Table 5, Figure 3). Non-significant differences were obtained between temperatures at both pH treatments after 62 days (Table 5), although the lowest concentrations of Chl-*a* were recorded in the combined treatment of low pH and high temperature (Table 5, Figure 3). In the case of *P. caribaeorum*, ANOVA analysis of differences in Chl-*a* concentration revealed significant differences in pH treatments, while no effects of temperature, time or the interaction between them were found (Table 4, Figure 3).

Bleaching status of the colonies, evaluated by frequency analyses of their colour score, showed significant differences between the combination of pH and temperature treatments for both *P. aff. clavata* ($\chi^2 = 51.95$; $p < 0.01$) and *P. caribaeorum* ($\chi^2 = 47.83$; $p < 0.01$). *Palythoa aff. clavata* exhibited low colour scores, representing bleaching, in colonies that were kept at the low pH treatment and under the combined treatment of low pH and high temperature (Figure 4). On the contrary, colonies kept at control pH and high temperature conditions showed values ranging from 4 to 6, which is

TABLE 3 Results of the two-way permutational ANOVAs analysing differences in colony coverage area of *P. caribaeorum* during the laboratory experiment at two different pH levels (7.5 and 8.1) and temperatures (22 and 26°C)

PERMANOVA					
Source	d.f.	SS	MS	Pseudo-F	p (perm)
Temperature (Te)	1	11.68	11.68	2.86	0.099
pH	1	125.60	125.60	30.79	0.001
Te \times pH	1	9.60	9.60	2.35	0.131
Total	38	299.69			

TABLE 4 Results of the three-way permutational ANOVAs analysing differences in zooxanthellae chlorophyll *a* concentration of *P. aff. clavata* (a) and *P. caribaeorum* (b) at two different pH levels (7.5 and 8.1 units) and temperatures (22 and 26°C), considering two periods of time during the course of the laboratory experiment (at 31 and 62 days of experimental conditions)

PERMANOVA					
Source	d.f.	SS	MS	Pseudo-F	<i>p</i> (perm)
(a) <i>P. aff. clavata</i>					
Time (Ti)	1	4,224.80	4,224.80	0.51	0.490
pH	1	26,493.00	26,493.00	3.21	0.092
Temperature (Te)	1	8,502.70	8,502.70	1.03	0.342
Ti × pH	1	12,757.00	12,757.00	1.55	0.239
Ti × Te	1	9,954.00	9,954.00	1.21	0.302
pH × Te	1	4,101.50	4,101.50	0.50	0.503
Ti × pH × Te	1	55,031.00	55,031.00	6.67	0.023
Total	22	2.6296E+05			
(b) <i>P. caribaeorum</i>					
Ti	1	7.06	7.06	0.42	0.526
pH	1	117.41	117.41	7.06	0.023
Temperature (Te)	1	2.43	2.43	0.15	0.711
Ti × pH	1	39.76	39.76	2.39	0.148
Ti × Te	1	43.13	43.13	2.59	0.127
pH × Te	1	0.97	0.97	0.06	0.805
Ti × pH × Te	1	69.02	69.02	4.15	0.067
Total	20	540.09			

TABLE 5 Results of pairwise tests examining the significant interaction of factors 'time × pH × temperature' obtained in the permutational ANOVA on chlorophyll *a* content of *P. aff. clavata* zooxanthellae during laboratory experiments

	31 days				62 days			
	pH 7.5		pH 8.1		pH 7.5		pH 8.1	
Pairwise tests	<i>t</i>	<i>p</i> (perm)	<i>t</i>	<i>p</i> (perm)	<i>t</i>	<i>p</i> (perm)	<i>t</i>	<i>p</i> (perm)
22 vs. 26°C	6.48	0.021	0.87	0.480	1.32	0.238	2.08	0.09
	22°C		26°C		22°C		26°C	
Pairwise tests	<i>t</i>	<i>p</i> (perm)	<i>t</i>	<i>p</i> (perm)	<i>t</i>	<i>p</i> (perm)	<i>t</i>	<i>p</i> (perm)
pH 7.5 vs. 8.1	1.084	0.399	2.36	0.137	0.24	0.814	2.52	0.052

associated with healthy colonies (Siebeck et al., 2006; Siebeck, Logan & Marshall, 2008) (Figure 4). *Palythoa caribaeorum* specimens also showed low colour scores with the pH treatment, while the highest values were obtained in colonies maintained at control pH and high temperature (Figure 4). All colonies of *P. caribaeorum* that grew at the combination of low pH and high temperature exhibited the lowest values on the colour card after 62 days (Figure 4).

3.3 | Predation experiments

Colonies of both *Palythoa* species showed an overall decrease in colony weight at experimental conditions with the predator *P. picta*,

regardless the experimental treatment of temperature and pH applied. Specimens in control conditions without the predatory crab showed more stable colony weights throughout the experimental period (Figure 5).

Results of the permutational ANOVA showed a significant interaction of factors '*P. picta* vs. control × pH' (Table 6) in *P. aff. clavata*, meaning that colony weight varied differently between conditions with and without the predator depending on the pH treatment (Figure 5). *A posteriori* pairwise analyses showed a significant less weight loss of colonies with crabs at pH 7.5 (-1.64 ± 0.56 g) compared with those that were kept at pH 8.1 (-4.26 ± 1.80 g; Figure 5, Table 7).

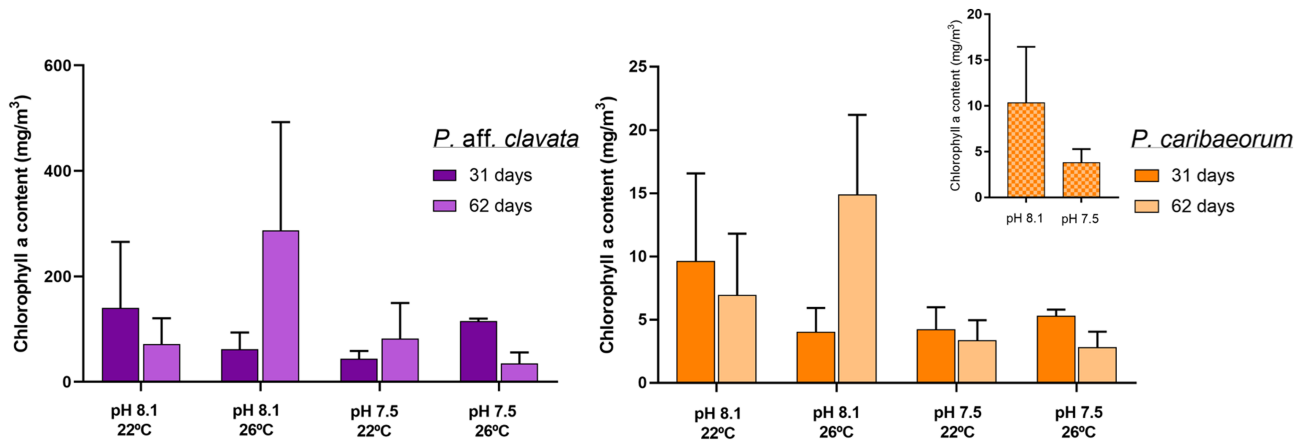


FIGURE 3 Mean concentration of chlorophyll *a* (\pm SD) in *P. aff. clavata* (left) and *P. caribaeorum* (right) at each combined treatment of temperature (22 and 26°C) and pH (7.5 and 8.1 units) in laboratory experiments. Overall mean concentrations of chlorophyll *a* at pH 7.5 and 8.1 (upper right) are also included (statistically different only for *P. caribaeorum*, $p < 0.05$)

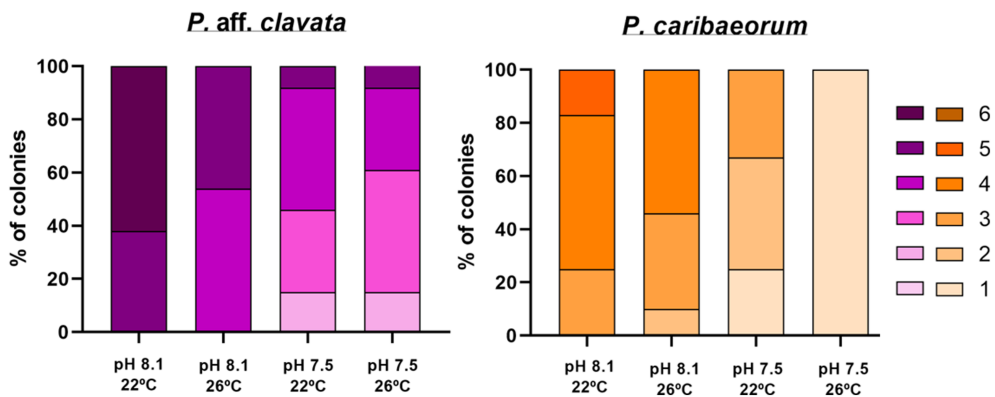


FIGURE 4 Cumulative percentage of colonies of *P. aff. clavata* (left) and *P. caribaeorum* (right) at each colour category ('CoralWatch Coral Health Chart' method) within each combined treatment of temperature (22 and 26°C) and pH (7.5 and 8.1), after 62 days of laboratory experiments. Colour score categories: from 6 to 1 increasing bleaching of colonies, associated with a loss of health

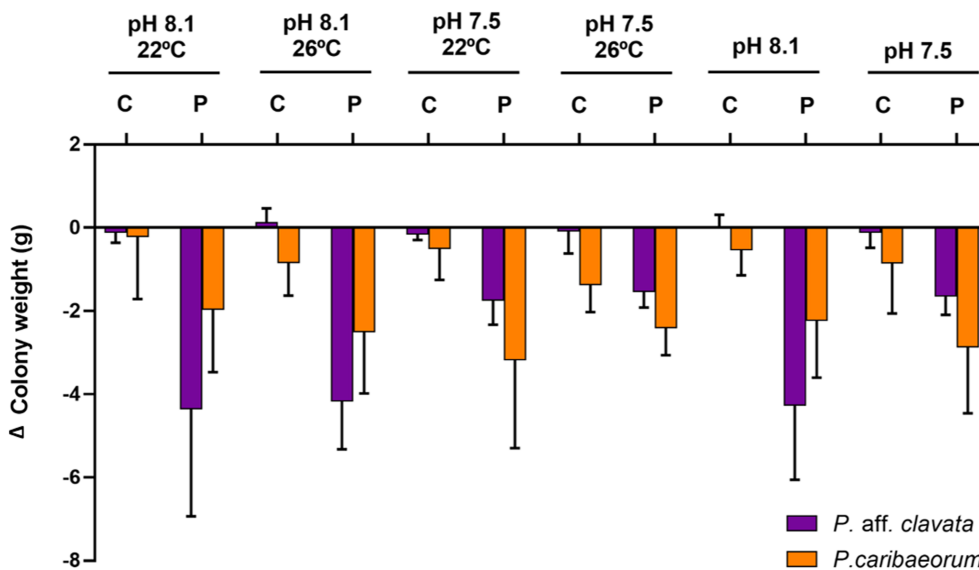


FIGURE 5 Results of predation experiments with *P. aff. clavata* and *P. caribaeorum*, showing mean differences in colony weight (\pm SD) after 6 days of exposure to the crab *Platypodiella picta* (P: experimental colonies) and to control conditions without crab (C: control colonies), at each combined treatments of temperature (22 and 26°C) and pH levels (7.5 and 8.1 units). Overall mean differences in colony weight at pH 7.5 and 8.1 for both species with each predation treatment are also included (statistically significant interaction of factors only for *P. aff. clavata*, $p < 0.001$)

In general, colonies of *P. caribaeorum* suffered a loss of weight in all experimental treatments when *P. picta* were present (-2.50 ± 1.44 g) compared with colonies without the crab, whose weight remained more stable (-0.73 ± 0.93 g; Figure 5). Results of the

three-way permutational ANOVA of the susceptibility of *P. caribaeorum* to be predated by *P. picta* at different combinations of pH and temperature confirmed these results, as only the factor '*P. picta* vs. control' showed significant differences, while no effect of

TABLE 6 Results of the three-way permutational ANOVA analysing differences in colony weight of (a) *P. aff. clavata* and (b) *P. caribaeorum* in predation experiments with the predatory crab *Platypodiella picta* ('*P. picta* vs. control', two levels), conducted at different pH levels (7.5 and 8.1 units) and temperature conditions (22 and 26°C) during 6 days

PERMANOVA					
Source	d.f.	SS	MS	Pseudo-F	p (perm)
(a) <i>P. aff. clavata</i>					
<i>P. picta</i> vs. control (Pp vs. C)	1	57.34	57.34	62.06	0.001
pH	1	10.78	10.78	11.66	0.002
Temperature (Te)	1	0.21	0.21	0.22	0.659
Pp vs. C × pH	1	12.88	12.88	13.94	0.001
Pp vs. C × Te	1	0.03	0.03	0.01	0.951
pH × Te	1	0.01	0.01	0.01	0.920
Pp vs. C × pH × Te	1	0.02	0.02	0.01	0.910
Total	27	96.77			
(b) <i>P. caribaeorum</i>					
<i>P. picta</i> vs. control (Pp vs. C)	1	16.87	16.87	11.16	0.005
pH	1	1.24	1.24	0.82	0.386
Temperature (Te)	1	0.53	0.53	0.35	0.569
Pp vs. C × pH	1	0.03	0.03	0.02	0.887
Pp vs. C × Te	1	1.01	1.01	0.67	0.434
pH × Te	1	0.40	0.38	0.25	0.628
Pp vs. C × pH × Te	1	0.79	0.79	0.52	0.477
Total	21	43.94			

TABLE 7 Pairwise tests examining the significant interaction of factors '*P. picta* vs. control × pH' in predation experiments with the predatory crab *Platypodiella picta* in *P. aff. clavata*

Pairwise tests	pH 7.5		pH 8.1	
	t	p (MC)	t	p (MC)
<i>P. picta</i> vs. control	6.4166	0.0003	6.1344	0.001
	<i>P. picta</i>		Control	
pH 8.1 vs. 7.5	t	p (MC)	t	p (MC)
	3.1194	0.0143	0.66311	0.526

temperature or pH or any interaction of these factors was detected on the outcomes of predation (Table 6).

4 | DISCUSSION

Results of this study revealed species-specific responses of *Palythoa* spp. to the combined effects of the temperature and pH ranges expected for a future climate change scenario within a subtropical region. High temperature and low pH seemed to affect the host and its symbiont in different ways, but colonies were greatly affected when grown in their combined effects. Contrary to what was expected for coral species without carbonate in their body wall, low pH had a greater effect than temperature on both organisms' fitness.

Despite the difficulty of cultivating *Palythoa* spp. in aquaria, colonies showed active polyps and healthy appearance after the acclimatization period. In fact, both *Palythoa* spp. had relatively high survival rates (>66%) to the climate change stressors set in the laboratory. However, while survival of *P. aff. clavata* was not affected by experimental treatments, the survival of *P. caribaeorum* was lower in the combined conditions of low pH and ocean warming. Intertidal habitats are one of the most stressful environments for marine organisms owing to the severe daily and seasonal fluctuations of abiotic conditions such as temperature, salinity and pH (Truchot, 1988; Helmuth et al., 2006). Although intertidal colonies of *P. aff. clavata* are adapted to such drastic changes, colony growth decreased after 62 days exposed to constant low sea water pH levels, regardless of the temperature.

Negative effects of reduced pH on growth rates were also evident in the case of subtidal colonies of *P. caribaeorum*, which also showed a reduction in colony area at both the control and elevated sea water temperatures. However, increases in sea water temperature alone had no effect on either coral species. In contrast to *P. caribaeorum*, experimental colonies of *P. aff. clavata* experienced weight loss at both control and temperature treatments, suggesting suboptimal conditions during the experiment. However, it has been demonstrated that size and weight greatly fluctuate in soft-bodied organisms owing to their physiological state or in response to environmental disturbances (Fabricius, 1995; Hellström & Benzie, 2011). Moreover, previous studies have highlighted that photochemical

efficiency in polyps that were cyclically emerged were lower than in polyps that were always immersed (Leal et al., 2015; Rosa et al., 2016). Nevertheless, under environmental stress the host can change its main feeding behaviour from autotrophy to heterotrophy (Gustafsson, Baird & Ralph, 2013).

Considering all of these factors, intertidal colonies of *P. aff. clavata* that experience daily exposure during low tide may need more energetic resources provided by heterotrophy compared with subtidal colonies of *P. caribaeorum*. Future studies should consider such habitat differences and more accurate growth measurements for soft-bodied organisms, for example measuring host protein content levels (Khalesi, Beeftink & Wijffels, 2009; Graham & Sanders, 2015). Despite this likely shortage in energetic requirements, colonies of *P. aff. clavata* kept at control and temperature treatments showed active polyps and retained the typical coloration of the species.

The main effect of ocean acidification on biological components of marine systems is related to the reduction of available carbonate to calcifying organisms (Andersson, Mackenzie & Bates, 2008; McCulloch et al., 2012; Agostini et al., 2018). Although *Palythoa* spp. do not have calcareous skeletons, growth rates of both species were strongly affected by the decrease in pH to 7.5 units. It has been shown that changes in pH conditions not only reduce calcification rates but also alter many physiological mechanisms in marine species (Arnold et al., 2012; Li & Gao, 2012). In fact, predicted models based on habitat suitability of *P. caribaeorum* in the West Atlantic suggested that 7.85 units of pH might be a barrier to its distribution pattern (Durante, Cruz & Lotufo, 2018). Biological performance of zooxanthellate zoantharians is highly mediated by the status of their symbionts, since species sustenance strongly depends on nutrients photosynthesized by Symbiodiniaceae. Thus, reduction in growth induced by low pH levels in *Palythoa* spp. could be related to a reduction in internal abundances and/or photosynthetic activity of symbiotic dinoflagellates, as has been previously found in both soft (Michalek-Wagner & Willis, 2001) and hard corals (Porter et al., 1989). In fact, the negative effect of low pH was also confirmed in this study by the loss of colour, the colony colour being correlated to zooxanthellae density and/or chlorophyll content with reference to the health chart (Siebeck et al., 2006; Siebeck, Logan & Marshall, 2008). It has been found that colour variation is naturally much more common in zoantharians than in scleractinian corals (Burnett et al., 1995; Reimer et al., 2004; López et al., 2019), which could make monitoring of populations with this method difficult (Parkinson et al., 2016). However, our results showed the colour health chart to be a useful tool to track changes of individual colonies through time (Hibino et al., 2013).

Within this study, symbionts of experimental colonies of both *Palythoa* species were more vulnerable to low pH than to high temperatures. In fact, the highest concentrations of Chl-*a* were reached at 26°C and control pH levels, and colonies showed higher colour scores on the health chart, which are related to higher concentrations of pigments (Reimer et al., 2007). Despite the general vulnerability of Symbiodiniaceae to changes in temperature beyond thermal tolerance limits typical of the different symbiont types (Glynn, 1996; IPCC, 2007), their intrinsic characteristics and relative abundances of

dinoflagellate types may play a crucial role in overcoming the effects of ocean warming and acidification (Fay & Weber, 2012; Yorifuji et al., 2017). It has been found that some autotrophic organisms are able to utilize both CO₂ and HCO₃⁻ by means of carbon-concentrating mechanisms catalysed by the enzyme carbonic anhydrase (CA) (Sültmeyer, 1998; Kaplan & Reinhold, 1999; Leggat, Badger & Yellowlees, 1999), when there are low levels of dissolved CO₂ available for photosynthesis (Badger & Price, 2003; Giordano, Beardall & Raven, 2005). Carbon-concentrating mechanisms vary in their efficiency depending on the taxonomic group (Tortell, 2000; Giordano, Beardall & Raven, 2005) and even among different genera of Symbiodiniaceae (Brading et al., 2011). Although symbiont types were not examined, owing to the fact that all *Palythoa* species inhabiting the Macaronesia and Cape Verde ecoregions host *Cladocopium* (López et al., 2019), it can be assumed that experimental colonies also had this genus, which is adapted to a wide range of temperatures and irradiances (LaJeunesse, 2005; LaJeunesse et al., 2018). Previous studies have shown that the activity of the CA of both *P. aff. clavata* and their *Cladocopium* sp. was not affected by high temperature and enzyme activity of the dinoflagellate decreased only at low pH (Graham et al., 2015), in concordance with the low concentrations of Chl-*a* found at pH 7.5. In the same way, the higher concentration of Chl-*a* detected in both species under the temperature treatment may be related to an increase in CA activity in symbiont cells as mentioned above.

In some cases, it has been shown that acidification reduces algal cell densities (Graham et al., 2015; Graham & Sanders, 2015; Manson, 2018) and may explain the decreased Chl-*a* content recorded at low pH in the present study for *P. caribaeorum*. In *P. aff. clavata*, increased levels of Chl-*a* were observed under climate change conditions compared with control temperature, but only after 31 days of experimental conditions. These results highlight that higher temperatures may compensate for the negative effects of lowered pH in Chl-*a* content. However, after 62 days no such effect persisted and detrimental effects were observed in *P. aff. clavata* colonies, with a drop in Chl-*a* content. Despite the tropical affinities of *P. caribaeorum*, the effect of pH was more severe and, regardless of time, no offset of temperature regimes on Chl-*a* concentrations was observed. The stable conditions of the subtidal zone might lead to *P. caribaeorum* hosting a symbiont subtype less adapted to drastic changes in abiotic conditions in comparison with *P. aff. clavata* inhabiting changing intertidal environments. Nevertheless, the highest concentrations of Chl-*a* were recorded in colonies of both species after 62 days growing at 26°C. Previous studies demonstrated that high temperatures can lead to zooxanthellate cell proliferations (Dimond & Carrington, 2007; Dimond & Carrington, 2008; Graham et al., 2015; Graham & Sanders, 2015), which can explain the high values of Chl-*a* observed. Over longer periods, these zooxanthellae proliferations may favour the expansion of colonies which, in the current context of ocean warming, could lead to negative consequences for local ecosystems.

Many sessile marine invertebrates produce toxic metabolites to avoid predation and outcompete other species, especially soft-bodied organisms, which lack structural protection (Tanner, 1995; Marty &

Pawlik, 2015). The fact that few predators are able to feed on zoantharian species might be related to the efficiency of their chemical defences that include nematocysts and potent toxins known as palytoxins (Chanas & Pawlik, 1995; O'Neal & Pawlik, 2002; Hines & Pawlik, 2012). However, coevolutionary processes may lead to certain predators being highly adapted to feeding on specific prey despite their toxicity, such as some nudibranch slugs that eat specific sponges (Proksch, 1994; Cheney et al., 2016) or obligate coral-feeding fish (McIlwain & Jones, 1997; Rotjan & Lewis, 2008). This seems to be the case for *P. picta*-zoantharia associations (Den Hartog & Holthuis, 1984; López & Clemente, 2019). In the current study, *P. picta* was able to actively feed on both *Palythoa* spp., with colonies showing significant decreases in weight in only 6 days, especially *P. caribaeorum*. Previous studies have shown that *P. aff. clavata* is 90 times more toxic than *P. caribaeorum* (Béress et al., 1983; Sawelew et al., 2018), which could explain the more active predation upon *P. caribaeorum* regardless of the experimental environmental conditions. Moreover, it was shown that the simulated low-pH conditions decreased the rate of consumption of *P. aff. clavata* by *P. picta*. Given that the species lacks structural defences that could be damaged by acidic conditions, this could be a result of changes in the palatability of colonies mediated by alteration of their chemical defences.

In recent years, land and sea water temperatures have been increasing owing to the effects of climate change. In the tropics, temperature is predicted to exceed the thermal threshold for many species and changes in species and coral reefs distributions will probably occur if they are not able to acclimate (Hoegh-Guldberg, 1999). Despite that, acidification levels forecast for the next century would probably have severe impacts in *Palythoa* spp., current ocean warming is enabling populations to expand, which might lead to irreversible consequences in local ecosystems, such as loss of biodiversity and functioning. In fact, phase-shift processes from coral reef to Zoantharia-dominated systems have already been reported (Cruz, Kikuchi & Creed, 2016; Cruz et al., 2016), which have even led on a reduction in fish assemblages in tropical areas (Cruz et al., 2015). Likewise, in the subtropical region of the Canary Islands *Palythoa* spp. are expanding and some populations of *P. caribaeorum* are replacing macroalgae benthic communities (López et al., 2020), with important implications for the conservation of highly productive benthic ecosystems.

Local conservation efforts can greatly enhance the resilience of marine communities, helping to mitigate the global issue of climate change. Detecting the most vulnerable species and communities to be replaced by zoantharians will undoubtedly help in any conservation decision making. In this sense, predicting the responses of combined climate-change stressors not only of a single species but also through indirect processes, which can lead to cascade effects (Brook, Sodhi & Bradshaw, 2008; Bellard et al., 2012), has become an urgent field of research in order to properly focus management decisions on the most vulnerable species/communities. However, this study highlights the difficulty of making accurate predictions, owing to the fact that warming ameliorates or aggravates the effects of acidification at different levels and on different time scales. Nevertheless, *Palythoa*

populations are expected to increase in a near future scenario favoured by ocean warming, especially considering that expected changes in ocean pH are more subtle. Population spread might lead in major changes and negative consequences for marine biodiversity and any future monitoring programme should include broad exploratory surveys, even in locations where *P. aff. clavata* and *P. caribaeorum* have not been found yet. Recording the establishment of new colonies could reveal areas degraded owing to climate change or other human impacts. This approach may be useful to understand the ecosystem effects of any protective measure implemented to preserve the sustainability and functioning of local ecosystems in upcoming years.

ACKNOWLEDGEMENTS

The authors have no conflict of interest to declare. We are grateful Carlos Aspiroz and Sergio Moreno for helping during laboratory experiments. CL was co-funded by the Canarian Agency for Research, Innovation and Information Society of the Ministry of Economy, Industry, Trade and Knowledge and by the European Social Fund integrated operational programme of the Canary Islands 2014–2020. This research was conducted within the framework of the 'INDICATROP' project conducted with the financial support of the 'CajaCanarias' Foundation.

ORCID

Cataixa López  <https://orcid.org/0000-0002-6877-9466>

REFERENCES

- Acosta, A. (2001). Disease in Zoanthids: Dynamics in space and time. *Hydrobiologia*, 460, 113–130. https://doi.org/10.1007/978-94-017-3284-0_10
- Acosta, A., Casas, M., Vargas, C.A. & Camacho, J.E. (2005). Lista de Zoantharia (Cnidaria: Anthozoa) del Caribe y de Colombia. *Biota Colombiana*, 6(2), 147–162. <https://doi.org/10.21068/c001>
- Agostini, S., Harvey, B.P., Wada, S., Kon, K., Milazzo, M., Inaba, J. & Hall-Spencer, J.M. (2018). Ocean acidification drives community shifts towards simplified non-calcified habitats in a subtropical-temperate transition zone. *Scientific Reports*, 8, 11354. <https://doi.org/10.1038/s41598-018-29251-7>
- Anderson, M.J. (2001). Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences*, 58(3), 626–639. <https://doi.org/10.1139/f01-004>
- Anderson, M.J. & Robinson, J. (2003). Generalized discriminant analysis based on distances. *Australian & New Zealand Journal of Statistics*, 45 (3), 301–318. <https://doi.org/10.1111/1467-842X.00285>
- Andersson, A.J., Mackenzie, F.T. & Bates, N.R. (2008). Life on the margin: Implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Marine Ecology Progress Series*, 373, 265–273. <https://doi.org/10.3354/meps07639>
- Araújo, R. & Freitas, M. (2003) A new crab record *Platypodiella picta* (A. Milne-Edwards, 1869) (Crustacea: Decapoda: Xanthidae) from Madeira Islands waters. Natural History Museum of Funchal ISSN: 0523-7904
- Arnold, T., Mealey, C., Leahey, H., Miller, W.A., Hall-Spencer, J.M., Milazzo, M. & Maers, K. (2012). Ocean acidification and the loss of phenolic substances in marine plants. *PLoS ONE*, 7, e35107. <https://doi.org/10.1371/journal.pone.0035107>
- Badger, M.R. & Price, G.D. (2003). CO₂ concentrating mechanisms in cyanobacteria: Molecular components, their diversity and evolution.

- Journal of Experimental Botany*, 54(383), 609–622. <https://doi.org/10.1093/jxb/erg076>
- Baker, A.C., Glynn, P.W. & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine and Coastal Marine Science*, 80, 435–471. <https://doi.org/10.1016/j.ecss.2008.09.003>
- Barton, E.D., Aristegui, J., Tett, P., García-Braun, J., Hernández-León, S., Nykjaer, L. et al. (1998). The transition zone of the Canary Current upwelling region. *Progress in Oceanography*, 41, 455–504. <https://doi.org/10.1016/j.pocean.2004.08.003>
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, 15(4), 365–377. <https://doi.org/10.1111/j.1461-0248.2011.01736.x>
- Béress, L., Zwick, J., Kolkenbrock, H.J., Kaul, P.N. & Wassermann, O. (1983). A method for the isolation of the caribbean palytoxin (C-PTX) from the coelenterate (zoanthid) *Palythoa caribaeorum*. *Toxicon*, 21, 285–290. [https://doi.org/10.1016/0041-0101\(83\)90013-2](https://doi.org/10.1016/0041-0101(83)90013-2)
- Bindoff, N.L., Willebrand, J., Artale, V., Cazenave, A., Gregory, J.M., Gulev, S. et al. (2007) Observations: Oceanic climate change and sea level. In: Solomon, S., Qin, D., Manning, M., Marquis, M., Averyt, K.B., Tignor, M., et al. (Eds.) *Climate change 2007: The physical science basis. Contribution of Working Group 1 to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press. pp. 385–432.
- Brading, P., Warner, M.E., Davey, P., Smith, D.J., Achterberg, E.P. & Suggett, D.J. (2011). Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae). *Association for the Sciences of Limnology and Oceanography*, 56(3), 927–938. <https://doi.org/10.4319/lo.2011.56.3.0927>
- Brook, B.W., Sodhi, N.S. & Bradshaw, C.J.A. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, 23(8), 453–460. <https://doi.org/10.1016/j.tree.2008.03.011>
- Burnett, W.J., Benzie, J.A.H., Beardmore, J.A. & Ryland, J.S. (1995). Patterns of genetic subdivision in populations of a clonal cnidarian, *Zoanthus coppingeri*, from the Great Barrier Reef. *Marine Biology*, 122, 665–673. <https://doi.org/10.1007/BF00350688>
- Caldeira, K. & Wickett, M.E. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research*, 110, C09S04. <https://doi.org/10.1029/2004JC002671>
- Chanas, B. & Pawlik, J.R. (1995). Defenses of Caribbean sponges against predatory reef fish. II. Spicules, tissue toughness, and nutritional quality. *Marine Ecology Progress Series*, 127, 195–211. <https://doi.org/10.3354/meps127195>
- Cheney, K.L., White, A., Mudianta, I.W., Winters, A.E., Quezada, M., Capon, R.J. et al. (2016). Choose your weaponry: Selective storage of a single toxic compound, Latrunculin A, by closely related nudibranch molluscs. *PLoS ONE*, 11, e0145134. <https://doi.org/10.1371/journal.pone.0145134>
- Cole, C., Finch, A.A., Hintz, C., Hintz, K. & Allison, N. (2018). Effects of seawater pCO₂ and temperature on calcification and productivity in the coral genus *Porites* spp.: An exploration of potential interaction mechanisms. *Coral Reefs*, 37, 471–481. <https://doi.org/10.1007/s00338-018-1672-3>
- Costa, C.F., Sassi, R. & Górlach-Lira, K. (2008). Uma abordagem metodológica para o estudo das zooxantelas de corais do Brasil. *Boletim Do Laboratório de Hidrobiologia*, 21, 83–94.
- Cruz, I.C., Loiola, M., Albuquerque, T., Reis, R., Jose de Anchieta, C.C., Reimer, J.D., et al. (2015). Effect of phase shift from corals to Zoantharia on reef fish assemblages. *PLoS ONE*, 10, e0116944. <https://doi.org/10.1371/journal.pone.0116944>
- Cruz, I. C. S., Kikuchi, R. K., & Creed, J.C. (2016) Ecological processes of phase shift to *Palythoa grandiflora* dominance on reefs of Todos-os-Santos Bay, Brazil. *Frontiers in Marine Science* Conference Abstract: XIX Iberian Symposium on Marine Biology Studies. <https://doi.org/10.3389/conf.FMARS.2016.05.00050>
- Cruz, I.C.S., Meira, V.H., Kikuchi, R.K.P. & Creed, J.C. (2016). The role of competition in the phase shift to dominance of the zoanthid *Palythoa* cf. *variabilis* on coral reefs. *Marine Environmental Research*, 11, 28–35. <https://doi.org/10.1016/j.marenvres.2016.01.008>
- Davies, P.S. (1991). Effect of daylight variations on the energy budgets of shallow-water corals. *Marine Biology*, 108, 137–144. <https://doi.org/10.1007/BF01313481>
- Den Hartog, J.C. & Holthuis, L.B. (1984). A note on an interesting association of the crab *Platypodiella picta* (A. Milne-Edwards, 1869) and species of Zoantharia. *Courier Forschungsinstitut Senckenberg*, 68, 21–29.
- Den Hartog, J.C. & Türkay, M. (1991). *Platypodiella georgei* spec. nov. (Brachyura: Xanthidae), a new crab from the island of St. Helena, South Atlantic Ocean, with notes on the genus *Platypodiella* Guinot, 1967. *Zoologische Mededelingen*, 65, 209–220.
- Depczynski, M., Gilmour, J.P., Ridgway, T., Barnes, H., Heyward, A.J., Holmes, T.H. et al. (2013). Bleaching, coral mortality and subsequent survivorship on a West Australian fringing reef. *Coral Reefs*, 32, 233–238. <https://doi.org/10.1007/s00338-012-0974-0>
- Dias, T.L.P. & Gondim, A.I. (2015). Bleaching in scleractinians, hydrocorals, and octocorals during thermal stress in a northeastern Brazilian reef. *Marine Biodiversity*, 6, 303–307. <https://doi.org/10.1007/s12526-015-0342-8>
- Dickson, A.G. & Millero, F.J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research Part A: Oceanographic Research Papers*, 34, 1733–1743. [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5)
- Dickson, A.G., Sabine, C.L., & Christian, J.R. (2007) Guide to best practices for ocean CO₂ measurement. Sidney, British Columbia, North Pacific Marine Science Organization (PICES Special Publication 3). <http://hdl.handle.net/11329/249>
- Dimond, J. & Carrington, E. (2007). Temporal variation in the symbiosis and growth of the temperate scleractinian coral *Astrangia poculata*. *Marine Ecology Progress Series*, 348, 161–172. <https://doi.org/10.3354/meps07050>
- Dimond, J. & Carrington, E. (2008). Symbiosis regulation in a facultatively symbiotic temperate coral: Zooxanthellae division and expulsion. *Coral Reefs*, 27, 601–604. <https://doi.org/10.1007/s00338-008-0363-x>
- Dixon, D.L., Munday, P.L. & Jones, G.P. (2010). Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters*, 13(1), 68–75. <https://doi.org/10.1111/j.1461-0248.2009.01400.x>
- Doney, S.C., Fabry, V.J., Feely, R.A. & Kleypas, J.A. (2009). Ocean acidification: The other CO₂ problem. *Annual Review of Marine Science*, 1, 169–192. <https://doi.org/10.1146/annurev.marine.010908.163834>
- Dupont, S., Dorey, N., Stumpp, M., Melzner, F. & Thorndyke, M. (2012). Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology*, 160, 1835–1843. <https://doi.org/10.1007/s00227-012-1921-x>
- Durante, L.M., Cruz, I.C.S. & Lotufo, T.M.C. (2018). The effect of climate change on the distribution of a tropical zoanthid (*Palythoa caribaeorum*) and its ecological implications. *PeerJ*, 6, e4777. <https://doi.org/10.7717/peerj.4777>
- Fabricius, K.E. (1995). Slow population turnover in the soft coral genera *Sinularia* and *Sarcophyton* in mid- and outer-shelf reefs of the Great Barrier Reef. *Marine Ecology Progress Series*, 126, 145–152. <https://doi.org/10.3354/meps126145>
- Fay, S.A. & Weber, M.X. (2012). The occurrence of mixed infections of *Symbiodinium* (Dinoflagellata) within individual hosts. *Journal of Phycology*, 48(6), 1306–1316. <https://doi.org/10.1111/j.1529-8817.2012.01220.x>
- 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. <https://doi.org/10.1126/science.1097329>
- Ferrari, M.C.O., Mc Cormick, M.I., Munday, P.L., Meekan, M.G., Dixon, D. L., Lonnstedt, O. & Chivers, D.P. (2011). Putting prey and predator into the CO₂ equation—qualitative and quantitative effects of ocean acidification on predator–prey interactions. *Ecology Letters*, 14, 1143–1148. <https://doi.org/10.1111/j.1461-0248.2011.01683.x>
- Gao, K., Aruga, Y., Asada, K. & Kiyohara, M. (1993). Influence of enhanced CO₂ on growth and photosynthesis of the red algae *Gracilaria* sp. and *G. chilensis*. *Journal of Applied Phycology*, 5(6), 563–571. <https://doi.org/10.1007/BF02184635>
- García, E., Clemente, S., López, C., McAlister, J. & Hernández, J.C. (2015). Ocean warming modulates the effects of limited food availability on *Paracentrotus lividus* larval development. *Marine Biology*, 162, 1463–1472. <https://doi.org/10.1007/s00227-015-2682-0>
- Gattuso, J.P. & Buddemeier, R.W. (2000). Ocean biogeochemistry: Calcification and CO₂. *Nature*, 407, 311–313. <https://doi.org/10.1038/35030280>
- Ghedini, J., Russell, B.D. & Connell, S.D. (2015). Trophic compensation reinforces resistance: Herbivory absorbs the increasing effects of multiple disturbances. *Ecology Letters*, 18, 182–187. <https://doi.org/10.1111/ele.12405>
- Giordano, M., Beardall, J. & Raven, J.A. (2005). CO₂ concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology*, 56, 99–131. <https://doi.org/10.1146/annurev.arplant.56.032604.144052>
- Glynn, P.W. (1996). Coral reef bleaching: Facts, hypotheses and implications. *Global Change Biology*, 2, 495–509. <https://doi.org/10.1111/j.1365-2486.1996.tb00063.x>
- Glynn, P.W., Maté, J.L., Baker, A.C. & Calderón, M.O. (2001). Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation Event: Spatial/temporal patterns and comparisons with the 1982–1983 event. *Bulletin of Marine Science*, 69(1), 79–109. <http://hdl.handle.net/10088/11920>
- Goldberg, J., & Wilkinson, C. (2004) Global threats to coral reefs: Coral bleaching, global climate change, disease, predator plagues and invasive species. In *Status of Coral Reefs of the World 2004*. Wilkinson, C. Townsville, Australia, pp. 67–92.
- González-Delgado, S., López, C., Brito, A. & Clemente, S. (2018). Marine community effects of two colonial zoanthids in intertidal habitats of the Canary Islands. *Regional Studies in Marine Science*, 23, 23–31. <https://doi.org/10.1016/j.rmsa.2018.03.006>
- Graham, E.R., Parekh, A., Devassy, R.K. & Sanders, R.W. (2015). Carbonic anhydrase activity changes in response to increased temperature and pCO₂ in *Symbiodinium*–zoanthid associations. *Journal of Experimental Marine Biology and Ecology*, 473, 218–226. <https://doi.org/10.1016/j.jembe.2015.08.017>
- Graham, E.R. & Sanders, R.W. (2015). Species-specific photosynthetic responses of symbiotic zoanthids to thermal stress and ocean acidification. *Marine Ecology*, 37, 442–458. <https://doi.org/10.1111/maec.12291>
- Gustafsson, M.S.M., Baird, M.E. & Ralph, P.J. (2013). The interchangeability of autotrophic and heterotrophic nitrogen sources in scleractinian coral symbiotic relationships: A numerical study. *Ecological Modelling*, 250, 183–194. <https://doi.org/10.1016/j.ecolmodel.2012.11.003>
- Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, J.B.C., Thornber, C.S. et al. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, 9, 228–241. <https://doi.org/10.1111/j.1461-0248.2005.00871.x>
- Harvell, D., Kim, K., Quirolo, C., Weir, J. & Smith, G. (2001). Coral bleaching and disease: Contributors to 1998 mass mortality in *Briareum asbestinum* (Octocorallia, Gorgonacea). *Hydrobiologia*, 450, 97–104. <https://doi.org/10.1023/A:1013169331913>
- Hellström, M. & Benzie, J.A.H. (2011). Robustness of size measurement in soft corals. *Coral Reefs*, 30, 787–790. <https://doi.org/10.1007/s00338-011-0760-4>
- Helmuth, B., Mieszkowska, N., Moore, P. & Hawkins, S.J. (2006). Living on the edge of two changing worlds: Forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 373–404. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110149>
- Heuer, R.M. & Grosell, M. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *AJP Regulatory Integrative and Comparative Physiology*, 307, R1061–R1084. <https://doi.org/10.1152/ajpregu.00064>
- Hibino, Y., Todd, P., Ashworth, C.D., Obuchi, M. & Reimer, J.D. (2013). Monitoring colony colour and zooxanthellae (*Symbiodinium* spp.) condition in the reef zoanthid *Palythoa tuberculosa* in Okinawa, Japan. *Marine Biology Research*, 9(8), 794–801. <https://doi.org/10.1080/17451000.2013.766344>
- Hines, D.E. & Pawlik, J.R. (2012). Assessing the antipredatory defensive strategies of Caribbean non-scleractinian zoantharians (Cnidaria): Is the sting the only thing? *Marine Biology*, 159, 389–398. <https://doi.org/10.1007/s00227-011-1816-2>
- Hirose, M., Obuchi, M., Hirose, E. & Reimer, J.D. (2011). Timing of spawning and early development of *Palythoa tuberculosa* (Anthozoa, Zoantharia, Sphenopidae) in Okinawa, Japan. *Biological Bulletin*, 220, 23–31. <https://doi.org/10.2307/20839322>
- Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, 50, 839–866. <https://doi.org/10.1071/MF99078>
- Holcomb, M., Cohen, A.L. & McCorkle, D.C. (2012). An investigation of the calcification response of the scleractinian coral *Astrangia poculata* to elevated pCO₂ and the effects of nutrients, zooxanthellae and gender. *Biogeosciences*, 9, 29–39. <https://doi.org/10.5194/bg-9-29-2012>
- Horta e Costa, B. & Gonçalves, E.J. (2013). First occurrence of the monrovia doctorfish *Acanthurus monroviae* (Perciformes: Acanthuridae) in European Atlantic waters. *Marine Biodiversity Records*, 6, e20. <https://doi.org/10.1017/S1755267213000055>
- Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C. et al. (2003). Climate change, human impacts, and the resilience of coral reefs. *Science*, 301, 929–933. <https://doi.org/10.1126/science.1085046>
- Iglesias-Prieto, R., Matta, J.L., Robins, W.A. & Trench, R.K. (1992). Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 10302–10305. <https://doi.org/10.1073/pnas.89.21.10302>
- IPCC. (2007) In: Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden and Hanson, C.E. (Eds.) *Climate change 2007: Impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University press.
- IPCC. (2013) *Climate change 2013: The physical science basis*. In: Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., et al. (Eds.) *Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.
- Israel, A. & Hophy, M. (2002). Growth, photosynthetic properties and rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO₂ concentrations. *Global Change Biology*, 8, 831–840. <https://doi.org/10.1046/j.1365-2486.2002.00518.x>
- Israel, A., Shlomit, K., Dubinsky, Z., Merrill, J.E. & Friedlander, M. (1999). Photosynthetic inorganic carbon utilization and growth of *Pophrhya linearis* (Rhodophyta). *Journal of Applied Phycology*, 11, 447–453. <https://doi.org/10.1023/A:1008122306268>

- Jiménez, C. (2001). Bleaching and mortality of reef organisms during a warming event in 1995 on the Caribbean coast of Costa Rica. *Revista de Biología Tropical*, 49, 233–238.
- Jokiel, P.L., Rodgers, K.S., Kuffner, I.B., Andersson, A.J., Cox, E.F. & Mackenzie, F.T. (2008). Ocean acidification and calcifying reef organisms: A mesocosm investigation. *Coral Reefs*, 27, 473–483. <https://doi.org/10.1007/s00338-008-0380-9>
- Kaplan, A. & Reinhold, L. (1999). CO₂ concentrating mechanisms in photosynthetic microorganisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 539–570. <https://doi.org/10.1146/annurev.arplant.50.1.539>
- Karlson, R.H. (1988). Growth and survivorship of clonal fragments in *Zoanthus solanderi*, Lesueur. *Journal of Experimental Marine Biology and Ecology*, 123(1), 31–39. [https://doi.org/10.1016/0022-0981\(88\)90107-4](https://doi.org/10.1016/0022-0981(88)90107-4)
- Khalesi, M.K., Beeftink, H.H. & Wijffels, R.H. (2009). Light-dependency of growth and secondary metabolite production in the captive zooxanthellate soft coral *Sinularia flexibilis*. *Marine Biotechnology*, 11, 488–494. <https://doi.org/10.1007/s10126-008-9164-z>
- Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S. et al. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, 19, 1884–1896. <https://doi.org/10.1111/gcb.12179>
- Kübler, J.E., Johnston, A.M. & Raven, J.A. (1999). The effects of reduced and elevated CO₂ and O₂ on the seaweed *Lomentaria articulata*. *Plant, Cell and Environment*, 2, 1303–1310. <https://doi.org/10.1046/j.1365-3040.1999.00492.x>
- LaJeunesse, T.C. (2005). 'Species' radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene–Pliocene transition. *Molecular Biology and Evolution*, 22(3), 570–581. <https://doi.org/10.1093/molbev/msi042>
- LaJeunesse, T.C., Parkinson, J.E., Gabrielson, P.W., Jeong, H.J., Reimer, J. D., Voolstra, C.R. & Santos, S.R. (2018). Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Current Biology*, 28(16), 2570–2580. <https://doi.org/10.1016/j.cub.2018.07.008>
- Langdon, C., Broecker, W.S., Hammond, D.E., Glenn, E., Fitzsimmons, K., Nelson, S.G. et al. (2003). Effect of elevated CO₂ on the community metabolism of an experimental coral reef. *Global Biogeochemical Cycles*, 17(1), 1011. <https://doi.org/10.1029/2002GB001941>
- Leal, M.C., Cruz, I.C., Mendes, C.R., Calado, R., Kikuchi, R.K.P., Rosa, R. et al. (2015). Photobiology of the zoanthid *Zoanthus sociatus* in intertidal and subtidal habitats. *Marine and Freshwater Research*, 67(12), 1991–1997. <https://doi.org/10.1071/MF15300>
- Leggat, W., Badger, M. & Yellowlees, D. (1999). Evidence for an inorganic carbon-concentrating mechanism in the symbiotic dinoflagellate *Symbiodinium* sp. *Plant Physiology*, 121, 1247–1255. <https://doi.org/10.1104/pp.121.4.1247>
- Lewis, E. & Wallace, D. (1998) Program developed for CO₂ system calculation. In: ORNL/CDIAC-105de América. Oak Ridge, TN: Carbon Dioxide Information Analysis Center
- Li, W. & Gao, K. (2012). A marine secondary producer respire and feeds more in a high CO₂ ocean. *Marine Pollution Bulletin*, 64, 699–703. <https://doi.org/10.1016/j.marpolbul.2012.01.033>
- López, C., & Clemente, S. (2019) Feeding behaviour of the clown crab *Platypodiella picta* upon two species of the genus *Palythoa*. XX Iberian Symposium on Marine Biology Studies. 10.3389/conf.fmars.2019.08.00160
- López, C., Clemente, S., Almeida, C., Brito, A. & Hernández, M. (2015). A genetic approach to the origin of *Millepora* sp. in the eastern Atlantic. *Coral Reefs*, 34, 631–638. <https://doi.org/10.1007/s00338-015-1306-y>
- López, C., Moreno-Borges, S., Álvarez, O., Brito, A. & Clemente, S. (2020). Distribution of zooxanthellate zoantharians in the Canary Islands: Potential indicators of ocean warming. *Estuarine, Coastal and Shelf Science*, 233, 106519. <https://doi.org/10.1016/j.jecss.2019.106519>
- López, C., Reimer, J.D., Brito, A., Simón, D., Clemente, S. & Hernández, M. (2019). Diversity of zoantharian species and their symbionts from the Macaronesian and Cape Verde ecoregions demonstrates their widespread distribution in the Atlantic Ocean. *Coral Reefs*, 38, 269–283. <https://doi.org/10.1007/s00338-019-01773-0>
- Loya, Y., Sakai, K., Yamazato, Y., Nakano, Y., Sambali, H. & Van Woesik, R. (2001). Coral bleaching: The winners and the losers. *Ecology Letters*, 4, 122–131. <https://doi.org/10.1046/j.1461-0248.2001.00203.x>
- Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.M., Siegenthaler, U. et al. (2008). High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature*, 453, 379–382. <https://doi.org/10.1038/nature06949>
- Luz, D.C., Zebal, Y.D., Klein, R.D., Marques, J.A., Marangoni, L.F.B., Pereira, C.M. et al. (2018). Oxidative stress in the hydrocoral *Millepora allicornis* exposed to CO₂-driven seawater acidification. *Coral Reefs*, 37(2), 571–579. <https://doi.org/10.1007/s00338-018-1681-2>
- Manson, R.A.B. (2018). Decline in symbiont densities of tropical and subtropical scleractinian corals under ocean acidification. *Coral Reefs*, 37, 945–953. <https://doi.org/10.1007/s00338-018-1720-z>
- Marty, M.J. & Pawlik, J.R. (2015). A fish-feeding laboratory bioassay to assess the antipredatory activity of secondary metabolites from the tissues of marine organisms. *Journal of Visualized Experiments*, 95, 1–6. <https://doi.org/10.3791/52429>
- McCulloch, M., Falter, J., Trotter, J. & Montagna, P. (2012). Coral resilience to ocean acidification and global warming through pH upregulation. *Nature Climate Change*, 2, 623–627. <https://doi.org/10.1038/nclimate1473>
- McIlwain, J.L. & Jones, G.P. (1997). Prey selection by an obligate coral-feeding wrasse and its response to small-scale disturbance. *Marine Ecology Progress Series*, 155, 189–198. <https://doi.org/10.3354/meps155189>
- Mehrbach, C., Culbertson, C.H., Hawley, J.E. & Pytkowicz, R.M. (1973). Measurement of the apparent dissociation constants of carbonic acid in sea water at atmospheric pressure. *Limnology and Oceanography*, 18, 897–907. <https://doi.org/10.4319/lo.1973.18.6.0897>
- Michaelidis, B., Ouzounis, C., Palaras, A. & Pörtner, H.O. (2005). Effects of long-term moderate hypercapnia on acid–base balance and growth in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, 293, 109–118. <https://doi.org/10.3354/meps293109>
- Michalek-Wagner, K. & Willis, B.L. (2001). Impacts of bleaching on the soft coral *Lobophytum compactum*. II Biochemical changes in adults and their eggs. *Coral Reefs*, 19, 240–246. <https://doi.org/10.1007/PL00006959>
- Mueller, E. & Haywick, D.W. (1995). Sediment assimilation and calcification by the Western Atlantic reef zoanthid, *Palythoa Caribaeorum*. *Bulletin. Institut Océanographique (Monaco)*, 14, 89–100.
- Muscatine, L., Falkowski, P.G., Porter, J.W. & Dubinsky, Z. (1984). Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proceedings of the Royal Society of London*, 222, 181–202. <https://doi.org/10.1098/rspb.1984.0058>
- Nagelkerken, I. & Munday, P.L. (2016). Animal behaviour shapes the ecological effects of ocean acidification and warming: Moving from individual to community-level responses. *Global Change Biology*, 22, 974–989. <https://doi.org/10.1111/gcb.13167>
- O'Neal, W. & Pawlik, J.R. (2002). A reappraisal of the chemical and physical defenses of Caribbean gorgonian corals against predatory fishes. *Marine Ecology Progress Series*, 240, 117–126. <https://doi.org/10.3354/meps240117>
- Ong, C.W., Reimer, J.D. & Todd, P.A. (2013). Morphologically plastic responses to shading in the zoanthids *Zoanthus sansibaricus* and *Palythoa tuberculosa*. *Marine Biology*, 160, 1053–1064. <https://doi.org/10.1007/s00227-012-2158-4>

- Parkinson, J.E., Yang, S.Y., Kawamura, I., Byron, G., Todd, P.A. & Reimer, J. D. (2016). A citizen science approach to monitoring bleaching in the zoantharian *Palythoa tuberculosa*. *PeerJ*, 4, 1815. <https://doi.org/10.7717/peerj.1815>
- Perry, A.L., Low, P.J., Ellis, J.R. & Reynolds, J.D. (2005). Climate change and distribution shifts in marine fishes. *Science*, 308, 1912–1915. <https://doi.org/10.1126/science.1111322>
- Polak, O., Loya, Y., Brickner, I., Kramarski-Winter, E. & Benayahu, Y. (2011). The widely-distributed Indo-Pacific zoanthid *Palythoa tuberculosa*: A sexually conservative strategist. *Bulletin of Marine Science*, 87, 605–621. <https://doi.org/10.5343/bms.2010.1088>
- Porter, J.W., Fitt, W.K., Spero, H.J. & Rogers, C.S. (1989). Bleaching in reef corals, physiological and stable isotopic responses. *Proceedings of the National Academy of Sciences of the United States of America*, 86(23), 9342–9346. <https://doi.org/10.1073/pnas.86.23.9342>
- Pörtner, H.O. & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315, 95–97. <https://doi.org/10.1126/science.1135471>
- Prada, C., Weil, E. & Yoshioka, P.M. (2010). Octocoral bleaching during unusual thermal stress. *Coral Reefs*, 29, 41–45. <https://doi.org/10.1007/s00338-009-0547-z>
- Proksch, P. (1994). Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. *Toxicon*, 32(6), 639–655. [https://doi.org/10.1016/0041-0101\(94\)90334-4](https://doi.org/10.1016/0041-0101(94)90334-4)
- Rabelo, E.F., Soares, M.O. & Matthews-Cascos, H. (2013). Competitive interactions among zoanthids (Cnidaria: Zoanthidae) in an intertidal zone of northeastern Brazil. *Brazilian Journal of Oceanography*, 61, 35–42. <https://doi.org/10.1590/S1679-87592013000100004>
- Raymundo, L.J., Burdick, D., Hoot, W.C., Miller, R.M., Brown, V., Reynolds, T. et al. (2019). Successive bleaching events cause mass coral mortality in Guam, Micronesia. *Coral Reefs*, 38(4), 677–700. <https://doi.org/10.1007/s00338-019-01836-2>
- Reimer, A.A. (1971). Observations on the relationships between several species of tropical zoanthids (Zoanthidea, Coelenterata) and their zooxanthellae. *Journal of Experimental Marine Biology and Ecology*, 7, 207–214. [https://doi.org/10.1016/0022-0981\(71\)90032-3](https://doi.org/10.1016/0022-0981(71)90032-3)
- Reimer, J.D., Ono, S., Fujiwara, Y., Takishita, K. & Tsukahara, J. (2004). Reconsidering *Zoanthus* spp. diversity: Molecular evidence of conspecificity within four previously presumed species. *Zoological Science*, 21, 517–525. <https://doi.org/10.2108/zsj.21.517>
- Reimer, J.D., Ono, S., Sinniger, F. & Tsukahara, J. (2008). Distribution of zooxanthellate zoanthid species (Zoantharia: Anthozoa: Hexacorallia) in southern Japan limited by cold temperatures. *Galaxea, Journal of Coral Reef Studies*, 10, 57–67. <https://doi.org/10.3755/galaxea.10.57>
- Reimer, J.D., Takishita, K., Ono, S. & Maruyama, T. (2007). Diversity and evolution in the zoanthids genus *Palythoa* (Cnidaria: Hexacorallia) based on nuclear ITS-rDNA. *Coral Reefs*, 26, 399–410. <https://doi.org/10.1007/s00338-007-0210-5>
- Riebesell, U., Schulz, K.G., Bellerby, R.G.J., Botros, M., Fritsche, P., Meyerhöfer, M. et al. (2007). Enhanced biological carbon consumption in a high CO₂ ocean. *Nature*, 450, 545–548. <https://doi.org/10.1038/nature06267>
- Rodríguez, A., Clemente, S., Brito, A. & Hernández, J.C. (2018). Effects of ocean acidification on algae growth and feeding rates of juvenile sea urchins. *Marine Environmental Research*, 140, 382–389. <https://doi.org/10.1016/j.marenvres.2018.07.004>
- Rodríguez, A., Hernández, J.C., Brito, A. & Clemente, S. (2017). Effects of ocean acidification on juveniles sea urchins: Predator–prey interactions. *Journal of Experimental Marine Biology and Ecology*, 493, 31–40. <https://doi.org/10.1016/j.jembe.2017.04.005>
- Rosa, I., Rocha, R.J.M., Lopes, A., Cruz, I.C.S., Calado, R., Bandarra, N., et al. (2016). Impact of air exposure on the photobiology and biochemical profile of an aggressive intertidal competitor, the zoanthid *Palythoa caribaeorum*. *Marine Biology*, 163, 222. <https://doi.org/10.1007/s00227-016-3002-z>
- Rotjan, R.D. & Lewis, S.M. (2008). The impact of coral predators in tropical reefs. *Marine Ecology Progress Series*, 367, 73–91. <https://doi.org/10.3354/meps07531>
- Ryland, J.S., Putron, S., Scheltema, R.S., Chimonides, P.J. & Zhadan, D.G. (2000). Semper's (zoanthid) larvae: Pelagic life, parentage and other problems. *Hydrobiologia*, 440, 191–198. <https://doi.org/10.1023/A:1004171127777>
- Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L. et al. (2004). The oceanic sink for anthropogenic CO₂. *Science*, 305, 367–371. <https://doi.org/10.1126/science.1097403>
- Santana, E.F.C., Alves, A.L., Santos, A.D.M., Cunha, M.D.G.G.S., Perez, C.D. & Gomes, A.P.B. (2015). Trophic ecology of the zoanthid *Palythoa caribaeorum* (Cnidaria: Anthozoa) on tropical reefs. *Journal of the Marine Biological Association of the UK*, 95(02), 301–309. <https://doi.org/10.1017/S0025315414001726>
- Santos, M.E.A., Kitahara, M.V., Lindner, A. & Reimer, J.D. (2016). Overview of the order Zoantharia (Cnidaria:Anthozoa) in Brazil. *Marine Biodiversity*, 46, 547–559. <https://doi.org/10.1007/s12526-015-0396-7>
- Sawelew, L., Gault, F., Nuccio, C., Perez, Y. & Lorquin, J. (2018). Characterisation of palytoxin from an undescribed *Palythoa* (Anthozoa: Zoantharia: Sphenopidae) with significant in vitro cytotoxic effects on cancer cells at picomolar doses. *BioRxiv*, 292219. <https://doi.org/10.1101/292219>
- Scheibling, R.E. & Robinson, M.C. (2008). Settlement behaviour and early post-settlement predation of the sea urchin *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology*, 365, 59–66. <https://doi.org/10.1016/j.jembe.2008.07.041>
- Sebens, K.P. (1982). Intertidal distribution of zoanthids on the Caribbean coast of Panama: Effects of predation and desiccation. *Bulletin of Marine Science*, 32, 316–335.
- Shirayama, Y. & Thornton, H. (2005). Effect of increased atmospheric CO₂ on shallow water marine benthos. *Journal of Geophysical Research*, 110, C09S08. <https://doi.org/10.1029/2004JC002618>
- Siebeck, U., Logan, D. & Marshall, N. (2008). CoralWatch: A flexible coral bleaching monitoring tool for you and your group. *Proceedings of the 11th International Coral Reef Symposium*, 1, 54–553.
- Siebeck, U., Marshall, N., Klüter, A. & Hoegh-Guldberg, O. (2006). Monitoring coral bleaching using a colour reference card. *Coral Reefs*, 25(3), 453–460. <https://doi.org/10.1007/s00338-006-0123-8>
- Somero, G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integrative and Comparative Biology*, 42, 780–789. <https://doi.org/10.1093/icb/42.4.780>
- Sültemeyer, D. (1998). Carbonic anhydrase in eukaryotic algae: Characterization, regulation, and possible function during photosynthesis. *Canadian Journal of Botany*, 76, 962–972. <https://doi.org/10.1139/b98-082>
- Sunday, J.M., Bates, A.E. & Dulvy, N.K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, 2, 686–690. <https://doi.org/10.1038/nclimate1539>
- Tanner, J.E. (1995). Competition between scleractinian corals and macroalgae: An experimental investigation of coral growth, survival and reproduction. *Journal of Experimental Marine Biology and Ecology*, 190(2), 151–168. [https://doi.org/10.1016/0022-0981\(95\)00027-O](https://doi.org/10.1016/0022-0981(95)00027-O)
- Tanner, J.E. (2002). Consequences of density-dependent heterotrophic feeding for a partial autotroph. *Marine Ecology Progress Series*, 227, 293–304. <https://doi.org/10.3354/meps227293>
- Tortell, P. (2000). Evolutionary and ecological perspectives on carbon acquisition in hytoplankton. *Limnology and Oceanography*, 45, 744–750. <https://doi.org/10.4319/lo.2000.45.3.0744>
- Truchot, J.P. (1988). Problems of acid–base balance in rapidly changing intertidal environments. *American Zoologist*, 28, 55–64. <https://doi.org/10.1093/icb/28.1.55>

- Tubaro, A., Durando, P., Del Favero, G., Ansaldi, F., Icardi, G., Deeds, J.R. & Sosa, S. (2011). Case definitions for human poisoning postulated to palytoxins exposure. *Toxicon*, 57, 478–495. <https://doi.org/10.1016/j.toxicon.2011.01.005>
- Ullah, H., Nagelkerken, I., Goldenberg, S.U. & Fordham, D.A. (2018). Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. *PLoS Biology*, 16(1), e2003446. <https://doi.org/10.1371/journal.pbio.2003446>
- Vélez, P., González, M., Pérez, M.D. & Hernández, A. (2015) Open ocean temperature and salinity trends in the Canary Current Large Marine Ecosystem. In: Valdés, J.L. and Déniz, G.I. (Eds.) *Oceanographic and biological features in the canary current large marine ecosystem*. Paris: IOC-UNESCO IOC Technical Series
- Vergés, A., Steinberg, P.D., Hay, M.A., Poore, A.G.B., Campbell, A.H., Ballesteros, E. et al. (2014). The tropicalization of temperate marine ecosystems: Climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society B*, 281, 20140846. <https://doi.org/10.1098/rspb.2014.0846>
- Wee, H.B., Reimer, J.D., Safuan, M., Saidin, J., Tan, C.H. & Bachokac, Z. (2017). Zoantharian abundance in coral reef benthic communities at Terengganu Islands, Malaysia. *Regional Studies in Marine Science*, 12, 58–63. <https://doi.org/10.1016/j.rsma.2017.02.005>
- Williams, J.E.H. & Bunkley-Williams, L. (1990). The world-wide coral reef bleaching cycle and related sources of coral mortality. *Atoll Research Bulletin*, 335, 1–71. <https://doi.org/10.5479/si.00775630.335.1>
- Winter, A., Appeldoorn, R.S., Bruckner, A., Williams, E.H. & Goenaga, C. (1998). Sea surface temperatures and coral reef bleaching of La Parguera, Puerto Rico (northeastern Caribbean Sea). *Coral Reefs*, 17, 377–382. <https://doi.org/10.1007/s003380050143>
- Yapici, S., Filiz, H. & Bilge, G. (2016). Northwards range expansion of *Sparisoma cretense* (Linnaeus, 1758) in the Turkish Aegean Sea. *Journal of Aquaculture Engineering and Fisheries Research*, 2(4), 201–207. <https://doi.org/10.3153/JAEFR16022>
- Yorifuji, M., Harii, S., Nakamura, R. & Fudo, M. (2017). Shift of symbiont communities in *Acropora tenuis* juveniles under heat stress. *PeerJ*, 5, e4055. <https://doi.org/10.7717/peerj.4055>

How to cite this article: López C, Bas-Silvestre M, Rodríguez A, Brito A, Clemente S. Effects of low pH and high temperature on two *Palythoa* spp. and predator–prey interactions in the subtropical eastern Atlantic. *Aquatic Conserv: Mar Freshw Ecosyst*. 2020;1–16. <https://doi.org/10.1002/aqc.3487>