


Article

Understanding *Balanophyllia regia* Distribution in the Canary Islands: Effects of Environmental Factors and Methodologies for Future Monitoring

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Abstract: The future of marine ecosystems is at risk due to climate change and other human impacts. Specifically, due to ocean warming, some tropical species are expanding their populations while populations of temperate species are in regression, making the establishment of conservation measures imperative to maintain local biodiversity. In this study we establish a baseline on the distribution and abundance of the temperate coral *Balanophyllia regia* from the Canary Islands. We found that the main environmental factors determining *B. regia*'s distribution and abundance were sea surface temperature and hydrodynamic conditions. Areas under large wave action and colder environments enhanced this warm-temperate species' development. Since its metabolic performance depends exclusively on the surrounding environment, we also propose a methodology to potentially monitor climate change on coastal habitats through this azooxanthellate calcified coral. Results of a tagging experiment showed that a concentration of 20 mg/mL of calcein during 6 h might be enough to in situ label polyps of *B. regia* without compromising corallite survival. Long-term monitoring of population abundances and growth rates of *B. regia* through calcein tagging will allow us to identify alterations in local ecosystems early and focus future conservation investments on the most vulnerable areas with higher ecological and economic value.

Keywords: azooxanthellate coral; ocean warming; calcein; distribution pattern; growth; Canary Islands; conservation management



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1. Introduction

Many marine ecosystems are currently under threat worldwide due to the effects of climate change. Warmer temperature regimens are enabling northward expansions and settlement of tropical species in new ecosystems ('tropicalization' process) [1–3]. Meanwhile, in subtropical and warm-temperate regions, populations of some native species with tropical affinities have been able to proliferate within their own geographic range ('meridionalization' process), given the more appropriate environmental conditions [4]. Both 'tropicalization' and 'meridionalization' can cause the displacement of native species with more temperate affinities [5,6], threatening their survival and persistence, especially when they already live close to their limits of maximum thermal tolerances [7,8]. Combined with ocean warming, lowered seawater pH levels make shell and skeleton formation in marine calcifiers difficult while promoting the dissolution of calcium carbonate (CaCO₃) [9,10]. Many marine organisms have become more susceptible to environmental pressures and predation [11]. In fact, numerous studies have revealed dramatic reductions in calcification

rates due to lowered CO_3^{2-} concentrations in several calcareous organisms, including coralline algae, corals, and other invertebrates [12,13].

The Canary Islands are an oceanic archipelago located where the Canary Current and the Saharian upwelling converge, leading to a great heterogeneity in ocean climate mainly driven by contrasting sea surface temperatures (SST). In this sense, SST at the eastern islands is up to 2 °C lower than at the western ones, while within each island, waters at the north and north-eastern coasts are cooler than at the south-western coasts [4,14]. This heterogeneity in SST influences biota organization and allows tropical and warm-temperate species to coexist in a relatively small region [15,16]. For instance, the marine biota of the easternmost island (Lanzarote) shows temperate affinities, sharing many species with the Mediterranean Sea and higher latitudes, while tropical species are abundant in ecosystems from the easternmost island (El Hierro), which highlights the large biological effects of even subtle temperature differences. Although consequences of ocean acidification due to climate change have not been noticed in ecosystems of the Canary Islands, the impact of ocean warming is already patent in many ways. SST has experienced an increase of 0.28 °C per decade since 1970, being more noticeable during summer months. Consequently, several species from tropical latitudes have been able to settle stable populations in the Archipelago [17,18], or increased in abundance [19] during the last decades, while warm-temperate species might be in current threat [20,21].

Balanophyllia regia (Dendrophylliidae: Anthozoa) is a shallow-water warm-temperate coral distributed from southern England to the Canary Islands, including the Mediterranean Sea [22,23]. This solitary scleractinian coral mostly lives in shaded habitats with high hydrodynamic regimens, usually within tidepools and rock crevices often covered by coralline crustose algae. *B. regia* is azooxanthellate, meaning that its source of energy for metabolic processes is provided only by heterotrophic feeding, and then corallites completely depend on the surrounding environment [24]. Since *B. regia* reaches its southernmost distribution limit in the Canary Islands, its intertidal populations may be especially susceptible to current ocean warming in the Archipelago [19]. Also, future ocean acidification might compromise growth rates in this calcareous organism. Less favorable environmental conditions, exacerbated by a greatly limited ability to migrate to other regions because of a deep water barrier surrounding the islands, might compromise the presence of *B. regia* in the Canary Islands.

The impacts of climate change on coastal environments are especially worrying since they host most of the species richness and marine resources on the planet, but they also have a high concentration of human activities [8]. For this reason, the aim of this study was to evaluate the current status of *B. regia* populations and determine the main local-scale environmental drivers that explain patterns in the abundance and distribution of the species in intertidal habitats. Additionally, we aimed to establish a suitable methodology for in situ monitoring growth rates of *B. regia* through calcein chemical tagging, which has been widely used in different coral species [25–27], including *B. regia* from the Atlantic coast of France [28]. However, as high concentrations of calcein can compromise the survival of corallites [26] while increasing methodological costs, our goal was to develop a suitable methodology for the long-term monitoring growth rates of intertidal populations of *B. regia* in the Canary Islands, optimizing the amount of product and time required for tagging.

2. Materials and Methods

2.1. Sampling Sites and Local-Scale Environmental Drivers

Due to the east-to-west flow of the Saharan upwelling, the westernmost island of El Hierro represents a more tropical environment. In contrast, the marine biota of the easternmost island of Lanzarote is more similar to temperate areas, with the central island of Tenerife being in an intermediate state. In this sense, to detect environmental factors that may control the distribution and abundance of *Balanophyllia regia* along the Archipelago, we counted the number of corallites on 6 rocky intertidal platforms of El Hierro, 6 of Lanzarote, and 20 around Tenerife (Figure 1).

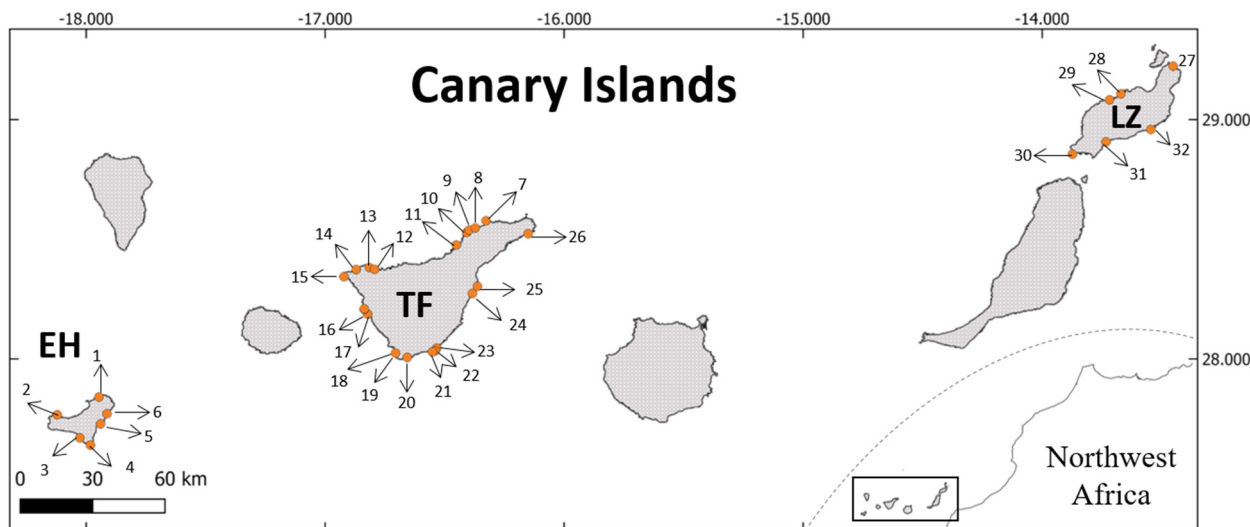


Figure 1. Rocky intertidal platforms where *B. regia* populations were sampled along the Canary Islands during the course of this study: 6 platforms at El Hierro (EH), 20 at Tenerife (TF), and 6 at Lanzarote (LZ).

Surveys consisted of visiting each location at low tide and as many times as needed to cover the whole platform extension by at least two researchers who recorded the number of *B. regia* individuals observed. The total rocky platforms surveyed comprised an area of 611,580 m² (16,880 m², 27,335 m², and 32,135 m² in El Hierro, Lanzarote, and Tenerife, respectively).

Although many parameters might be determining *B. regia* distribution and abundance at the local scale, i.e., competition with space with other benthic organisms, algae cover, and/or presence of predators, in this study, we focused on abiotic parameters that might be influencing its populations at a larger scale. In this sense, ocean temperature, chlorophyll a concentration (Chl-a) as a proxy of seawater productivity, wave exposure, and the area of the rocky platform were chosen as potential predictors to explain the distribution and abundance of intertidal populations of *B. regia*. Mean (SST_{mean}) and maximum (SST_{max}) values of SST and Chl-a concentration for each location were obtained from Bio-Oracle raster layers with 0.008° resolution (=1 km²) [29,30]. Mean ocean wave power at each location was inferred from a time series from 1985 to 2020 (=0.5 km²), provided by the Environmental Hydraulics Institute of the University of Cantabria. Finally, the extension areas of rocky platforms (m²) were calculated from aerial ortho-photographs at low tide, using image processing software (ImageJ 1.52a).

2.2. Calcein Tagging

A total of 148 polyps of *Balanophyllia regia* were collected in Punta del Hidalgo, Tenerife (site 7 in Figure 1) with the aid of a spatula and immediately transported to the laboratory in wet conditions. Specimens were glued in artificial substrates and maintained for 10 days in aquariums with air bubbling for acclimatization to laboratory conditions. Afterward, corallites were healthy and without external signals of damage, and all were used for tagging experiments. A total of 109 specimens were randomly distributed among four tagging aquaria: two at concentrations of 20 mg calcein/L and two at 50 mg/L. To apply three different soaking times, around 8 to 10 specimens were subsequently moved away from each treatment aquaria at 6, 12, and 24 h. Corallites soaked in calcein were rinsed in seawater and placed into control aquaria with fresh seawater together with unlabeled polyps as a control treatment (n = 39). In both acclimatization and experimentation, salinity and temperature (WTW Cond 3110) and pH (Hanna HI 98919) were monitored every two days, and specimens were fed weekly with a mix of Phyto and Zooplankton (AF Phyto Mix, Aquaforest). In addition, seawater at each aquarium was completely replaced once a week.

Corallite survival and viability after tagging were evaluated after 5 months by calculating growth rates after the experiment. To do so, we measured the largest corallite diameter at the beginning and at the end of the experiment with the aid of a digital vernier caliper with a precision of ± 0.01 mm. Polyps were then cleaned with sodium hypochlorite (10% household bleach diluted in seawater) for 12 h and then left to dry before further processing. Corallites were then observed under UV light using a binocular microscope (NIKON AZ 100 with FITC filter cube, Tokyo, Japan) to assess the success of tagging. We also measured the intensity of the mark that was categorized as no-tagged when no fluorescence was observed, diffuse when fluorescence was partially and slightly detected, faint when fluorescence was partially but well-detected, and bold when the whole polyp was fluorescent (see Figure 2).

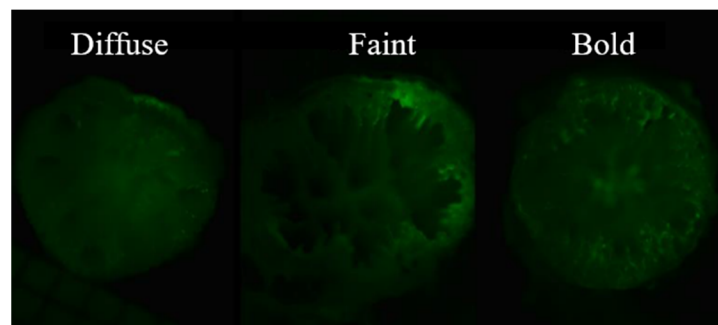


Figure 2. Corallites of *Balanophyllia regia* observed under UV light in a binocular microscope showing the intensity of the label; from left to right, the mark was categorized as diffuse, faint, and bold.

2.3. Data Analyses

We evaluated the variation in the abundance of *B. regia* throughout the Canary Islands using Generalized Linear Models (GLMs), which assessed the contribution of the environmental predictors: SSTmean, SSTmax, Chl-a, wave power and area of the rocky platform. Firstly, and through Spearman correlation coefficients, we tested for collinearity among predictors to choose those uncorrelated variables of biological significance. In this sense, because SSTmean, SSTmax, platform area, Chl-a, and wave power were highly correlated (Figure 3), only SSTmean was included in terms of modeling. Then, models were fitted to obtain the parameter estimates (coefficients) using a negative binomial error distribution since we dealt with overdispersed count data [31]. Estimates indicate the change in the expected value of the response variable for a one-unit change in the predictor variables, assuming all other variables are held constant. After modeling, variance inflation factors (VIF) were calculated to check for collinearity among the selected variables, and since none of them showed VIF values > 10 [32], both SSTmean and wave power were retained. Models were performed using the “lme4” and “MASS” R packages [33] and collinearity analyses throughout the “car” package [34].

For model selection, we compared the models in increasing complexity using the Akaike Information Criterion corrected for the small sample (AICc) to select the most suitable model explaining the distribution of *B. regia*. In this sense, model performance with only one predictor variable was compared to the model combining both parameters, SSTmean and wave action. Finally, we used the “MuMIn” R package [35] that ranks candidate models according to the AICc and included a multimodel averaging that incorporates model selection uncertainty. The same package was used to estimate the relative importance of each predictor variable, as the sum of Akaike weights over all possible models.

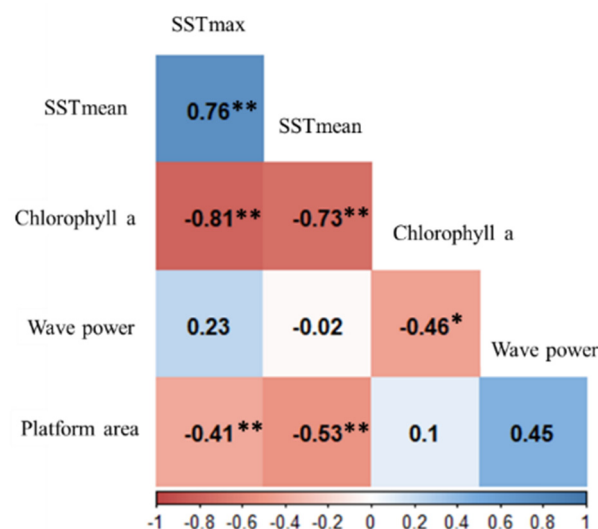


Figure 3. Correlation matrix between predictor variables for the 32 replicates analyzed. SST max = Maximum Sea Surface Temperature, SST mean = Mean Sea Surface Temperature. ** p -value < 0.01 and * p -value < 0.05.

Calcein mark intensity observed in corallites of *Balanophyllia regia* after 5 months was also analyzed by GLMs. We used a multinomial logistic regression through the “nnet” package [36], an extension of binary logistic regression which is applied when the response variable has more than two categories. Then, an estimation of the probability of each category (no-tagged, diffuse, faint, and bold) was modeled as a linear combination of the concentration of calcein (2 levels: 20 and 50 mg/L) and soaking times (3 levels: 6, 12 and 24 h). In the same way, a multinomial logistic regression was performed to evaluate if the probability of detecting each mark intensity varied according to corallite size, which was categorized into 3 groups according to polyp height (3 levels: 0–3 mm, 3–6 mm and >6 mm).

3. Results

3.1. Distribution and Abundance of *Balanophyllia regia*

A total of 1995 corallites of *Balanophyllia regia* were recorded at 14 of the 32 surveyed intertidal platforms (Figure 4). Higher population densities were found in the easternmost island (Lanzarote), followed by the central island (Tenerife) (158.5 ± 281.306 and 52.2 ± 86.14 , mean \pm SD, respectively), while no specimens were found in the warmest westernmost island (El Hierro) (Figures 4 and 5). Wave power and SSTmean were the most relevant variables to explain *B. regia* distribution; the most parsimonious model included both predictors, according to the AICc (Table 1). SSTmean showed a statistically significant estimated coefficient of -1.61 (Table 2), which means that for each one-unit increase in SSTmean, the expected log count of the number of corallites decreases by -40.74 . Likewise, for each one-unit increase in wave power, the expected log count number of corallites significantly increased by 1.45 (Table 2). In fact, within the same island, higher population densities of *B. regia* were recorded in northern locations, exposed to large oceanic swells from the north, such as La Santa ($n = 726$), El Sauzal ($n = 254$), or Punta del Hidalgo ($n = 247$) (sites 28, 11 and 7 in Figure 1, respectively), and this was more noticeable in Tenerife Island (Figures 4 and 5).

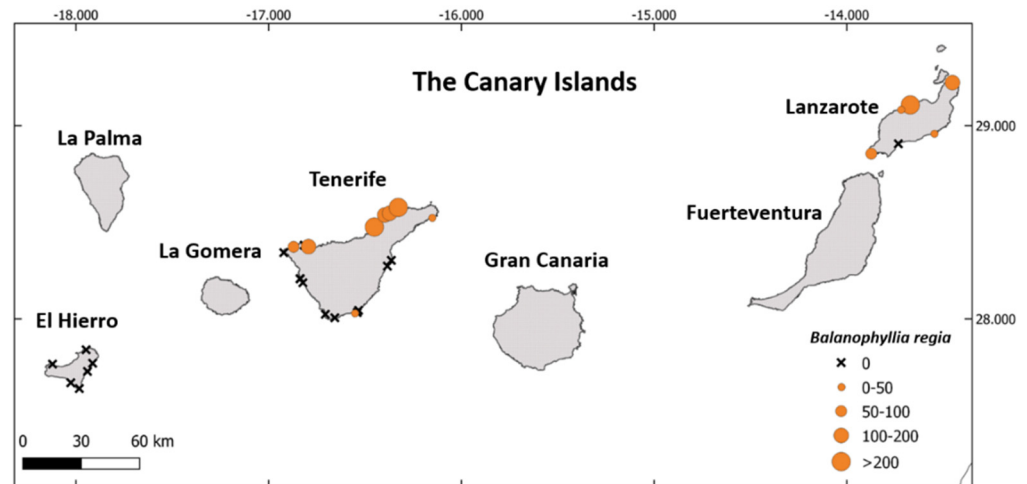


Figure 4. Distribution and abundance of *Balanophyllia regia* populations around intertidal habitats throughout the Canary Islands.

Table 1. Model selection results from multimodel averaging, according to the AICc. Akaike weights are shown. The log-likelihood (logLik) measures how well the model fits observed data; higher values indicate a better fit.

| Model | df | logLik | AICc | Weight |
|------------------------------------|----|---------|---------|--------|
| Model 1: SSTmean, Wave power | 4 | −133.78 | 2685.50 | 1 |
| Model 2: Wave power | 3 | −178.37 | 3574.30 | 0 |
| Model 3: SSTmean | 3 | −235.88 | 4724.50 | 0 |

Table 2. Relative importance of predictor variables, mean SST and wave power, affecting *Balanophyllia regia* abundances from multimodel averaging of GLMs. Estimates indicate the change in the log-odds of the outcome for a one-unit change in the predictor.

| Predictor | Estimate (Coefficient) | Adjusted SE | z Value | Pr (> z) | Relative Importance |
|-----------|---------------------------|-------------|---------|----------------------|------------------------|
| | 0.16 | 0.0041 | 37.44 | $<2 \times 10^{-16}$ | 1 |
| SSTmean | −1.61 | 0.0549 | 29.27 | $<2 \times 10^{-16}$ | 1 |

3.2. Calcein Labeling Experiment

All *Balanophyllia regia* specimens survived experimental calcein treatments. During the 5 months kept in running seawater at constant salinity, temperature, and pH (36.2 ± 0.2 UPS, 20.5 ± 0.5 °C, and 8.0 ± 0.1 , respectively), the corallites showed healthy conditions, with open and active tentacles that reacted in the presence of food. Corallite diameter grew on average (\pm SD) 0.31 ± 0.67 mm after 5 months, showing higher growth rates of large and smaller polyps than intermediate sizes (mean \pm SD) (0.51 ± 0.53 mm, 0.36 ± 0.58 mm and 0.16 ± 0.77 mm, respectively). In this sense, the average growth rate per day and size category class were 2.37 ± 3.87 μ m for polyps between 0.3 mm, 1.05 ± 5.14 μ m for polyps between 3–6 mm, and 3.39 ± 3.55 μ m for polyps larger than 6 mm, with an average of 2.05 ± 4.48 μ m.

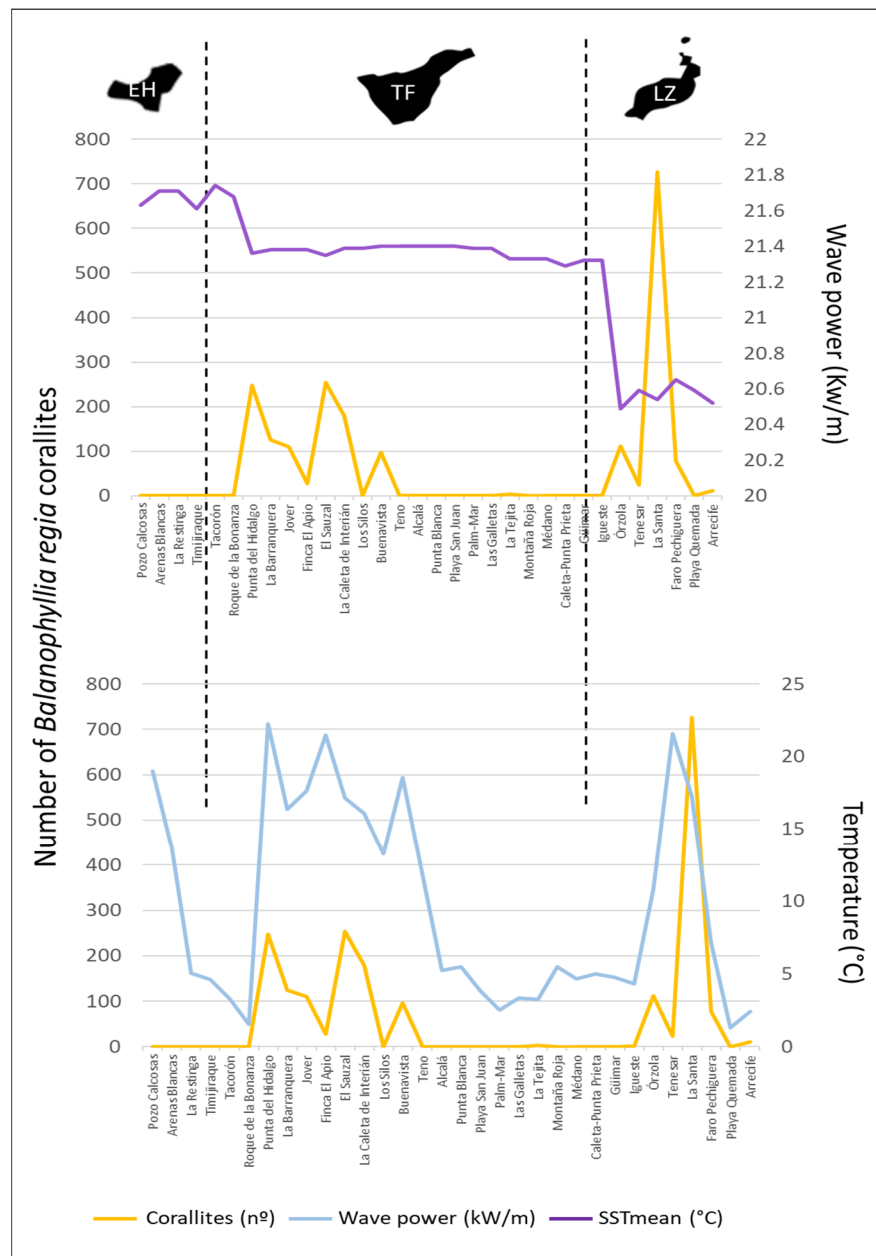


Figure 5. Number of *Balanophyllia regia* corallites (orange lines) and their relationship with wave power (blue line in upper graph) and SSTmean (purple line in bottom graph) at each study site across islands of El Hierro (EH), Tenerife (TF) and Lanzarote (LZ). Numbers represent sampling sites and they match those in Figure 1.

A green mark with different fluorescence intensities was observed in 97 out of the 109 (89%) individuals exposed to calcein (Figure 6), while none of the 39 polyps in the control treatment showed fluorescence. The results showed no significant differences in mark intensity between calcein concentrations (20 and 50 mg/mL), nor in the interaction of the factors time and calcein. Mark intensity depended only on soaking times applied (6, 12, and 24 h) (Table 3). Around 70% of the polyps of *B. regia* exposed to calcein during 6 h showed a diffuse mark, regardless of the concentration of calcein, while the intensity of the resulting tag increased with time (Table 3, Figure 6). In fact, the number of individuals that showed a faint mark after 12 and 24 h of soaking in calcein significantly increased from those with faint marks after 6 h (Table 3, Figure 6).

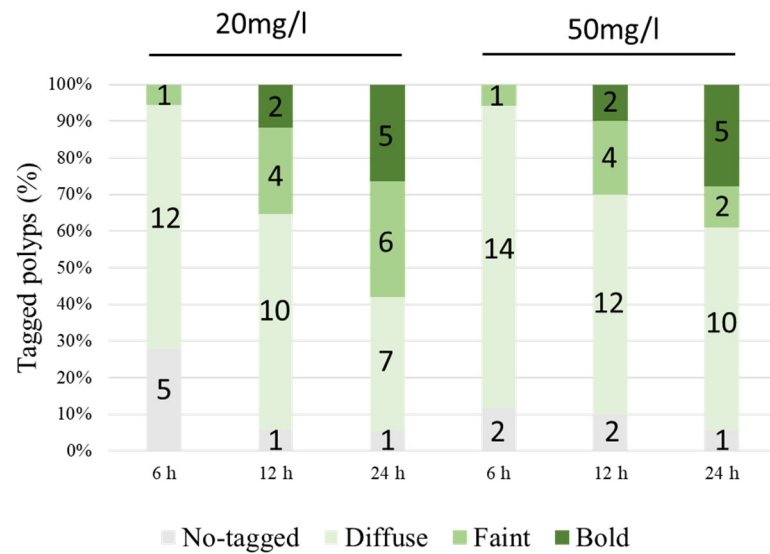


Figure 6. Number (in percentage) of *Balanophyllia regia* corallites showing a non-tagged, diffuse, faint, or bold mark depending on soaking times (6, 12, and 24 h) and calcein concentration (20 and 50 mg/L). Numbers within columns represent the number of individuals in each category.

Table 3. Multinomial logistic regression results analyzing calcein mark intensity observed according to calcein concentrations (20 and 50 mg/mL) and soaking times (6, 12, and 24 h) applied in experimental treatments of *Balanophyllia regia*. Results of the 2-tailed z-test are shown: * $p < 0.05$.

| Mark Intensity | Intercept | 50 mg/L | 12 h | 24 h | 50 mg/L * 12 h | 50 mg/L * 24 h |
|----------------|-----------|---------|--------|--------|----------------|----------------|
| Diffuse | 0.86 | 10.70 | 1.43 | 1.07 | −1.58 | −0.71 |
| Faint | −16.08 | 0.92 | 2.99 * | 3.40 * | −1.61 | −20.13 |
| Bold | −122.80 | −25.67 | 12.97 | 13.89 | 1.88 | 25.68 |

The multinomial logistic regression results showed no significant differences among polyp sizes in the mark intensity observed, despite the wide range of polyp sizes analyzed (2–19 mm in height). Nevertheless, the faint and bold marks were more frequent in polyps smaller than 6 mm in height, while a diffuse mark was more abundant as increasing in size (60, 63 and 83% for 0–3 mm, 3–6 mm, and >6 mm, respectively, Figure 7).

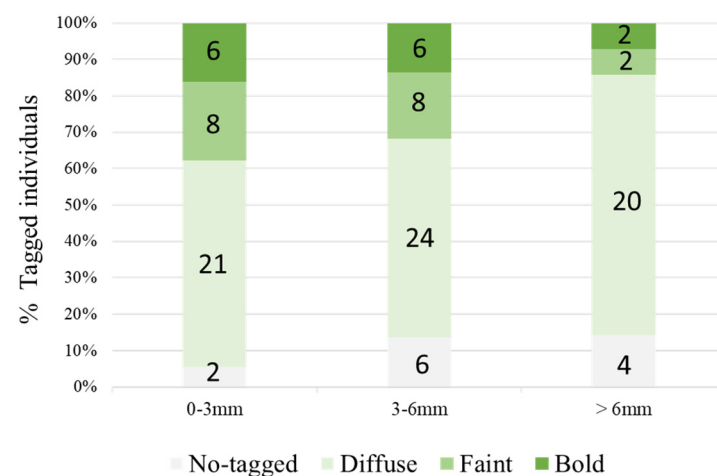


Figure 7. Number in percentage of *Balanophyllia regia* corallites showing a non-tagged, diffuse, faint or bold mark depending on the 3 size categories established. Numbers within columns represent the number of individuals in each category.

4. Discussion

This study demonstrated that wave exposure, operating within island-scale, and sea surface temperature (SST), mainly operating at larger scales, influence the abundance and distribution of *Balanophyllia regia* populations in the Canary Islands. High population densities were frequent in the most wave-exposed intertidal platforms of Lanzarote and Tenerife, where lower values of SST are recorded through the year. Our results also indicated that a concentration of 20 mg/L of calcein for 6 h is enough to tag most of the corallites, regardless of polyp size. This study establishes a baseline for the distribution and abundances of *B. regia* in the Canary Islands and introduces a suitable methodology for in situ evaluation of corallite growth rates. This approach can be implemented for long-term monitoring alterations in population abundances, distribution and corallite growth, which may vary across locations and be influenced by ocean warming and other human impacts. Predicting species distribution is imperative for marine biodiversity management and environmental conservation [37,38]. In the Canary Islands, marine communities are mainly distributed in relation to the longitudinal gradient created by the influence of the Saharian upwelling and the Canary Current [4,14]. In this context, species with warm-temperate affinities are frequently found in Lanzarote and Fuerteventura, while tropical species are more abundant in El Hierro and La Palma islands [19,39]. The results of our study demonstrated that populations of *Balanophyllia regia* followed the same pattern as warm-temperate species such as the fish *Symphodus mediterraneus* and the sea anemone *Actinia equina*, which reach their higher abundances in Lanzarote but are absent in El Hierro (Figure 7) [40,41]. Importantly, not only SST follows this longitudinal gradient, as the upwelling provides nutrients to the euphotic layer and leaves a general pattern of decreasing nutrients from the east to the west of the Archipelago [42,43]. Since Chl-a concentrations are usually a proxy of phytoplankton biomass and primary productivity [44], the influence of the upwelling in nutrient distribution might explain the results of the correlation analyses, in which values of SST and Chl-a were highly correlated.

During our surveys, we found denser populations of *B. regia* in the northern locations of Lanzarote and Tenerife, characterized by extensive rocky platforms that receive more energy from breaking waves [45]. Previous studies highlight that high ocean energy produces the movement of feeding particles and facilitates the growth of filter-feeding azooxanthellate corals [46,47]. In fact, due to its dependence on heterotrophic nutrient availability, corallites of *B. regia* display a slighter and smaller size than its zooxanthellae sister species, *B. europea* [48]. The combination of cold environments and higher food availability, due to the upwelling and wave action, might be enhancing the development of *B. regia* populations in Lanzarote and Tenerife, as this is a warm-temperate species whose source of energy comes from heterotrophic feeding [23].

The large and wide rocky platforms in the intertidal habitats of Lanzarote and Tenerife, compared to their smaller area extensions in El Hierro, provide more substrate available for the settlement and development of intertidal organisms [49,50]. These differences are related to the origin of the Canary Islands, which also follows an east-to-west gradient, with the eastern and central islands being the oldest and then the most eroded of the Archipelago [51,52]. This explains the high correlation between the rocky platform area and the SST and Chl-a. Although our results did not find a direct effect of platform area owing to model variable exclusion due to correlation, a combination of environmental processes acting at island (wave energy and platform area) and at regional (SST and Chl-a) scales, with varying substrate availability, may be playing an important role in the distribution and abundance of *B. regia* across the Canary Islands. Beyond the influence of large-scale environmental factors, future studies should address the role of micro-alterations in the environment at local scales, such as those within different tide pools during low tide. Additionally, examining biotic interactions between *B. regia* and other intertidal organisms is crucial, as these alterations might regulate their populations on large rocky platforms. For example, sea urchins have been shown to facilitate the survival of *B. elegans* by controlling macroalgae growth [53].

Not only changes in marine species distributions but also alterations in their growth rates can provide useful insights into the health status of their populations. In our experiment, *B. regia* grew at a rate of $2.05 \pm 4.48 \mu\text{m}$ per day on average, with the polyps of larger size the ones that grew the fastest ($3.39 \pm 3.55 \mu\text{m}$). Although a high deviation of polyp sizes can be seen in our estimates of growth, this could be due to our experimental design, which intentionally included a wide range of polyp sizes (from 1.5 mm up to 10 mm). The growth rates obtained are slightly lower and contrary to previous results from a short-term experiment, in which *B. regia* juveniles grew faster than adults ($3.5 \pm 1.8 \mu\text{m}$ and $3.0 \pm 1.3 \mu\text{m}$ per day, respectively) [28]. However, in the experiment by Brahmi et al. [28], the specimens were kept at temperatures of 12–16 °C. Considering the temperate affinities of *B. regia*, the higher temperatures in our experiment (~20.5 °C) might have hindered corallite growth, which could be more detrimental in its early stages of life. Future studies should focus on specimens in the natural environment and consider the effects of different environmental parameters to estimate the growth rates of this species more reliably. However, monitoring *B. regia* corallite growth in situ can be very challenging. Its cryptic behaviors, i.e., that it is mainly found in holes and crevices on tide-pools, and its small size complicate access with cameras or scales to take appropriate measurements and, therefore, we needed a different approach.

Our results showed that calcein tagging is a reliable methodology to study growth rates of *B. regia*, since all the specimens survived and showed high percentages of tagging success (89%). Furthermore, they maintained a healthy appearance after 5 months of tagging, with open polyps and active tentacles that reacted to the presence of food. Most polyps showed a diffuse mark that increased in intensity with exposure times to the fluorochrome (6, 12, and 24 h), while no effect of calcein concentration was found (20 vs. 50 mg/L). A concentration of 100 μM of calcein (62 mg/L) for 2 h was used to tag primary polyps of *Acropora digitifera* and to study initial skeletal growth in live tissues [26], while 3 h at 50 μM of calcein (31 mg/L) was enough to label corallites of *B. regia* in a previous study [28]. However, it has been found that high concentrations of calcein may compromise the survival of early stages of coral species [26]. In this sense, the results of this study greatly reduced the concentration of calcein needed (20 mg/L) and demonstrated that it is suitable for tagging specimens for long-term studies.

In agreement with previous studies [28], fluorescence increased with exposure time to the fluorochrome, so that almost 40% of the corallites showed a faint or bold mark after 12 h, in contrast to 5% after 6 h. However, the exposure times required greatly limit the applicability of this technique for in situ intertidal tagging *B. regia* populations in the Canary Islands, a region that is under a semidiurnal tidal regimen to maximum equinoctial spring tides. In these conditions, certain tide pools may be isolated for approximately 6 h. Although ~70% of the corallites showed a diffuse fluorescence at 20 mg/L after 6 h of soaking time, this could be an adequate methodology since mark intensity was evaluated in the whole corallite. To improve detectability, histological analyses could be performed only when fluorescence is first detected in raw samples. Furthermore, these results were irrespective of polyp size, despite juveniles usually allocating most of the metabolic energy to growth [28,48], while adults spend more energy in reproduction [54]. Therefore, we believe that the methodology proposed here could be applied to accurately assess growth rates of *B. regia* under contrasting conditions by direct labeling in tide pools since it has already been proven to be harmless for different kinds of calcareous organisms, such as mollusks [55,56], echinoderms [57,58] and corals [26,59].

Long-term monitoring programs are very important for detecting trends in marine communities, improving the interpretation of many ecological processes that usually act at a broad scale [60]. Programs involving intertidal organisms are especially relevant, since coastal acidification and temperature are more dynamic than in open oceans, due to the influence of freshwater runoff, tides, eutrophication and other human disturbances [61,62]. However, funding agencies are often unwilling to support long-term projects, as they require a large amount of financing. For these reasons, it is necessary to optimize techniques

to assess the effects of anthropogenic disturbances on marine ecosystems and populations over long timeframes and at low cost. *B. regia* has restricted mobility and showed small-sized corallites, which were easy to identify by their bright orange or yellow color, and its azooxanthellate condition that facilitates the interpretation of alterations in the environmental parameters [63]. For these reasons, we established a baseline on the distribution and abundance of *B. regia* in the Canary Islands and propose long-term monitoring of its populations and growth rates through calcein tagging, in order to detect alterations that might indicate ecosystem degradation [64–66]. An early warning of alterations in corallites growth rates and intertidal population abundances responding to environmental changes could help managers to focus future investments in specific locations with higher ecological and economic relevance, since local management can greatly enhance the resilience of marine ecosystems.

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