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Assessing the expansion and success of a restored population of *Gongolaria barbata* (Stackhouse) Kuntze (Fucales, Phaeophyceae) using high-precision positioning tools and size distribution frequencies

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Abstract

Ongoing human pressures over recent decades have caused the loss and ensuing impoverishment of the complexity and diversity of Mediterranean habitats dominated by algal species of the order Fucales. *Gongolaria barbata*, a habitat-forming Fucales species that has disappeared in several places across the Mediterranean, was reintroduced in a cove (Cala Teulera, Maó Bay, Menorca) where it was known to have been locally extinct for more than 40 years. Reintroduction was performed in 2011 using innovative non-destructive techniques. Here we describe the expansion patterns of the population ten years after its reintroduction, and we look at the size-structure distribution of the restored population compared to one of the only natural populations known in Spain (Fornells Bay, Menorca). Newly settled individuals exhibit a progressive dispersal pattern from restoration sites, favouring rocky substrates at the shallowest level parallel to the shore. The area occupied by *G. barbata* has increased by almost three orders of magnitude in ten years, from approximately 3.6 m^2 of the initially restored area to a current restored area of 2093 m^2. Recruits and juveniles dominate the restored population but some individuals have reached large size classes, the overall size distribution resembling the reference population. The high-resolution cartography included in our study enables an accurate mid to long-term assessment of the expansion of *G. barbata*. Incorporating such tools in restored marine forests would facilitate the implementation of efficient management policies that will help reinforce their conservation.

Keywords: Cartography; *Cystoseira sensu lato*; Fucales; marine forests; monitoring; rocky infralittoral.

Introduction

Mediterranean marine macroalgal forests dominated by *Cystoseira sensu lato* species (including *Ericaria* Stackhouse, 1809, *Gongolaria* Boehmer, 1760, and *Cystoseira* C. Agardh, 1820; see Orellana *et al*., 2019 and Molinari-Novoa & Guiry, 2020) constitute structurally complex habitats in the infralittoral and upper circalittoral zones. Members of *Cystoseira s. l.* are actively involved in coastal primary production (Ballesteros, 1989, 1992; Sales & Ballesteros, 2012) and provide food, shelter, and nursery areas for a wide variety of associated organisms (i.e. algae, invertebrates, and fishes) (Pinnegar & Polunin, 2000; Chemello & Milazzo, 2002; Cheminée *et al*., 2013; Piazzi *et al*., 2018). Moreover, they are highly sensitive to environmental changes, and thus their presence and relative abundance are used as indicators of ecological status in Mediterranean coastal water bodies (Arévalo *et al*., 2007; Ballesteros *et al*., 2007; Pinedo *et al*., 2007; Sales & Ballesteros, 2009; Sfriso & Facca, 2011; Blanfuné *et al*., 2016; Badreddine *et al*., 2018; de la Fuente *et al*., 2018; Bahbah *et al*., 2021).

Cystoseira s. l. forests are amongst the most representative, diverse, and structurally complex Mediterranean habitats (Rodríguez-Prieto *et al*., 2013). Nevertheless, in recent decades, these forests have experienced an anthropogenic-related decline across the basin through water pollution and direct habitat destruction (Thibaut *et al*., 2005; Mangialajo *et al*., 2008; Sales & Ballesteros, 2009; Perkol-Finkel & Airoldi, 2010; Thibaut *et al*., 2015; Mancuso *et al*., 2018; Mariani *et al*., 2019), being replaced by less complex and low-diversity habitats.

Gongolaria barbata (Stackhouse) Kuntze [formerly known as *Cystoseira barbata* (Stackhouse) C. Agardh; Molinari-Novoa & Guiry, 2020], an engineering species growing in shallow and sheltered environments, has seen its already limited distribution in the Mediterranean reduced over time (Orfanidis *et al*., 2021). This macroalga is currently considered as threatened by the Barcelona Convention (Annex II; UNEP/MAP, 2013). Its distribution in westernmost Mediterranean coastal waters is extremely reduced (Gómez-Garreta *et al*., 2000), and thus preserving or enhancing the viability of the current populations deserves prioritization.

On the Spanish coast, until recently, *G. barbata* populations only thrived in Fornells Bay (NW Mediterranean, Spain) (Sales & Ballesteros, 2009), yet there is historical evidence that they were also once present in Cala Teulera (Maó Harbour, Menorca, NW Mediterranean) (Rodríguez-Femenías, 1889). During the 20th century, increasing pollution levels coming from the continuous dumping of sewage in Maó Harbour (del Hoyo-Bernat, 1981) led to the local extinction of *G. barbata* in Cala Teulera (Sales *et al*., 2011). Regrettably, despite subsequent improvement in seawater quality, the population never recovered naturally (Sales *et al*., 2011), probably hindered by the low dispersal capacity of *Cystoseira s. l.* zygotes (Clayton, 1992; Sales, 2010). These traits and circumstances made *G. barbata* a suitable target for taking active policies to promote its recovery at Cala Teulera. Within this context, a reintroduction project was started in 2011 using novel non-destructive restoration methods based on recruitment-enhancement techniques (culturing small portions of fertile branchlets from wild individuals and letting the dispersal process take its natural course), and using the Fornells Bay population as the zygote donor population (Verdura *et al*., 2018).

Once the species was successfully reintroduced, the subsequent monitoring of the restored populations became crucial for assessing its progress under the recovered environmental conditions. Developing a high-resolution map, in particular, could help to accomplish this objective. Despite the proven utility of cartography for visualizing habitat destruction and implementing conservation actions (Thibaut *et al*., 2005, 2015; Mariani *et al*., 2014, 2019), no mapping initiatives have been developed in restored marine populations. Using fine-scale cartography in post-restoration monitoring programmes could provide accurate data on how the newly settled population evolves in space and time. Here, we apply high-spatial-resolution cartography to the restored *G. barbata* population and accompanying species from Cala Teulera over a time frame of ten years in order to assess *G. barbata*'s expansion. We also determine the size-structure distribution of the individuals in the restored population and compare it with a natural reference population in order to assess whether they show a similar pattern.

Materials and Methods

Study site

The study was conducted in two locations in Menorca (Balearic Islands, NW Mediterranean, Spain), Cala Teulera and Fornells Bay (Fig. 1). Cala Teulera is a sheltered, non-urbanized cove located within Maó Harbour, on the east coast of the island. In 2011, a restoration experiment involving *G. barbata* recruits obtained using non-destructive techniques (Verdura *et al*., 2018) was started at two different sites in Cala Teulera (RS1, RS2; Fig. 1A). Since then, several individuals settled on rocks scattered

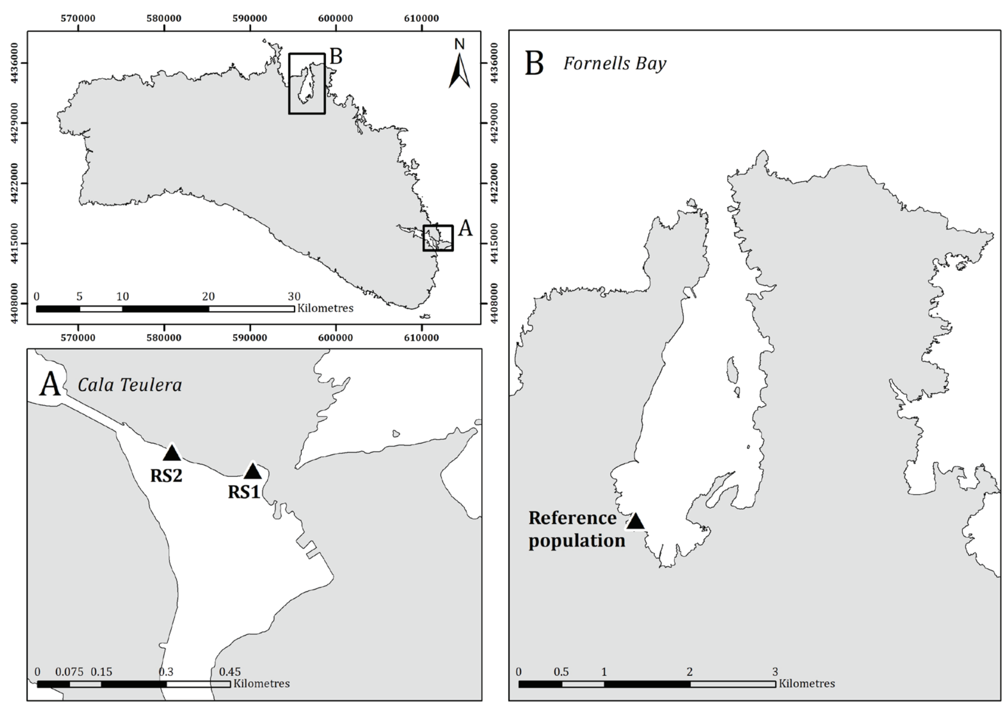

Fig. 1: A) Location of the *Gongolaria barbata* restoration sites in Cala Teulera (RS1, RS2) and B) the reference population in Fornells Bay.

at a depth of up to one meter in an area consisting predominantly of cobble and seagrass [*Cymodocea nodosa* (Ucria) Ascherson] meadow (Verdura *et al*., 2018).

Fornells Bay is a well-preserved area within the North Menorca Marine Reserve and the only locality with important populations of *G. barbata* on the Spanish coast (Gómez-Garreta *et al*., 2000; Sales & Ballesteros, 2009). The reference population was located close to the southernmost part of the bay (Fig. 1B), at the same depths as the Cala Teulera restoration was carried out. Here, *G. barbata* grows together with the fucoids *Cystoseira foeniculacea* (Linnaeus) Greville f. *tenuiramosa* (Ercegovic) Gómez-Garreta *et al*., *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, and *Cystoseira pustulata* (Ercegovic) Neiva & Serrao. The rest of the cove is covered predominantly by a *C. nodosa* habitat and a photophilic algal bed, similar conditions to those of Cala Teulera (see Results section).

Sampling was carried out during June 2021, coinciding with the beginning of *G. barbata*'s vegetative resting period (Falace & Bressan, 2006), when the perennial cauloid has reached a steady, annual maximum height and has still not surpassed its top temperature tolerance, which could limit its survival (Orfanidis, 1991).

Cartography and description of the **Gongolaria barbata** *restoration zone*

Topography in Cala Teulera

A topographic survey was performed in order to produce an exhaustive map of the *G. barbata* restoration zone. A total area of 2276 m^2 was delimited based on the current range of the restored population (Fig. 2). Topographical survey measurements were carried out using a Leica RTK GNSS DGPS (Differential GPS) SmartRover that ensured high-precision positioning results (± 0.03 m in position or height). Coordinates (latitude, longitude) were collected at every single meter along the perimeter. In addition, 64 reference points were also recorded: 9 geographical landmarks located parallel to the coastline and 55 underwater points previously marked with tagged pegs to provide a spatial reference in the field (Fig. 2). Lastly, habitat boundaries were determined with the corresponding orthophoto (SITIBSA; *Ortofoto*, 2500, 2015 *de les Illes Balears*, ETRS89) serving as a guide (Fig. 2). The resulting dataset was georeferenced in a Geographic Information System (GIS) for future use.

Spatial distribution and habitat characterisation

The phytobenthic communities of Cala Teulera were characterised along linear transects, placed parallel and equidistant every 3 m throughout the defined area (Fig. 3). To estimate the abundance of *G. barbata* and other dominant species, 50 x 50 cm quadrats (n = 1353) subdivided into 10 x 10 cm subquadrats were positioned consecutively along each transect, and the number of 10 x 10 cm subquadrats occupied by every macroalgal and seagrass species was recorded. The number of subquadrats per quadrat where a particular species was present was used as a measure of abundance, which was finally expressed as a relative percentage (% of subquadrats where the species was present relative to the total number of subquadrats sampled) (Sala & Ballesteros, 1997; Sant *et al*., 2017; Teixidó *et al*., 2018). Sampling was differentiated into three areas (RS1, RS2 and the expansion area; see Cartography Results section).

Simultaneously, the total abundance of *G. barbata* individuals composing the restored population was determined. To do this, each individual sighted within the interval between transects and up to the surveyed outer boundary of the study area was counted individually (Fig. 3).

Fig. 2: Topographical survey of the study area corresponding to the restored population in 2021. The map also shows the location of the repopulation experiments (RS1, RS2) carried out during 2011. Each black tree symbol indicates the location of six 0.05 m^2 stones that contained recently settled individuals.

Fig. 3: Abundance sampling transects and subdivisions used to facilitate the IDW interpolation.

Spatial analysis

The cartographic representation of the restoration zone was carried out using spatial analysis tools. Prior to mapping, every transect was divided into 5-meter segments (Fig. 3). Each segment was considered as a single value and represented on the georeferenced map as a point, where the respective mean abundance values (as abundance percentage per 1 m^2) were introduced. The resulting abundance dataset was plotted (illustrated as a polygon) using the Inverse Distance Weighting (IDW) interpolation method and contour lines. Distances and different surface areas were measured using proximity and geometric calculation tools, respectively. All data digitalization and analyses were performed in ArcMap 10.4.1 (Esri ArcGIS Engine).

Restored* vs. *reference populations

The size structure of both the restored and reference populations was determined by measuring the length of the main axis (Ballesteros *et al*., 1998, 2009) of a minimum of 100 individuals. This was carried out at each area from Cala Teulera (RS1, RS2 and expansion area) and compared with the reference population from Fornells Bay. In all cases, size-structure distributions were represented by 2-cm size classes, from 0 to 28 cm.

Statistical analysis

The statistical significance of the size structure of the restored *G. barbata* population (as a whole) in comparison to the reference population was assessed. First, the dataset was tested for normality and homogeneity of variance using the Kolmogorov-Smirnov (KS) and Bartlett tests, respectively. As the transformations were insufficient to satisfy normality, a non-parametric Welch's t-test was performed. All statistical analyses were performed with R software (R Core Team, 2021).

Results

Habitat characterisation

A total of 19 macroalgal and seagrass species were identified during the surveys (Table 1). The main habitat consisted of a dense meadow of the seagrass *C. nodosa* thriving on soft bottom (61.4%), with scattered individuals of the seagrass *Zostera noltii* Hornemann. The hard substrate encompassed a photophilic algae habitat–devoid of fucoids–composed of 13 species, and a *Cystoseira s. l.* habitat that contained four species: *G. barbata*, *C. compressa*, *C. pustulata* and *C. foeniculacea* f. *tenuiramosa*, with the species complex including *C. compressa* and *C. pustulata* (difficult to distinguish in the field) the most abundant (Table 1). With regards to the photophilic algal assemblage, *Padina pavonica* (Linnaeus) Thivy was the most abundant alga throughout the study area (19.8%). Furthermore, other brown algae such as *Dictyota* spp. [encompassing *D. mediterranea* (Schiffner) G. Furnari, *D. implexa* (Desfontaines) J.V. Lamouroux and possibly other taxa], *Halopteris scoparia* (Linnaeus) Sauvageau, and green algae such as *Cladophora* cf. *coelothrix* Kützing and *Ulva prolifera* O.F. Müller, were also common (see Table 1).

Cartography of the* Gongolaria barbata *restoration zone

Ten years after restoration, the *G. barbata* population covered an area of 2093 m^2 along 235 m of shoreline, exhibiting a dispersal pattern defined by the prevalence of shallower areas with greater rocky substrate availability. The new population was composed of a total of 1821 individuals of the restored species. On average, *G. barbata* abundance represented 1.4% of the surveyed area (Table 1) but individuals were concentrated heterogeneously throughout the study area (Fig. 4).

The cartography clearly depicted two areas with higher abundance values, corresponding to the initial resto-

Table 1. Macrophyte species abundances (%) resulting from the surveys in Cala Teulera.

Species	Abundance (%)
Cymodocea nodosa (Ucria) Ascherson	61.4
Padina pavonica (Linnaeus) Thivy	19.8
Cladophora cf. *coelothrix* Kützing	7.4
Dictyota spp.	7.2
Cystoseira compressa (Esper) Gerloff & Nizamuddin + *Cystoseira pustulata* (Ercegovic) Neiva & Serrao	6.9
Halopteris scoparia (Linnaeus) Sauvageau	4.6
Ulva prolifera O. F. Müller	2.5
Sphacelaria cirrosa (Roth) C. Agardh	1.6
Acetabularia acetabulum (Linnaeus) P. C. Silva	1.4
Gongolaria barbata (Stackhouse) Kuntze	1.4
Amphiroa rigida J. V. Lamouroux	1.2
Haliptilon virgatum (Zanardini) Garbary & H. W. Johansen	1.1
Cystoseira foeniculacea (Linnaeus) Greville f. *tenuiramosa* (Ercegovic) Gómez-Garreta *et al*.	0.6
Zostera noltii Hornemann	0.6
Caulerpa cylindracea Sonder	0.4
Dasycladus vermicularis (Scopoli) Kraser	0.3
Symploca hydnoides Kützing ex Gomont	0.1
Ulva compressa Linnaeus	0.1

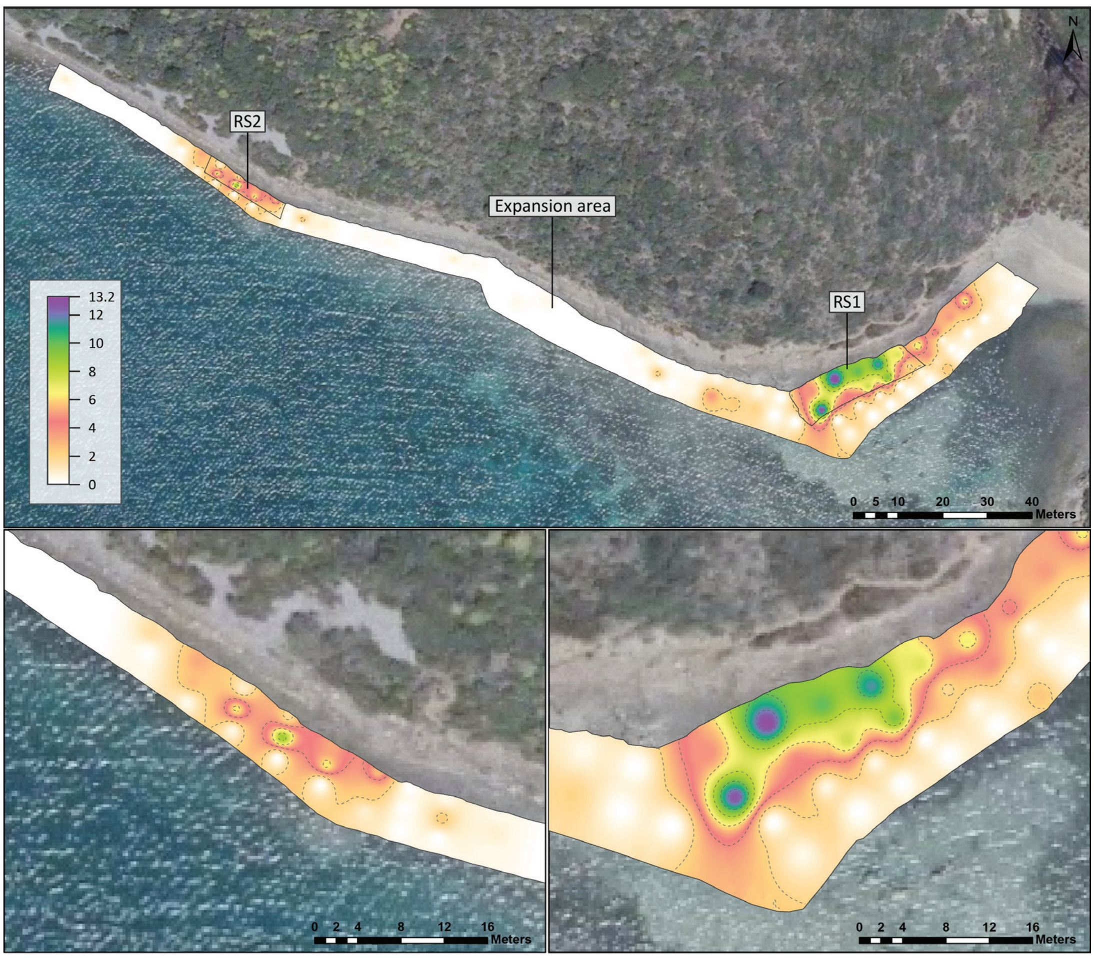

Fig. 4: Abundance (%) of the *Gongolaria barbata* restored population throughout the study area of Cala Teulera, showing restoration sites 1 (RS1; bottom right) and 2 (RS2; bottom left) in greater detail. Colours indicate % abundance according to the scale included in the upper part of the figure. Contour lines delimit the abundance at 2% intervals.

ration sites RS1 (east) and RS2 (west), which gradually decreased at greater distances, and decreased more acutely with depth. It also showed differences in the abundance rate and breadth of the two sites. RS1 covered an area of 217 m^2, reaching a maximum abundance value of 13.2% and a minimum of 1.8%, even though it did not drop below 6% in approximately two-thirds of its area. Conversely, RS2, with a steeper bathymetry, occupied less than a third of the area of RS1 (70 m^2). Moreover, its abundance ranged between 8.8% and 0.8% and had a sharper descent in comparison to the smoother decline of RS1.

The map also shows the *G. barbata* population extending beyond these restoration sites, occupying a remarkably large area (1806 m^2), yet with much lower abundance, described here as the expansion area. Although expansion occurs in all directions around the two initial restoration sites, the settlement of new individuals is higher in the area closest to RS1, with maximum abundance values of 6%. In general, however, *G. barbata* appears very infrequently in most of the expansion area (<2%) compared to both restoration sites.

Restored* vs. *reference populations

The mean size structure of the entire restored population resembled that of the reference population (Welch t-test, $t_{1484.18} = -0.32485$, P = 0.7454). Furthermore, when the restoration (RS1, RS2) and expansion area datasets were analysed, in 2-cm size classes, alongside the natural population, most size classes of *G. barbata* were observed in all four distributions, with recruits (size class 0-2 cm) and small-sized individuals (2-8 cm) predominating (Fig. 5). Despite the similarities, distributions displayed slight yet notable differences: the expansion area showed the highest percentage of recruits (22%), while RS2 contained individuals that reached sizes of up to 27 cm, the highest recorded in our surveys.

Discussion

Continuous monitoring of a population after a restoration initiative is essential to assessing its success. Unfortunately, studies on the expansion of marine restored populations are scarce as such projects often lack long-term commitment (Borja *et al*., 2010; Cebrian *et al*., 2021). Monitoring of re-established structuring species usually covers only the early stages (Borja *et al*., 2010; Cebrian *et al*., 2021) and does not last longer than two years (Bayraktarov *et al*., 2016), which may be insufficient to confirm the full recovery of structural and ecological functionality. The cartographic methodology employed here is time-consuming and limited in space and time, but it provides robust and accurate mapping of expansion patterns.

In Cala Teulera, *G. barbata* now thrives in an environment of scattered rocks amongst a *C. nodosa* seagrass meadow. According to the benthic algal list obtained in the surveys (Table 1), most rocky habitats from Cala Teulera belong to the category “Sheltered infralittoral rock, well illuminated, without Fucales, with *Padina pavonica*” (code 0301030701), which after the restoration shifted to the habitat “Extremely sheltered infralittoral rock, well illuminated with *Gongolaria barbata* and *Cystoseira foeniculacea* f. *tenuiramosa*” (code 0301030601) (habitats have been assigned according to the *Spanish Inventory of Marine Habitats and Species* hierarchical classification by Templado *et al*., 2012). *G. barbata* presented high abundances at both restoration sites, hence proving the effectiveness of the restoration methodology used (Ver-

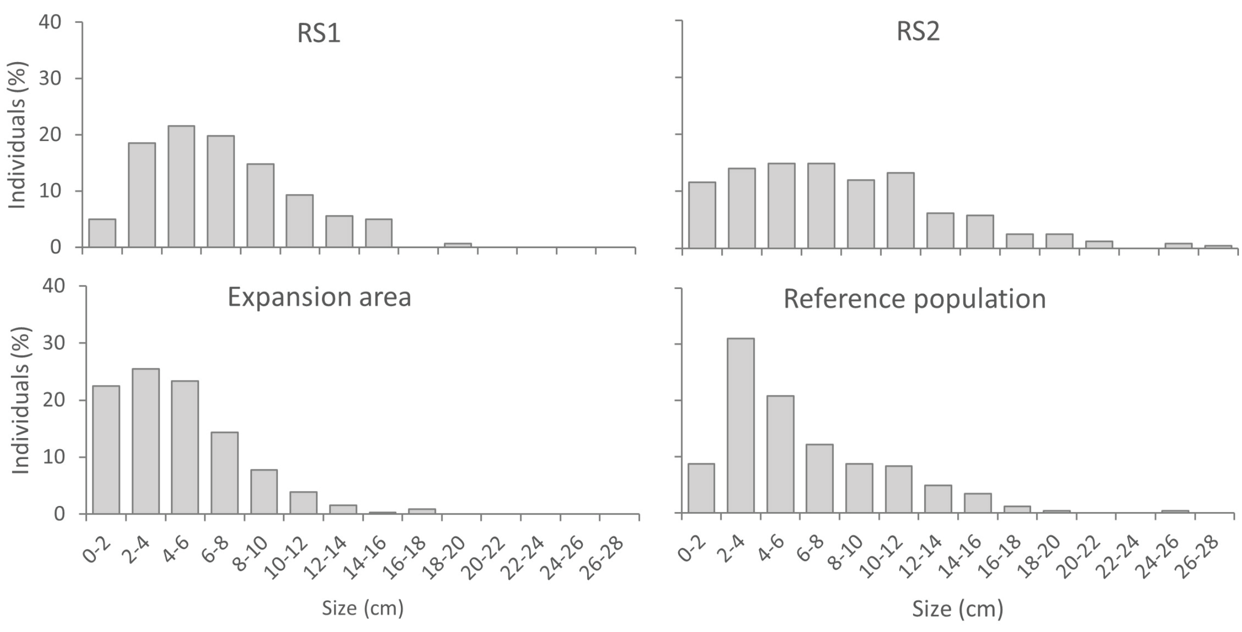

Fig. 5: Size structure (cm) of *Gongolaria barbata* individuals (%) in restoration site 1 (RS1; n = 162), restoration site 2 (RS2; n = 242) and expansion area (n = 334) from Cala Teulera, as well as the reference population from Fornells Bay (n = 264).

dura *et al*., 2018). The success and perseverance of the restored population were probably maximised by the similarity in the geomorphology (sheltered area, shallow depth and rocky substrate) and other environmental characteristics, such as similar temperatures, suitable for the persistence of *G. barbata*, between the restored and donor sites (Orfanidis, 1991). Despite the limited dispersal ability of *Cystoseira s. l.* zygotes (Clayton, 1992; Schiel & Foster, 2006; Verdura *et al*., 2018), the restored population increased its area by three orders of magnitude in 10 years, from the approximate original area of 3.6 m^2 to the current 2093 m^2. As expected, in terms of abundance, the population is mostly concentrated in restored sites, declining gradually with distance from these points. *G. barbata* individuals propagate parallel to the shoreline, with a preference for shallower areas with greater rocky substrate availability. The patchy distribution of *G. barbata* individuals reflects the patchy distribution of the isolated rocks and stones spread over the *C. nodosa* bed.

The cartography revealed that individuals spread widely in the RS1 site, which could be related to the more suitable environmental conditions for the species: greater habitat availability due to the presence of an extensive shallow, rocky area as opposed to the narrow rocky strip that characterises RS2 (Fig. 4). This would ease recruit settlement and subsequent growth.

G. barbata managed to coexist with the three other fucoids present in both restoration areas, but with some differences: RS1 displayed a higher abundance of *Cystoseira s. l.* species in comparison to RS2. This supports our previous conclusions about the better establishment of individuals at RS1 due to more ideal environmental conditions for *G. barbata*. Nonetheless, the cartography revealed that each member of the Fucales has its own microhabitat preferences (field observations by the authors). The ability of restored individuals to propagate in a linear and continuous pattern in contrast to the patchy distribution of *C. compressa* and *C. pustulata* would make *G. barbata* a better competitor, in accordance with observations by Mangialajo *et al*. (2012). The species *C. foeniculacea* f. *tenuiramosa* was more abundant in deeper areas. Such small-scale distribution patterns may help to reduce interspecific competition (Ballesteros & Sant, 2022).

Population size structure was dominated by recruits and small individuals in all surveyed areas; in most sites (Fornells Bay, RS1, Expansion area), however, even if small individuals were abundant, the size distribution did not follow a negative exponential function as would be expected for populations at equilibrium (e.g., Lorimer, 1980; Berg & Hamrick, 1994). Negative exponential distributions have been found in several populations of fucoids (Cousens, 1985; Russell, 1990; Fernández *et al*., 1990; Ang, 1991), but not in others (e.g., Ballesteros *et al*., 1998, 2009; Medrano *et al*., 2020b, c). In fact, some authors have detected a high variability of distribution sizes at small geographical scales (e.g., Hereu *et al*., 2008; Navarro *et al*., 2011), suggesting the interplay of different environmental factors in shaping these distributions (Ballesteros *et al*., 1998, 2009; Hereu *et al*., 2008; Navarro *et al*., 2011). The expanded population of *G. barbata* only showed a negative exponential size distribution immediately after the beginning of the restoration project, becoming log normal after five years (Verdura *et al*., 2018). No major differences in size distribution patterns are evident after ten years, with the exception of the presence of large-sized individuals in both the restored populations and in the expansion area, with length axes similar to those of the natural, reference population. Thus, we can assert that the size distribution of the new populations is similar to the reference population (Fig. 5).

Our study confirms the medium-term success of a restoration project of a species of the order Fucales (with the restored population now on a par with a natural population), and, in particular, ten years into the restoration project, it highlights *G. barbata's* great capacity for expansion. It also emphasises the utility of the regular monitoring of this population, supporting its continued implementation.

By mapping the restored forest of Cala Teulera, we have provided a powerful tool for its monitoring. The high-resolution cartography used enhances the accuracy of data on *G. barbata* expansion dynamics in space and time by reducing the bias from data-interpolation. Marine forest restoration projects are a growing field (e.g., de la Fuente *et al*., 2019; Tamburello *et al*., 2019; Medrano *et al*., 2020a; Orlando-Bonaca *et al*., 2021; Savonitto *et al*., 2021), but suffer from a lack of long-term monitoring. An accurate spatial assessment of the evolving restored population and the size-frequency distribution of individuals over time may help in understanding the population performance in the mid to long-term and in the implementation of further restoration actions. For logistical reasons, however, this methodology is only workable for reintroduced species growing in shallow and sheltered habitats, as its implementation would be very costly in areas where diving was necessary. Nevertheless, where feasible, this approach may prove to be a useful tool for improving and implementing appropriate management actions, and thus maximising the success of restoration projects.

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