Stranded seaweeds (Gongolaria barbata): an opportunity for macroalgal forest restoration

(Article begins on next page)

Stranded seaweeds (*Gongolaria barbata*): an opportunity for macroalgal forest restoration

Running head: New protocol for *Gongolaria barbata* restoration

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Abstract

Macroalgal forests play a crucial ecological role, providing important ecosystem services, but are also among the most vulnerable marine habitats. In the Mediterranean Sea the forests of *Cystoseira sensu lato* (*s.l.*) are undergoing a drastic decline due to the presence of multiple stressors and, among these species, *Gongolaria barbata* is one of the most threatened. Despite the various attempts to restore these macroalgal forests, the success of the interventions is limited by the availability of fertile apices to promote zygotes release and the embryo development for subsequent replacement *in situ*. Here we propose a new approach based on the use of *G. barbata* stranded on the beach, for the restoration of these marine habitats. We developed a protocol based on the collection of stranded macroalgae to collect their fertile apices and produce healthy zygotes and embryos, whose recruits can be then returned at sea (through *ad hoc* hard substrates). We show that 3 months of incubation in mesocosms allow the recruits to reach an average length of 1 – 2 mm with an average density of 50 – 80 recruits per tile. We demonstrate that these recruits can survive and grow vigorously both in mesocosms, and after outplanted at sea. The protocol presented here has the double advantage: a) obtaining recruits without impacting the natural populations, and b) providing to a second life to macroalgal fragments (through ecological restoration) that would be otherwise lost.

Key-words: macroalgal restoration; *ex situ* cultivation; *Gongolaria barbata*; *Cystoseira s.l.*; Mediterranean Sea.

Implications for Practice and Technique

- A new protocol is described for the restoration of the seaweeds (*Gongolaria barbata*), having the advantage of obtaining recruits from stranded individuals without any damage to the natural populations.
- The protocol exploits stranded fertile adults which produce healthy recruits, thus representing a potential source of fertile apices. Stranded adults could be thus used in restoration actions instead of being lost.
- The results obtained applying this protocol, in terms of new recruits survival % and growth, are similar to those obtained with other approaches known for this species.

Introduction

Cystoseira sensu lato (*s.l.*) (Phaeophyceae, Fucales) species, including the genera *Ericaria*, *Gongolaria* and *Cystoseira*, are foundation species playing a key role as habitat-formers on the Mediterranean rocky bottoms (Orlando-Bonaca et al. 2021). They ensure high primary production, food sustaining grazers, and nurseries for a large variety of species (Chiarore et al. 2019; Bianchelli & Danovaro 2020). Moreover, they can support high biodiversity levels, due to their three-dimensional structure providing a relevant "ecological volume" and contributing to ecosystem services (Thibaut et al. 2016).

In the last decades, the decline of *Cystoseira s.l.* populations was documented along many Mediterranean coasts (Thibaut et al. 2015; Iveša et al. 2016), due to multiple natural and anthropogenic stressors (e.g., habitat destruction, pollution, overgrazing, alien species; Sales & Ballesteros 2009; Marletta & Lombardo 2020), exacerbated by the effects of climate change, including marine heatwaves (Lejeusne et al. 2010; Bevilacqua et al. 2019; Garrabou et al. 2022).

A dramatic loss of fucoid species was reported also in the Adriatic Sea (Rindi et al. 2020), with their replacement with less-structured turf-forming algae, preventing in turn canopy-forming-species recruitment (Orlando-Bonaca et al. 2021). This phenomenon was widely documented along the Conero Riviera (North-Eastern Adriatic Sea) for four fucoid species (*Fucus virsoides*, *Cystoseira foeniculacea*, *Cystoseira humilis*, *Sargassum acinarium*). The main driver of this forest loss is the human impact on coastlines, increasing sediment resuspension, high nutrient concentrations and loads (Rindi et al. 2020). As a result, only *Cystoseira compressa* and *Gongolaria barbata* are still present, even though the latter species is in regression, due to their greater tolerance to environmental stress (Orlando-Bonaca et al. 2022).

In the last years, the possibility of restoring these macroalgal forests (e.g., *G. barbata*) was explored in the Adriatic Sea (Savonitto et al. 2021; Orlando-Bonaca et al. 2022). The restoration of fucalean forests is carried out either using *in situ* and *ex situ* (cultivated in mesocosms) recruitment approaches or using hybrid methods (combining cultivation in mesocosm and culture in the natural environment, also coupled with passive restoration measures (Verdura et al. 2018; Orlando-Bonaca et al. 2022; Bianchelli et al. 2023a; 2023b).

Nevertheless, climate change and thermal anomalies might jeopardise conservation and restoration efforts, compromising the future viability of brown algae of *Cystoseira s.l.* species in the Mediterranean (Iveša 2019; Orlando-Bonaca et al. 2021). Recent studies highlighted that variability in the magnitude of marine heat waves can lead to local extinctions of already fragmented populations in the Mediterranean Sea (Verdura et al. 2021). Yet, the increasing storm frequency and intensity promote the detaching of macroalgae from substrates and the stranding on the beach (Pisano et al. 2020). The Adriatic Sea is particularly exposed to storm surges due to the scirocco winds, which produce the largest wave heights (Pomaro et al. 2017), thus fostering the fragmentation or local extinction of Adriatic populations.

Moreover, seawater warming can alter physiology and phenology of *G. barbata*, impairing reproductive timing and recruitment (Eggert 2012; Bevilacqua et al. 2019). Altogether, these factors might have a negative synergistic effect (Bevilacqua et al. 2019; Garrabou et al. 2022), compromising

G. barbata zygote availability, and reducing its reproductive potential (Savonitto et al. 2021). Therefore, the interactions of these factors might become limiting for the success of a restoration intervention (Verdura et al. 2018).

Recently, the use of plants fragments naturally detached from the meadows after storms has been suggested as a valid non-destructive method for seagrass restoration, not only to limit the impact on natural meadows, but also for the low probability of flowering of Mediterranean seagrass species (Ferretto et al. 2021). However, to our best knowledge, the implementation of detached fertile fragments from fucoid species in restoration interventions has never been reported.

Here we explored the potential use of *G. barbata* stranded along the shores after a storm, for the restoration of this species. The aim of this study was to assess the efficacy of an approach based on the collection of fertile stranded specimens of *G. barbata*, otherwise lost from the marine habitats, to generate vital and healthy offspring than can promote the recovery of this species.

Methods

Sample collection

In April 2023, fertile thalli of *Gongolaria barbata* were found, stranded along the Sassonia beach and two locations of Fano shoreline (Lido 1 and Lido 2) (North-Eastern Adriatic Sea; Figure 1A-B). Fano is a touristic city hosting a marina and breakwaves along the shoreline. The sampling sites are subjected to rough sea weather conditions and storms usually occur from autumn to spring (Rindi et al. 2020). The presence of *G. barbata* along the Fano shore was never reported before. However, during a previous survey in the area, we detected scattered individuals both on the artificial breakwaters and on *Sabellaria* reefs in the area.

For each location, 2 thalli were collected and their sizes (length of the thalli, receptacles and aerocysts) were measured. Subsequently the thalli were brought to the laboratory and stored in 40 L tanks at the Fano Marine Centre. Three receptacles from each thallus were taken and observed at the stereomicroscope. Longitudinal and transversal sections were made through a razor blade to verify their fertility and evaluate the receptacles and gametes morphometric variables (length of the receptacles, antheridia and oogonia). The reproductive period of *G. barbata* in the Adriatic Sea generally occurs mostly in spring and early summer (Rindi et al. 2023). The fertility was then checked and assessed by counting the number of released zygotes in 3 receptacles taken from the different thalli, positioned above slides and placed within a petri with filtered seawater.

G. barbata *maintenance and reproduction*

The macroalgae were transferred to the aquarium facility at the Polytechnic University of Marche (Ancona city) and acclimatized before their cultivation in mesocosms, by slow mixing the seawater used for the re-hydration and transport and the seawater from the tanks. Three mesocosms were used, one for each sampling location. For determining the efficiency of the reproductive thalli in producing healthy offspring, 4 clay tiles were located in each tank under the 2 fertile specimens (i.e., two individuals for each sampling location) to allow the zygotes and embryos to settle. The tiles were 7 cm in diameter and 1.5 cm in thickness, with a hole at the center, to be later mounted on a steel structure to be out planted at sea.

To optimize the maintenance of algae, we used the LSS (Life Support System) consisting in 3 x 50-L mesocosms, a reserve in which there are 3 socks of 100 μm for mechanical filtration, immersed razor clams for biological filtration. Fluorescent lamps produced 260-nm (λ) UV-C rays, sterilizing the water, damaging nucleic acids and preventing microbes' proliferation. A Teco TK 500 cooler was used for maintaining the temperature. The light intensity was generated by 2 LED lamps (SilverMoon Marine 10 thousand Kelvin and SilverMoon Universal 6.5 thousand Kelvin) 40 cm away from the water head. Irradiance was measured with Photometer of the apogee Model MQ-500. Light intensity and photoperiod were selected to reflect typical seasonal conditions during the reproductive phase of *G. barbata* (Savonitto et al. 2021). The photoperiod was set to a 15:9 h light : dark cycle, and light intensities was 80-100 μmol photons m^{-2} s^{-1}. The cultivation medium was Von Stosch's enriched filtered seawater renewed every 2 weeks, with addition of germanium dioxide to prevent microalgal proliferation. Temperature, salinity, pH and light intensity were monitored over the duration of the experiments. Furthermore, for routine maintenance of the system, water loading and unloading, lights, movement pumps, cooler, any water leaks at the pipe joints were checked. To assure the sterilization of the system, once a week the socks were washed, tubs were siphoned to remove organic debris and 10% of the seawater was exchanged every week.

Two weeks after, when the recruits were visible at the stereomicroscope, their growth was assessed in term of height and density (as number of recruits per tile) for 3 months. Recruits height was measured on 15 recruits and the density as number of recruits on 4 standard areas of each tile. Data were collected using a stereomicroscope (magnification 6.4x), an Olympus TG-6 camera and were then analyzed with the software ImageJ.

Outplanting and monitoring recruits in the field'

After 3 months, the tiles were transported to La Vela location, Portonovo (43.55° N – 13.60°E, ca-58-km distant from Fano), where the historical presence of *G. barbata* was previously documented (Perkol-Finkel & Airoldi 2010). *G. barbata* current occurrence along the Conero Riviera has been recently documented (Orfanidis et al. 2021). Portonovo is nearby two Sites of Community Importance (SCI IT5230005 "Coast between Ancona and Portonovo" and IT5320006 "Portonovo and calcareous cliff"; Bianchelli et al. 2023a).

The tiles were mounted on 3 45-cm-long steel structures and fixed to the sea bottom by four screws, at about 2 m of water depth. The tiles from each of the 3 locations were placed on a single structure, about 2 cm distant one from each other within each structure. The 3 structures were approximately 5 m apart from each other.

The tiles were monitored for 4 weeks after outplanting and photographed through an Olympus TG-6 camera. To estimate the recruits density and survival % for each tile, 3 photos were analyzed. The height of recruits was measured on 5 specimens per 3 tiles (n = 15) using a ruler. The survival % of recruits was calculated as n. recruits at 4th week / n. recruits at the beginning of the outplant x 100.

Statistical analyses

Due to the non-independence among the 3 mesocosms, data on the recruits height and density from the 3 locations (Lido 1, Lido 2 and Sassonia) in the lab phase were treated only with descriptive statistic. For data collected in the field on recruits height, density and survival %, two-way ANOVA was used to test for significant effect of the factors: "time" (fixed, 2 levels: first and fourth week, corresponding to the beginning and the end of the monitoring) and "location" (fixed, 3 levels: Lido 1, Lido 2 and Sassonia). Assumptions of normality (Shapiro-Wilk) were previously checked. When significant effect of the 2 factors was observed, post-hoc analyses were carried out by Tukey test. All statistical analyses were performed through the software Jamovi 2.3 (Jamovi project, 2022).

Results

Stranded macroalgal fragments

The specimens stranded were 20 – 30 cm in length (Figure 2A). The receptacles, brought on terminal branchlets, were sickle-shaped, solitary or branched (Figure 2B-C). The single or two-in-chain aerocysts were located at the base of the branchlets (Figure 2B).

All the collected specimens were fertile. In longitudinal section, inside the receptacles the number of conceptacles were 10-15, in parallel rows (Figure 2D). In cross-section, dark and oval oogonia and pigmented and poorly-branched antheridia were, respectively, in groups on the floor and on the roof of the conceptacle (Figure 2E). The morphometric and fertility variables are summarised in Table 1.

Table 1: Morphometric variables of the thalli, receptacles, aerocysts and gametes from the different specimens.

Location	Specimen length (cm)	Receptacle length (cm)	Aerocyst length (cm)	Oogonia length (µm)	Antheridia length (µm)	Number of released zygotes
Lido 1	27.49 ± 0.27	2.87 ± 0.42	1.03 ± 0.16	121.2 ± 30.0	30.5 ± 10.2	105.00 ± 2.02
Lido 2	26.60 ± 0.22	1.82 ± 0.48	0.77 ± 0.13	116.70± 26.8	26.6 ± 12.4	91.00 ± 2.61
Sassonia	20.40 ± 0.18	1.47 ± 0.30	0.64 ± 0.08	90.0 ± 20.0	20.0 ± 9.0	78.00 ± 3.02

Recruits height and density in mesocosms

Across maintenance in mesocosms, the average heights were 2.10 ± 1.13, 2.06 ± 0.95 and 1.79 ± 0.87 mm, for recruits deriving respectively from Lido 1, Lido 2 and Sassonia. Recruits from Lido 1 and Sassonia grew progressively over the 3 months (i.e., from month 1 to 2 and from month 2 to 3), whereas those from Lido 2 grew from month 1 to month 2 (Figure 3A).

The average density was 78.3 ± 4.57, 67.5 ± 3.66 and 47.5 ± 3.51 recruits per tile, respectively for Lido 1, Lido 2 and Sassonia (Figure 3B). Recruits density decreased progressively over the 3 months (i.e., from month 1 to 2 and from month 2 to 3), for Lido 1 and Sassonia, whereas decreased form month 2 to 3 for Lido 2 (Figure 3B).

Recruits growth in the field

When recruits were out planted in the field, they measured 2.94 ± 0.34, 2.49 ± 0.27 and 2.49 ± 0.25 mm, respectively for Lido 1, Lido 2 and Sassonia. After 4 weeks in the field, the recruits continued to grow reaching 8.93 ± 0.77, 7.10 ± 1.26 and 4.38 ± 0.40 mm, respectively for Lido 1, Lido 2 and Sassonia (Figure 4A). Two-way ANOVA revealed significant effects of “time” and “location” on recruits heights ($p < 0.05$; Table 2).

Table 2: Results of ANOVA on recruits length and density.

	Factors	df	F	p
Recruit length	Time	1	7.55	0.008
	Location	2	11.58	< 0.001
	Time x Location	2	1.14	0.33
Recruit density				
	Time	1	7.55	0.018
	Location	2	10.14	0.008
	Time x Location	2	1.35	0.29

When recruits were out planted in the field, the density were 56.7 ± 5.22, 53.20 ± 5.20 and 27.1 ± 4.43 per tile, respectively for Lido 1, Lido 2 and Sassonia. After 4 weeks, the density decreased, being 26.3 ± 3.79, 35.7 ± 14.8 and 12.7 ± 5.51, respectively in the structures with recruits from Lido 1, Lido 2 and Sassonia (Figure 4B). A significant effect of "time" and "location" was observed on recruits density (ANOVA, $p < 0.05$; Table 2).

The recruits showed a high survival % in the field, with 59.0 ± 1.2 %, 54.0 ± 6.7 % and 39.0 ± 2.7 %, respectively for the structure Lido 1, for Lido 2 and Sassonia (Figure 4C). The Tukey test revealed significant lower values for Sassonia than the Lido 1 and Lido 2 ($p < 0.05$).

Discussion

Stranded individuals of *G. barbata* offer an opportunity to promote habitat restoration, producing healthy and viable recruits. Indeed, stranded individuals can be successfully rescued and maintained in the laboratory and, if their fronds bear fertile apices, they may produce vital embryos and zygotes. In particular, we report here that after three months in mesocosms, the new recruits reached an average height of 1-2 mm and an average density of 50-80 recruits on each tile used as substrate, corresponding to ca. 13-21 new recruit per 10 cm^{-2}.

The use of stranded individuals represents a new approach to obtain vital recruits for macroalgal restoration. Previous macroalgal restoration approached, indeed, were based on the collection of the on *Cystoseria s.l. in situ* or their *ex situ* reproduction (or a combination of both). The recruits growth rates (on average 0.2-0.7 mm $week^{-1}$) and densities (up to 7 recruits 10 cm^{-2}) reported here from the stranded seaweed *G. barbata* are similar to those previously reported in the Adriatic Sea and in other Mediterranean regions using the standard approaches (Verdura et al. 2018; Savonitto et al. 2021; Orlando-Bonaca et al. 2022). The same applies when we compare the data presented here with previous studies conducted in the same area, i.e. the Conero Riviera (Central-eastern Adriatic Sea; Bianchelli et al. 2023a). Although we need further experiments to ascertain the efficacy of out planting interventions over the long term and at a large spatial scale, these findings suggest that this approach could represent a promising tool for restoration purposes.

The collected stranded fragments showed a large variability in the length of receptacles, aerocysts and gametes, but also in the number of released zygotes. During the maintenance in mesocosms and in the field after the outplant, we detected a difference in the recruits growth and density depending on the sampling location. Such differences could be due to the variability among different populations or some specimens may have been exposed to desiccation for a longer time, limiting their reproductive capacity. To better understand the driver of these difference, further studies are needed to ascertain which are the factors influencing the reproductive potential of stranded individuals, which may rely on source population health and distance, maturity and fertility of the individuals, floating and stranding period, but also environmental conditions, as season or water temperature (being the gametes' release temperature-dependent).

Moreover, we don't know the exact location of the source population of individuals stranded. We can hypothesize that the individuals coming from populations facing the shore, likely colonizing the breakwaters nearby. This aspect is particularly important, since *Cystoseira s.l.* populations are often fragmented and genetically disconnected also at small spatial scales, and connected by very low migration rates, along Mediterranean coasts (Buonuomo et al. 2016; Verdura et al. 2023). In turn, this has important implications for the identification of relevant conservation and management measures: each population, indeed, should be considered as separated units with dedicated conservation and restoration efforts (Riquet et al. 2021). On the other hand, dispersal of these species could be facilitated by rafting (Riquet et al. 2021). Moreover, detachment, rafting and stranding of adults may be the effect of extreme events, such as heatwaves or storms, which are predicted to be more and more frequent in the future, due to climate change (Blanfuné et al. 2019).

The observed variability among *G. barbata* stands is consistent with previous studies, which reported two main morphologies: i) long, sickle-shaped receptacles with numerous chained aerocysts, and ii) small, oval or spindle-shaped receptacles with no or few aerocysts (Orlando-Bonaca et al. 2022). Different morphologies and reproductive success suggest the presence of a morphological plasticity of *G. barbata,* possibly driven by different ecological conditions (Orlando-Bonaca et al. 2022). Testing the reproductive potential and recruits performance from different *G. barbata* populations could be suggested as a complementary action (Cebrian et al. 2021) to enhance the success of *G. barbata* restoration.

This study shows the feasibility of using fertile thalli stranded on the beach to obtain recruits for restoration, without damaging natural populations and habitats (Ferretto et al. 2021). In this regard, also stranded stems of *Posidonia oceanica* are used for its restoration (Piazzi et al. 2021). This approach has the double advantage of obtained recruits without any damage to the natural populations, and promoting a sort of "circular economy" of the re-use of the stranded seagrass/macroalgae that would be otherwise lost. This approach showed a high recruitment success and growth during the cultivation in mesocosms and after the outplant. Indeed, the recruits continued to grow in length and showed a survival up to 80% after one month in the field.

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Figure 1: A) Study area and location of the sampling locations in the Adriatic Sea; B) locations where the stranded fragments of Gongolaria barbata *were collected. Sassonia 43.84° N – 13.02° E; Lido 1 and Lido 2 43.85° N - 13.01° E. The distance between Sassonia beach and Lido 1 is about 750 m, whereas Lido 1 and Lido 2 are about 250 m apart.*

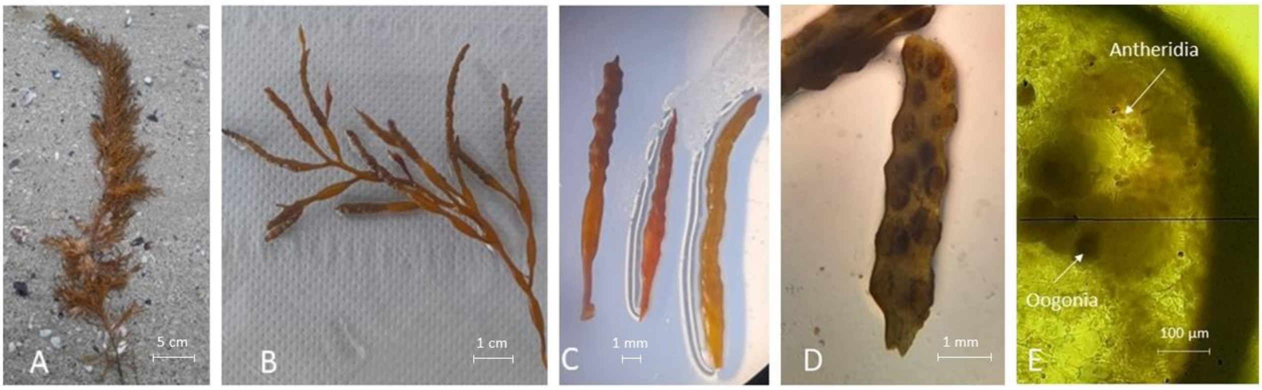

Figure 2: A) Stranded specimen of G. barbata*; B) Receptacles on terminal branchlets; C) Variability of receptacles; D) Longitudinal section of a receptacle showing the conceptacles arranged in parallel rows; E) Cross section of a receptacle showing oogonia and antheridia located in the conceptacle.*

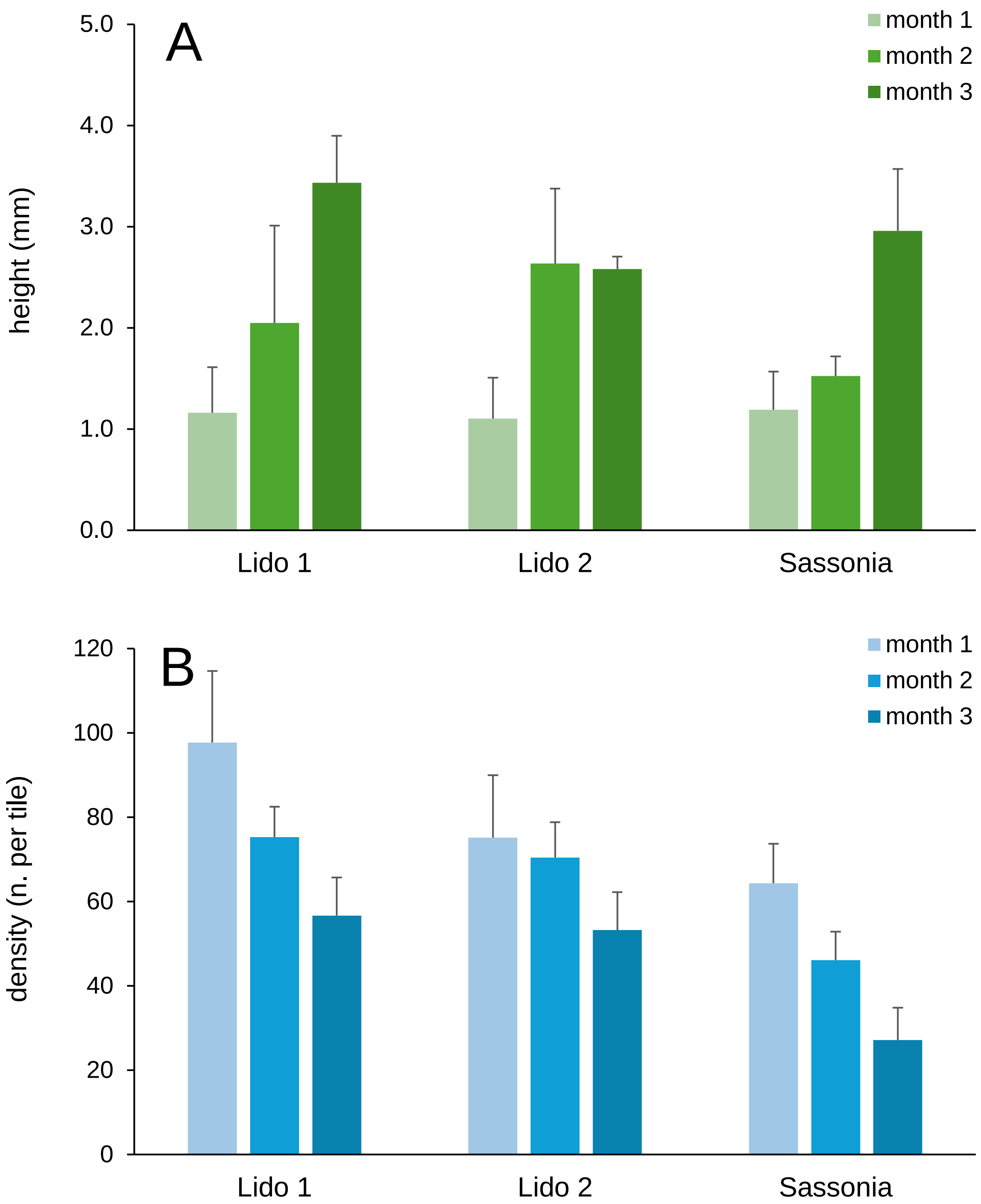

Figure 3: Mean heights (A) and density (B) of Gongolaria barbata *recruits on tiles placed below the fertile adults, across the 3 months in mesocosms. Data are reported as mean ± standard error.*

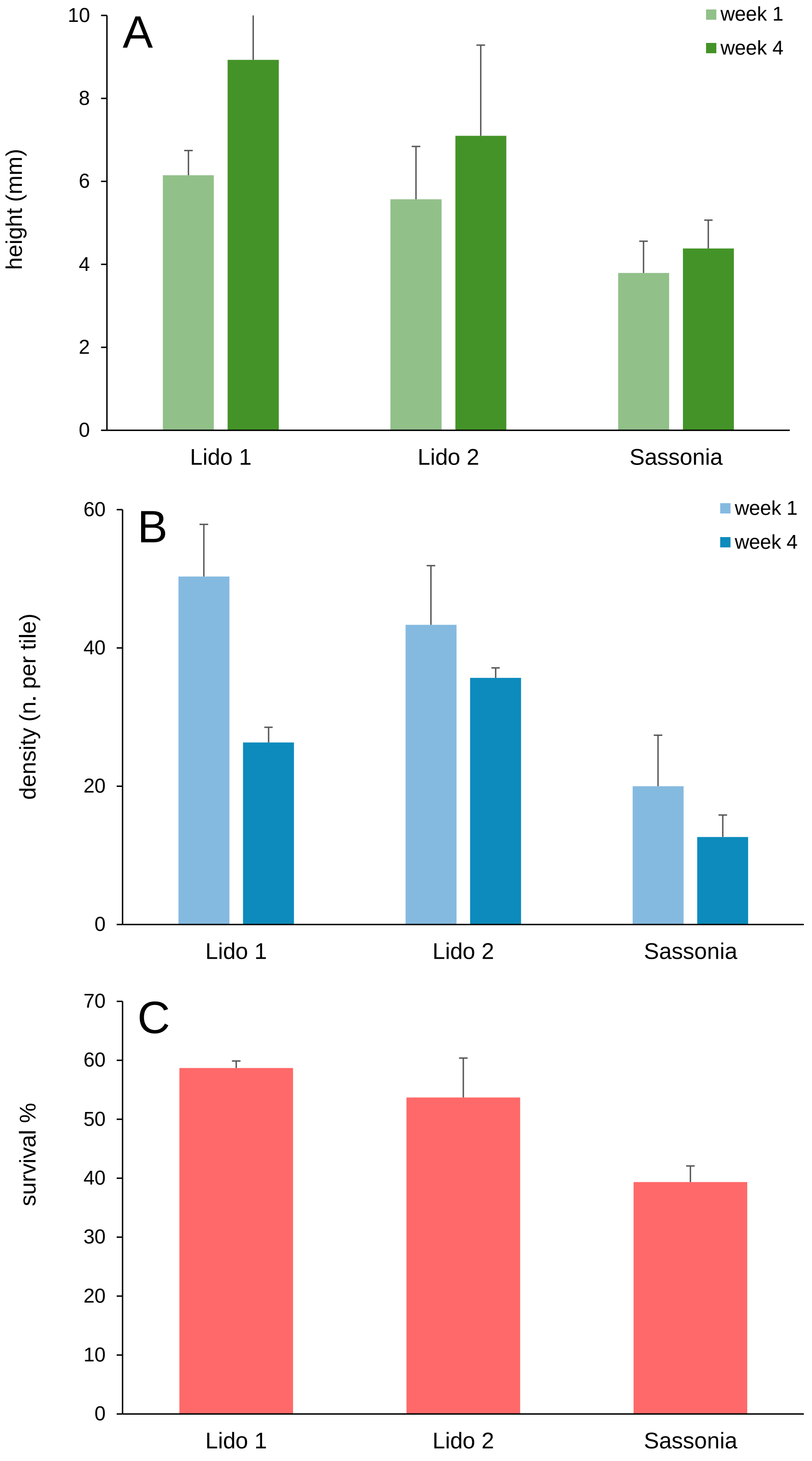

Figure 4: Mean heights (A), density (B) and survival % (C) of Gongolaria barbata *recruits after outplant at sea, across the 4 weeks of monitoring. Data are reported as mean ± standard error.*