UNIVERSITÀ POLITECNICA DELLE MARCHE

Dipartimento di Scienze della Vita e dell'Ambiente DISVA

PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Giulia Bellanti

Title of PhD research: Assessment of biodiversity metrics for restoration of macroalgal forests.

Name of PhD supervisor: Fabio Rindi
Research lab name: Botanica Marina

Cycle:
[ ] XXXVII
[x] XXXVIII

PhD Curriculum::
[x] Marine biology and ecology
[ ] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[x] Actea Mobile Laboratory
[x] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):
There is a growing attention to *Cystoseira s.l.* forests in terms of conservation and restoration, because the decline of these communities may lead to the loss of important ecosystem services.
Juvenile stages are more sensitive to environmental stressors than adults and their loss may affect the long-term viability of canopies; therefore it is important to achieve a better knowledge of their biology. Manipulative experiments carried out in the field and in the laboratory have tested hypotheses concerning factors that may affect recruitment and viability of juveniles.
Identification of patterns in community structure of *Cystoseira s.l.* forests may allow the definition of standardized indicators of health status for these ecosystems. The epiphytic vegetation is an important component of these communities and may be potentially suitable for this purpose. The macroalgal epiphytic vegetation was studied from 5 sites of the Conero Riviera hosting canopies in different health conditions.

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

BACKGROUND
Macroalgal forests are habitats of critical importance in coastal areas due to the many ecosystem services they provide. Large-sized macroalgae form complex tridimensional habitats that act as nursery areas; they are also

important primary producers and bioindicators[1–7]. In the Mediterranean, most macroalgal forests consist of *Cystoseira sensu latu* populations (from now on indicated as *Cystoseira s.l.*). These ecosystems are threatened both by natural and anthropogenic stressors and regressed in the last decades with consequent habitat fragmentation[1,7,8]. Interactions between multiple impacts can result in shifts from complex ecosystems to habitats formed by turf forming algae of lesser ecological and societal value[9], which hamper the natural recovery of canopies[3]. Adult specimens of *Cystoseira s.l.* may not be affected by impacts which can instead impair the survival ad growth of juvenile stages. Successful management of canopies indeed require knowledge of impacts on all life stages[9]. Moreover, efficient, and non-invasive active restoration plans for *Cystoseira s.l.* are based on recruitment enhancement. Accordingly, assessment of the stressors causing failure of recruitment would by extension give us information about the conditions needed to facilitate recruitment and possible habitat restoration[8]. *Gongolaria barbata* is a species of the *Cystoseira s.l.* complex occurring along the Conero Rivera both in continuous and fragmented populations. *G. barbata* is regarded as a threatened species (Barcelona Convention)[6] and should be considered a conservation priority[9]. Conservation and restoration of canopies also require experimental tests that focus on addressing and reducing local scale stressors to improve the resilience of foundation species to global scale stressors[9]. The effects of algal blooms on seaweeds have been rarely studied, even though some cases of seaweeds populations affected by HABs are known[10] and, in general, little information is available about interactions between macroalgae and benthic dinoflagellates. Along the Conero Riviera, summer blooms of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata* are a recurrent phenomenon characterized by abundances among the highest in the whole Mediterranean[10–12]. *O.* cf. *ovata* produces putative palytoxins together with high amounts of ovatoxin-a which have noxious effects on humans and are known to cause death of benthic organisms[12]. Since *Ostreopsis* reaches higher concentrations in sheltered sites[12,] where recently settled juveniles of *G. barbata* are often present, the potential negative impact of the toxic bloom on juveniles growth and survival must be investigated. High sediment load is also well known for its detrimental effects on *Cystoseira s.l.* juveniles, triggering the shift from canopies to opportunistic, fast-growing algae[8,9], which represent a further obstacle to the successful settlement of recruits. Removing excess of sediments and limiting the spread of opportunistic macroalgae may represent a form of positive feedback that facilitates self-recruitment and maintenance of forests, providing available space for recruits to settle[8]. *Cystoseira s.l.* assemblages support highly diverse communities[2]; however, studies on the associated epiphytic species are rare[13]. Interactions between habitat-forming macroalgae and their associated biota have a crucial ecological relevance and may result in a key-interplay, which should be considered to plan conservation programs in coastal habitats[14]. Epiphytes respond faster to anthropogenic stressors than canopy forming algae, and the assessment of common patterns in epiphytic community structure and composition would allow the use of epiphytic assemblages as tools for the evaluation of habitat changes in hard bottom communities[13] and as indicators of the health status of habitat forming species, such as *Cystoseira s.l.* species, according to changes in the environmental quality.

SCIENTIFIC AIMS

The main aims of the work presented in this report are: 1) investigate potential factors impairing the success of macroalgal forest restoration and management, by experiments carried out in the field and in the laboratory; 2) identify components of macroalgal forests that may potentially be used as indicators of conservation of natural populations and recovery of restored populations.

WP1.

Objectives: identify the potential negative effect of *Ostreopsis* cf. *ovata* blooms on the survival of *G. barbata* juveniles by laboratory experiments. These experiments are also designed to discriminate separately possible detrimental effects of *Ostreopsis* mat formation and toxin production on germlings.

Methods: reproductive phenology of the donor population of *Gongolaria barbata* was monitored at Passetto (Ancona) for 2 months (February-March 2023) to assess the maturity stage in receptacles collected from fertile adult specimens. Mature receptacles were kept in dark and cold conditions for 24h to stimulate the fertilization. Receptacles were then left for 24h in 20 mL petri dishes filled with sterilized filtered local seawater and 0.3 mg/L of GeO_2 to avoid diatoms contamination. The presence of zygotes was visually checked, and receptacles were removed from the petri dishes and discharged. 16 petri dishes with several zygotes were obtained. Recruits grew for 1 month before to start the experiment with daily visual checks and weekly water refill. At the end of the first month *G. barbata* germlings were exposed for 4 weeks to 2 treatments with respective controls:

i. concentrations of *O*. cf. *ovata* of 1000 cell/mL, equivalent to the local bloom peak. As matching control germlings were exposed to the same concentration of the non-toxic dinoflagellate *Coolia monotis*, which has similar morphology and similar capacity of mucilage production as *Ostreopsis*. This allowed to assess the possible negative effects of *Ostreopsis* only considering the stress produced by the mat coverage.

ii. toxins produced by *O.* cf. *ovata* obtained by filtration of the culture. The filtrate was inoculated in the amount producing a concentration of 1000 cell/mL; as matching control, germlings were kept in sterilized filtered seawater. This allowed the investigation of possible negative effect of *Ostreopsis* produced only by the toxins.

During the exposure period, daily checks were made to assess mortality of juveniles and weekly checks were made to assess the grow rates by measuring the germlings length.

Expected/obtained results: no juvenile mortality was observed during all the experiment in either of the treatments. The growth pattern was very similar in the treatments and controls (Fig. 1), indicating absence of negative effects in terms of both mat production and toxicity of the microalgae. This experiment seems to exclude the local seasonal bloom of *O*. cf. *ovata* as a stressor impairing the survival of recruits.

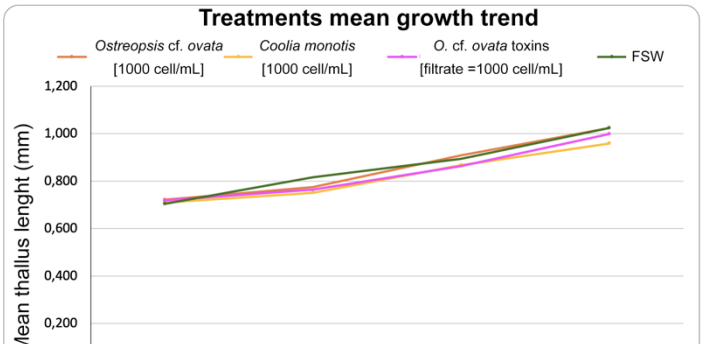

Fig. 1 *G. barbata* germlings thallus mean growth over 4 weeks where: orange and yellow represent the treatment and control **i**; pink and green represent treatment and control **ii**.

WP2.

Objectives: understand the possible role of competition for space by fast-growing macroalgae and high sediment loads in the recruitment success of *G. barbata*. The objective is to assess, through field manipulative experiments, if availability of substratum free of sediment and opportunistic macroalgae can enhance the settlement and survival of *G. barbata* juveniles.

Methods: in the period March-July 2023, 4 patches of *Gongolaria barbata* were selected at Passetto (Ancona). For each patch 3 20x20cm quadrats were scraped and cleaned from both sediment and opportunistic algae. For each treated quadrat, an untreated control quadrat was also selected at a minimum distance of 20 cm.

After scraping, 3 surveys were carried out. During the surveys both visual and photographic checks for recruit survival and density were performed.

Expected/obtained results: treatments showed significantly higher densities of recruits than the controls, which were instead completely occupied by *Ulva* and turfs, as shown in the barplots (Fig. 2). At the times of the second and third surveys, the results were also likely influenced by the start of the tourist summer season, which involved a possible death of juveniles due to trampling (Fig. 2-C). However, this experiment highlights the importance of the competition for space between different macroalgal species as well as the critical role played by the substrate availability in the outcome of a conservation/restoration action.

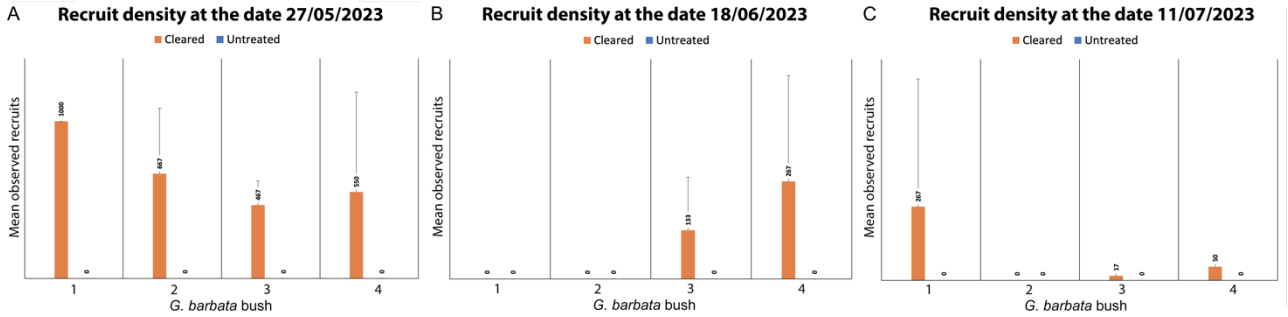

Fig. 2 observed recruitment on cleared quadrats (orange) and untreated quadrats (blue). *A)* first survey after the treatment, on each cleaned quadrat dense cover of juveniles was observed; *B)* second survey after the treatment, the quadrats around the first bush have not been found, the treated quadrats around the second bush were found covered with sediment with no juveniles alive, *C)* the quadrats corresponding to the first bush were recovered, the overall number of recruits started to decline.

WP3.

Objectives: compare the epiphytic assemblages associated with *G. barbata* forests in different health conditions (continuous and fragmented). The data are used for the identification of potential patterns in species composition and abundances potentially related to the health status of these ecosystems. This could allow to define standardized indices to be used as early signals of habitat recovery/decline to be applied in conservation management and restoration actions.

Methods: the epiphytic vegetation associated to *G. barbata* forests was studied using 30 branches collected from 5 sites along the Conero Riviera: Spiaggia delle Due Sorelle, Spiaggia del Frate and Spiaggia dei Sassi Neri (considered in a good status); and while Scalinata del Passetto and Spiaggia della Scalaccia (considered in fragmented status). Sampling was carried out following a nested design: 2 plots distant a few m to some tens of m from each other were randomly chosen in each site; 3 thalli close together were selected in each plot; and 1 branch (~ 20 cm) was collected from each thallus. Length, width (maximum extension of secondary branches), number of

secondary branches and surface were measured for each branch. Epiphytes were manually removed and identified based on morphological features. Finally the epiphytes were quantified in terms of branch surface covered. The data were analyzed by Nested Anovas, Permanovas, and non-parametric multidimensional scaling using R Studio.

Expected/obtained results: total percentage cover and total species richness of epiphytes resulted significantly different between sites ($p<0.001$). Further Post-Hoc pairwise comparisons revealed that Scalinata del Passetto was the only site significantly different from all other sites with lower values for each variable ($p<0.001$), this result was also confirmed considering the differences between sites in terms of epiphyte species composition investigated using distance matrices and non-parametric multidimensional scaling (Fig. 3).

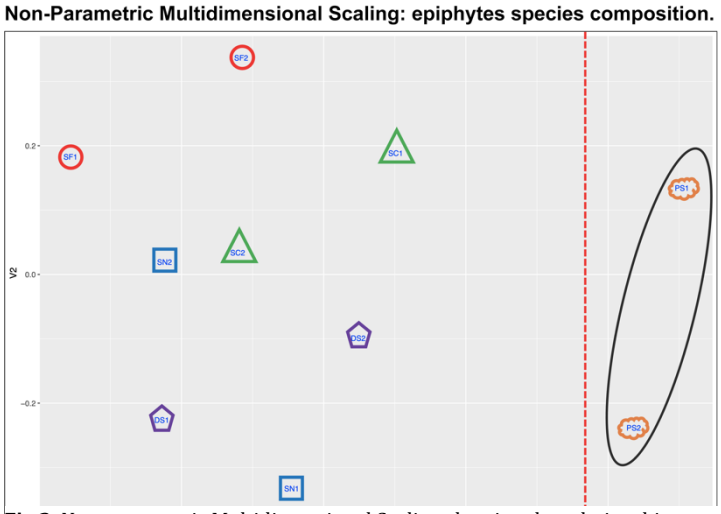

Fig 3. Non-parametric Multidimensional Scaling showing the relationships between each plot and sites considering the epiphytes species composition. **SF** = Spiaggia del Frate; **SN**= Spiaggia dei Sassi Neri; **SC**= Spiaggia della Scalaccia; **DS**= Spiaggia delle due Sorelle; **PS**= Scalinata del Passetto.

Scalinata del Passetto is an urban site characterized by high wave exposure, with a small population of *G. barbata* (consisting in 4-5 bushes) which is also characterized by a vegetative and reproductive phenology temporally shifted compared to other populations of the Conero Riviera; these features can explain the differences highlighted in these analyses. This preliminary study did not reveal obvious differences in the epiphytic vegetation among sites that can be directly related to the health status of the macroalgal forests. The study will be repeated at different times of the year to clarify if this pattern is consistent in time.

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Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Design of Research: European projects (UNIVPM).
2. Technology transfer and innovation (UNIVPM).
3. Elements of Marine Policy (UNIVPM).
4. Analisi di regressione mediante Microsoft Excel (UNIVPM).
5. Getting started with R: Environmental Computing (UNIVPM).
6. CAS SciFinder Training (UNIVPM)
7. Corso di base di statistica e analisi multivariata (Società Botanica Italiana)
8. Formazione generale salute e sicurezza sul lavoro (UNIVPM)

List of periods spent abroad

List of conferences/workshops attended and of contributions eventually presented

1. Biodiversa+ Forescue Kick-off meeting (Ancona).
2. Riunione annuale dei Gruppi di Biologia Cellulare e Molecolare, Biotecnologie e Differenziamento della Società Botanica Italiana (Ancona)
3. Riunione scientifica annuale del Gruppo di lavoro per l'Algologia della Società Botanica Italiana (Napoli). Contribution presented: "Can epiphytic vegetation be used as an indicator of the recovery state of *Cystoseira s.l.* forests? A study carried out along the Conero Riviera (N Adriatic)".
4. NFBC Spoke 2 annual meeting (online).

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

List of publications on conference proceedings

List of other publications (books, book chapters, patents)

[11/11/2023]

Student signature

Giulia Bellanti

Supervisor signature

Firmato digitalmente da Fabio Rindi
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