Better together: *ex situ* co-culture of seaweed and two mediterranean demosponge species

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***Abstract*— The interest in sponges is growing due to their environmental and economic potential such as bioremediation and aquariology and, therefore, so are the efforts to culture them. However, at the Mediterranean scale, few sponge species have been successfully reared under controlled conditions. Owing to their inorganic nutrient excretion, their co-culture with seaweeds (capable of assimilating these compounds) seems promising. In this work, the two Mediterranean demosponges *Hymeniacidon perlevis* and *Aplysina cavernicola* and the seaweed *Cladophora prolifera* have been successfully co-cultured *ex situ* for over three months. *H. perlevis* and *A. cavernicola* increased their total Projected Surface Area (PSA) 34.9% and 9.46% at the end of the trial, respectively, while *C. prolifera* showed a significant wet-weight increasing trend at a mean monthly growth rate of 6% (± 4.4 SE). The presence of the seaweed served to maintain low levels of nitrates, nitrites and phosphates during the duration of the experiment. This work represents a step forward in the knowledge of the cultivation practices of these species in *ex situ* systems.**

Keywords—aquariology, Porifera, macroalgae, IMTA, Mediterranean, growth rate, inorganic nutrients, photogrammetry.

I. INTRODUCTION

With over 9,600 recognized species, sponges are among the most abundant benthic invertebrates, thriving in diverse habitats and depths worldwide [1,2]. As filter feeders, they efficiently capture a wide range of organic particles, from 0.1 to 50 µm, including viruses, bacterioplankton, picoflagellates, and microphytoplankton [3-5], except for the carnivorous cladorhizid demosponges that prey on small crustaceans. To sustain themselves, sponges filter enormous volumes of seawater daily (up to 1,000 times their body volume) retaining and digesting highly efficient food particles smaller than 10 µm [5]. In addition to particulate food, sponges can absorb dissolved organic matter (DOM) from seawater, converting it into particulate organic matter through a process known as the "sponge loop." This conversion is essential for the marine food web, as it makes organic matter and nutrients accessible to other organisms [6-9].

Sponges also release inorganic nutrients through their metabolic processes, playing a crucial role in nutrient cycling [6, 7]. This nutrient cycling is further enhanced by the symbiotic microbial communities they host, which vary between low microbial abundance (LMA) and high microbial abundance (HMA) sponges. These sponge holobionts are dynamic systems that respond to environmental changes, with shifts in their microbial communities potentially affecting sponge health and their ability to adapt to stress [10].

The first attempts to cultivate these organisms in the field date back to more than 200 years ago and have only been increasing [11,12], due to their great environmental benefit and economic potential [13]. Thus, they have been proposed as emerging candidates for species polyculture systems such as Integrated MultiTrophic Aquaculture (IMTA) systems, in which fed aquaculture species are co-cultured with other organisms able to extract organic and/or inorganic substances from seawater [13]. Even so, challenges still exist in the pursuit of efficient and profitable culture of these animals and not so many species are satisfactorily farmed.

Despite many successful *in situ* sponge mariculture cases, leveraging the sponges' remarkable ability to regenerate from fragments, challenges arise as the growth rates of sponge explants decline across asexual generations, making this method unsustainable over time. To ensure sustainability, a renewable seed stock from sexually propagated sponges is necessary, thus *ex situ* cultivation method has also been tested without several problems in maintaining these invertebrates under controlled conditions [10].

Laboratory conditions offer higher survival rates for juvenile sponges but slower growth, while field conditions result in relatively lower survival but faster growth [14]. This research suggests that while juvenile breeding in the laboratory can produce higher survival rates, the challenge remains to balance these advantages with the faster growth observed in field conditions. Developing a feasible, low-cost, and scalable mariculture pattern that combines the benefits of both environments is crucial for supporting a sustainable large scale sponge cultivation.

On a Mediterranean scale, among the species studied in mariculture systems, the bioremediation capacity of *Hymeniacidon perlevis* (Montagu, 1814) (Porifera, Demospongiae), abundant in intertidal coastal areas, has already been analyzed [15, 16], although there is little data on its growth and cultivation [17,18]. On the other hand, *Aplysina cavernicola* (Vacelet, 1959) (Porifera, Demospongiae), being a deeper scyaphilous sponge [19], has not yet been tested and there is no information about its growth under controlled conditions. Macrolgae, on the other hand, are able to assimilate nutrients (e.g. ammonia, nitrates and phosphates) from the water and among the Mediterranean species *Cladophora prolifera* (Roth) Kützing, 1843 (Chlorophyta, Ulvophyceae) has shown a remarkable preformance [20].

Therefore, being species with high potential as bioremediators and despite the very different natural habitats of the species, the objective is to demonstrate the possible co-culture in aquaria of *H. perlevis* and *A. cavernicola* by creating and optimizing the system through the integration of the alga *C. prolifera*.

II. MATERIALS AND METHODS

A. *Sponge and seaweed collection*

Specimens of *H. perlevis* and *A. cavernicola* were collected by scuba diving at 2 and 30 meters at Mar Piccolo of Taranto (northern Ionian Sea) and along the Adriatic coast of Apulia, respectively (Fig. 1). Sponges were cut carefully leaving part of the sponge attached to the substrate in order to minimize impact on natural populations and promote regeneration and transported cooled and aerated in seawater to the laboratory, where they underwent a climatization period of one week. *C. prolifera* was collected together with *H. perlevis* specimens at 3m depth and maintained in a separated tank until the initiation of the experiment.

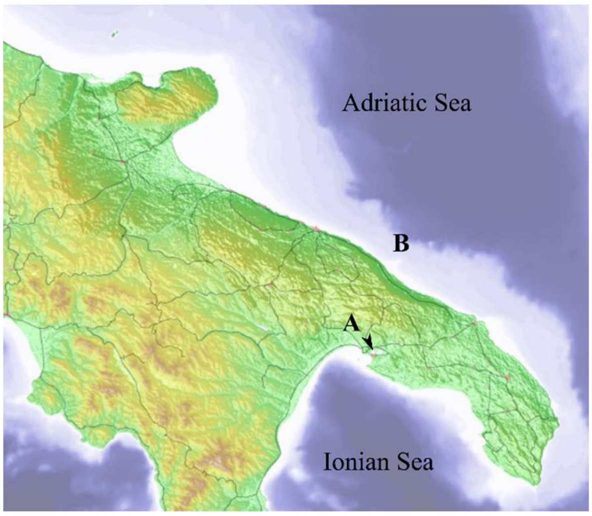

Fig. 1. *Hymeniacidon perlevis* (A) and *Aplysina cavernicola* (B) sampling stations along the Apulian coast.

B. *Experimental design*

A 100L tank was prepared with Artificial Sea Water, continuous aeration, 12:12h light-dark regime and room temperature, daily monitoring the latter and salinity. *H. perlevis* and *A. cavernicola* individuals were cut into 5 and 7 similar sized explants (total Projected Surface Area, PSA: 30.3 and 25.3 cm^2, respectively) and, at the beginning of the experiment, placed on a 1 cm^2 mesh support at 1 cm off the bottom and maintained immobile to monitor them individually. After 5 days, two net bags were suspended at 10 cm from the surface in order to achieve 10 $g \cdot L^{-1}$ of *C. prolifera* (Fig. 2). Sponges were fed daily with Sponge Power (Korallen-Zucht) following manufacturer's instructions for more than 3 months, from April to July 2022.

C. *Growth and nutrient concentration assessment*

Sponge and macroalgae growth was monitored monthly. An identical photo of the explants from the top was taken to calculate the PSA changes using ImageJ [21], which implies no stress to the explants. Seaweed biomass was calculated as wet-weight (WW). Three times a week, on alternate days and before feeding, nutrients were monitored using Handheld Colorimeter reagent kits (Hanna Instruments) measuring NO_2^-, NO_3^- and PO_4^{3-} levels.

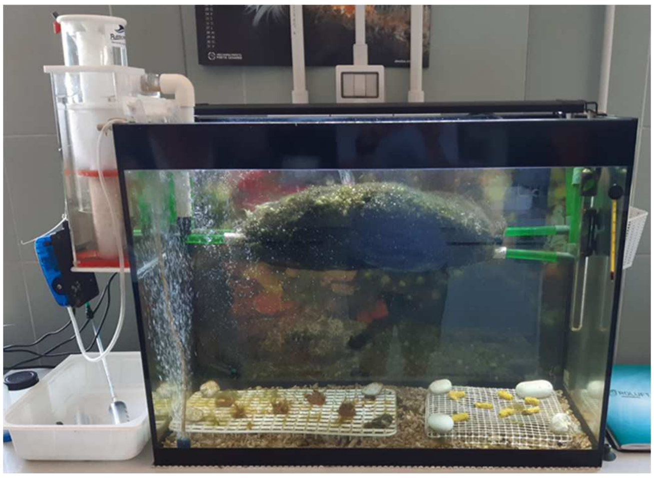

Fig. 2. Experimental tank used for the experiment. Note the two net bags suspended near the surface containing *C. prolifera* and the two mesh supports at the bottom with *H. perlevis* (left) and *A. cavernicola* (right) explants.

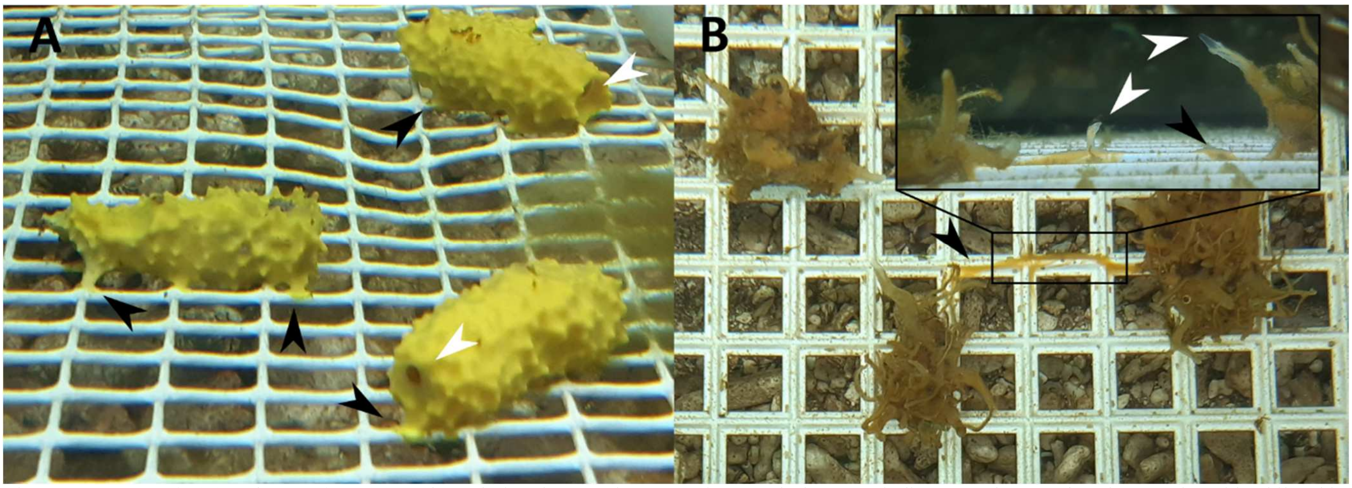

Fig. 3. *A. cavernicola* (A) and *H. perlevis* (B) explants during the co-culture experiment. Black arrows indicate attachment of explants to the grid, while white arrows point to functional oscula. Note the new osculum developed from the newly grown tissue (box in B). Grid squares measure 1 cm2.

D. *Data analysis*

The monthly growth rate (MGR) was calculated as expressed in (1), where B is the biomass of the explants or net bags in PSA or WW, respectively.

$$MGR\ (\%) = [(B_t / B_{t-1}) / B_{t-1}] \times 100 \qquad (1)$$

Temporal trend of salinity, temperature and growth was analyzed by means of Mann-Kendall trend test [22] using Past (Paleontological Statistical) software (v_4.03) [23].

III. RESULTS

All individuals showed no sign of stress, fast recovery from cutting (attaching to the support grid) and survived until the end of the trial; in fact, *A. cavernicola* showed open oscula few days after the start of the trial and *H. perlevis* growth was visible with development of a new osculum after less than a month (Fig. 3).

During the co-culure, salinity was maintained at 37 – 41 psu with no significant temporal trend ($Z = 0.33$, $p = 0.74$), while temperature significantly increased during summer period ($Z = 3.37$, $p < 0.001$), reaching a maximum value of 29 ºC in June (Fig. 4).

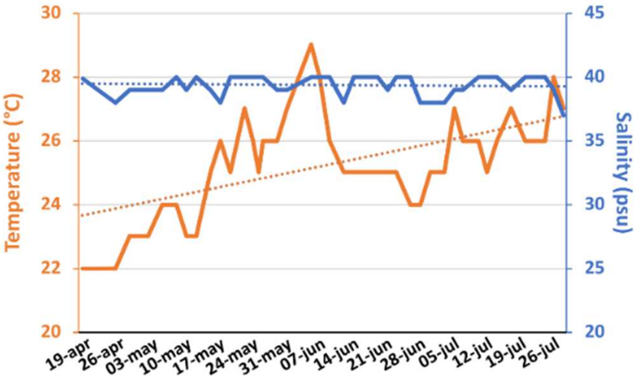

Fig. 4. Daily measures of temperature and salinity during the three-month experimental trial with their linear trends (dashed lines).

A. *Growth estimation*

The photogrammetric method used to monitor sponge growth proved successful as *A. cavernicola* explants were of a regular form and *H. perlevis* grew mainly by expanding through the supporting grid (Fig. 3). In fact, the total PSA of *H. perlevis* explants increased 34.9 % in 3 months with a mean monthly growth rate of 11.08 % (± 7.95 SE) (Fig. 5), showing, although not significant, an increasing trend probably conditioned by the variability among explants. ($Z = 1.72$, $p = 0.086$). On the other hand, *A. cavernicola* showed no significant temporal trend ($Z = 0.7$, $p = 0.48$) despite growing monthly at an average rate of 5.28 % (± 15.46 SE) and reaching at the end of the trial a total PSA increase of 9.46% (Fig. 5). Lastly, *Cladophora prolifera* biomass showed a significant increasing trend ($p < 0.05$) up to a 118.5% of the initial biomass (Fig. 5). The mean monthly growth rate was 6% (± 4.4 SE), higher during June.

B. *Nutrient levels*

Regarding inorganic nutrients (Fig. 6), the integration of *C. prolifera* into the system resulted in a total reduction of nitrates in the water from more than 15 mg/L to 0, which remained the same for the rest of the experiment.

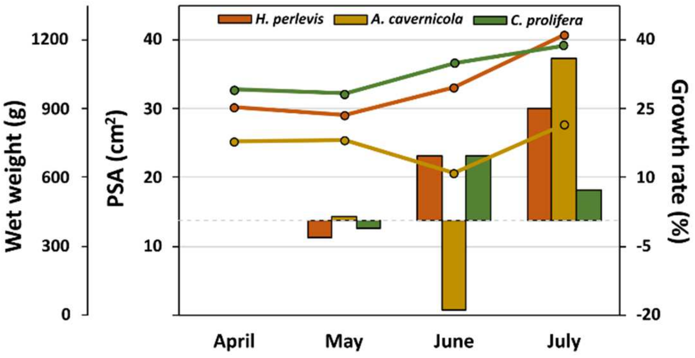

Fig. 5. Temporal growth of the co-cultured species in terms of total area (for sponges) or weight (for seaweed) (dots) and monthly rate (bars).

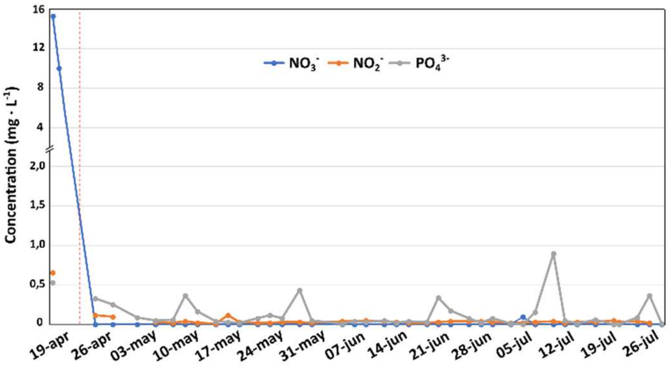

Fig. 6. Nutrient concentration levels during the co-culture period. The vertical red dotted line indicates the addition of *C. prolifera* to the system. Note the periodic increase in phosphate levels.

Similarly, nitrites also suffered a decrease of more than 83 %; during the rest of the experimental period, their levels in the aquarium varied between 0.01 and 0.05 mg/L except for a day when 0.12 mg/L was recorded (Fig. 6). Phosphate, however, showed an interesting pattern, with maximum values between 0.34 and 0.9 periodically almost every 19 days and remaining below 0.2 the rest of the time (Fig. 6).

IV. DISCUSSION AND CONCLUSIONS

Being an object of interest for different civilizations for thousands of years [24], the culture of marine sponges arises from a need to change the methodology of obtaining these animals, especially due to the decline of natural populations of some species in recent decades [25]. Thus, there are species such as *Dysidea avara* (Schmidt, 1862) that are considered endangered and of great conservation value for which specific protection measures have been recommended (Annex I of the Berne Convention, [25]).

The main economic interest of marine sponge culture is its capacity to produce bioactive compounds due to its high commercial value, which can reach thousands of dollars [12,26]. However, it has been shown that aquariology also presents a profitable and socially accepted economic potential [27], opening new challenges for the development and improvement of their maintenance and care in aquaria. Thus, the present study has demonstrated the survival and growth capacity of *Aplysina cavernicola* and *Hymeniacidon perlevis* in a tank and the usefulness of co-culture with the alga *Cladophora prolifera* to maintain low levels of inorganic nutrients.

The non-invasive methodology chosen to monitor sponge growth was adequate for the objectives of the study. Since it is based on photos, it facilitates its use and makes the monitoring of these animals accessible to anyone who owns an aquarium. Thus, although it does not serve to precisely quantify the increase in biomass, for species such as *H. perlevis* whose growth was based mainly on their expansion on the support grid, it proved to be adequate. On the other hand, despite being naturally found in deeper and scyphilic environments (with more pressure and less luminosity), *A. cavernicola* was stable throughout the experimental period. In the first two months, since the explants had regular shapes, it was possible to quantify the decrease in PSA, visible as a thinning of the explants (Fig. 3A), also characteristic of its congener *Aplysina aerophoba* (Nardo, 1833) [28].

The cultivation of sponges together with algae is not a very developed activity and up to date, to the best of our knowledge, there are no previous *ex situ* studies on the co-culture of algae with more than one sponge species. In *in situ* IMTA systems, the co-culture of filter-feeding organisms such as sponges or polychaetes and macroalgae has been proposed with excellent environmental and production results [29]. In *ex situ* systems, however, the conditions of the closed system pose a challenge with regard to the accumulation of nutrients that in high concentrations can be harmful to the species present in the aquarium. In this context, algae emerge as a natural solution to maintain adequate levels due to their high removal efficiency [20]. For its part*, H. perlevis* has already been cultured in aquariums in the presence of algae to simulate the natural conditions of the species and to quantify enzyme expression, yet without the objective of studying growth [30]. On the other hand, there is a lack of information on *A. cavernicola*; however, the culture and adaptation of its congener *A. aerophoba* has been more studied, although always in monocultures [e.g. 28,31,32].

All in all, this work represents the first successful attempt to maintain two sponge species with promising growth rates. The increase of their biomass in addition to that of algae as a by-product is a step forward in the sustainable, ecofriendly and profitable development of integrated aquaculture systems to exploit the cultured biomass.

Ex situ cultivation of sponges is a rewarding yet challenging endeavor that requires careful attention to the specific needs of the species being cultivated. With proper setup and maintenance, sponges can thrive in an aquarium environment, offering valuable opportunities for research, conservation and education.

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