PHD COURSE IN
LIFE AND ENVIRONMENTAL SCIENCES

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

Name of PhD student: Chiara Gregorin

Title of PhD research: Competition versus cooperation: Predation upon large prey as a driver of gregarism in cnidarian polyps

Name of PhD supervisor: Prof. Stefania Puce (UnivPM), Prof. Luigi Musco (UniSalento), Dr. Tomas Vega Fernandez (SZN)

Research lab name: Laboratorio di Zoologia

Cycle:
[X] XXXVI
[ ] XXXVII

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

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

Abstract

Protocooperation, a facultative interaction among small-sized cnidarian polyps to capture a large prey (LP), is hypothesized a driver of gregarism. My project aims to evaluate the benefits of protocooperation and at investigate the feeding behavior of polyps when aggregated, by deepening these aspects: i) the role of cognition in LP predation; ii) the aggregation's success of LP capture; iii) the individual and population growth with LP diet; iv) Study of protocooperation in the natural environment. Results: i) polyps remember the LP predatory behavior when stimulated with the LP homogenate for up to 26 days; ii) LP diet led to increased individual and population growth; iii) Predation success depends on several factors (*e.g.*, distance between polyps, hydrodynamic conditions); iv) Preys are >10 times larger than the single polyp, from 3 to >14 polyps cooperate, often of different colonies, the capture occurs in few seconds, the phase of the prey partitioning is not known yet.

PART 1: Scientific project

BACKGROUND

Cnidarian polyps are suspension-feeders, that benefit from the uptake of zooplanktonic small preys suspended in the water column [1]. Although, several papers report unusual feeding strategies of cnidarian polyps, engulfing preys that are way larger than their mouth diameter, such as portions of sea urchins, sea slugs, jellyfish and salps [2; 3; 4; 5; 6]. These papers describe a single polyp while feeding, but many other observations report several small-sized polyps that cooperate to capture a large prey, often gelatinous zooplankton [7; 8; 9] The collaborative predation played by polyps has been referred to as protocooperation by [7], indicating an unvoluntary cooperation not mediated by cognition. The coordination of polyps to capture large prey make jellyfish become an ephemeral but sometimes abundant (*e.g.*, during outbreaks) food resource. This strategy also requires aggregated polyps, close to each other to access the large prey. Since vicinity is necessary to ensure a successful protocooperation, it has been speculated that protocooperation itself may be a driver of gregarism both for solitary species that spend part or the whole life in a group, and, from an evolutionary point of view, for the formation of colonies. My PhD project aims at investigating the advantages of protocooperation of cnidarian polyps over intra-specific competition for space and resources, at evaluating the possible role of cognition in the predatory behavior played to catch and ingest a large prey, at further observing this phenomenon in the lab and in the field for its characterization, with the final aim to make a step forward the evaluation of protocooperation as a driver for gregarism in benthic cnidarian species.

SCIENTIFIC AIMS:

i)Environmental and within-population: investigate the advantages of protocooperation for cnidarian polyps over competition for space and resources, in term of growth and reproduction rates at individual and population levels, the costs:benefits ratio of protocooperation, the predation success of the aggregations, the phases of the predation of a large prey, which requires high metabolic costs. The aim is to understand why cnidarian polyps choose to involve into protocooperation, and what extent do protocooperation advantage their fitness.

ii)Within Aggregation: Investigate on the large prey predatory success of the aggregation of polyps, based on the distance between individuals and the number of polyps in the aggregation. The aim is to understand to what extent the large prey predation success is influenced by the aggregation level of polyps.

iii)Behavioral and physiological: investigate on the feeding behavior of the single individuals, on their capacity to develop memory on the large prey predation, and to adjust their feeding behavior to increase the predation efficiency. Test whether the learned behavior lasts over time and lead to habituation/sensitization processes. Try to identify a cue possibly produced by the polyps to communicate and the receptors to detect it. The aim is to understand whether protocooperation could be intended as an ancestral trait.

WORKPLAN AND RESEARCH ACTIVITIES:

WP 1 _ The role of learning in the predatory behavior to capture a large prey

ii) Objective: The predatory behavior to capture a large prey is different to the "sit-and-wait" suspension feeding. We tested the ability of *A. coerulea* polyps to learn the predatory active behavior in relation to the presence of the large prey, that requires active predation.

Methods: I observed polyps under the stereomicroscope while feeding on small zooplankton prey (*A. salina*) and large prey (*S. prolifera*), to build the ethogram for behaviors. I set up 65 experimental units (E.U.) with 5 polyps/E.U. (n=5) in a group. I provided alive preys to *A. coerulia* polyps: 20 E.U. received *A. salina* **(A)**, 20 E.U. received *S. prolifera* **(S)**, 20 received new (*i.e.*, never eaten) small prey *Brachionus plicatilis* Müller, 1786 **(B)**. After complete digestion, the 20 E.U. were subdivided in 4 groups of 5 E.U. each (5 replicates). To each of these groups, homogenates (hom.) of the 3 preys were provided in a crossed scheme (Fig. 1). Hom.s were prepared in 1 ml of FSW by selecting and withdrawing animals on the base of their dry weight to standardize the quantity of food (0.22 mg ±0.147). Animals were grinded and centrifuged (13.500 rpm, 20°C, 5 min.). Additional treatment with water flow (**wf**) was used to test the mechanical injection of the liquid. Polyps were observed under a stereomicroscope for 10 minutes and behaviors were categorized within the ethogram each 30 seconds [11]. The total of tentacles contractions and rapid mouth movements (TC/MM) were counted in the whole E.U. and used as a proxy for feeding excitement.

Results: WF treatment did not induce feeding excitement (p = 0.393, Fig. 2). TC/MM was of the treatment S-s (large prey + large prey homogenate) was significantly different to all the other prey+homogenate combinations (p<0.001 for all, Fig. 3). Behaviors were expressed in percentage of occurrence: the FM behavior (referring to the active predation) was tested with Repeated Measure ANOVA against all the other behaviors. This test highlighted significant effects of the treatment (df = 12; p < 0.001), the time (df = 20; p <0.001) and the interaction of the two (df = 240; p=0.18e-4). The FM is showed only in S-s treatment up to more than 80% over the whole observation period (Fig. 4a). The polyps that fed upon the worm showed the same feeding behavior in the presence of the S-hom., thus, it may confirm that they are able to recognize it in the form of homogenate. S-hom. triggered weaker feeding excitement and FM, RE-M and AR-M behaviors also in polyps that have never tasted it (Fig. 4b). This reaction is also triggered by A-hom. and was described by Ewer & Fox [12] with the name of "feeding reaction".

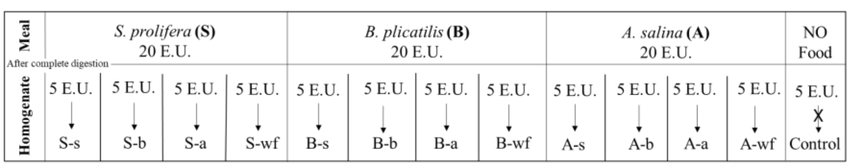


Fig. 1

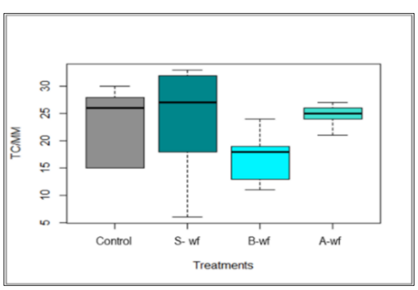
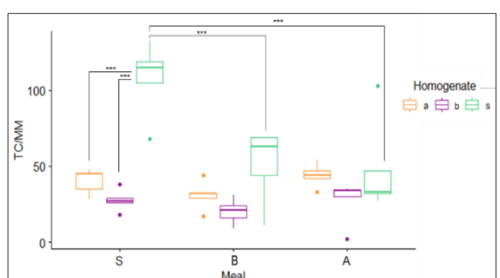


Fig. 2



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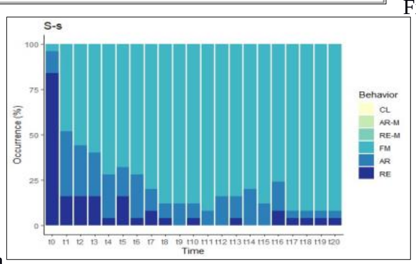
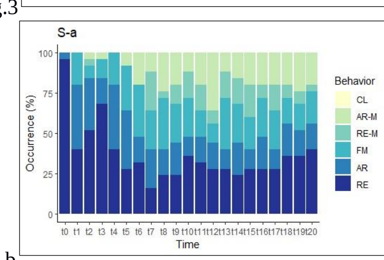
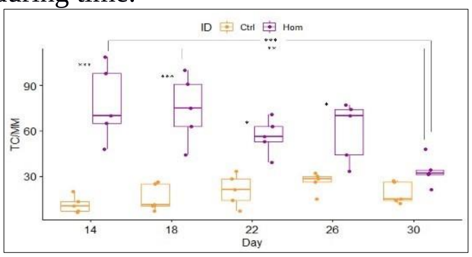


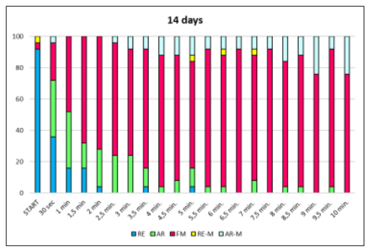
Fig. 4 a



b

ii) In relation to the results of the previous experiment, I wanted to test how long polyps remember the last meal (the alive large prey) by testing the S-s treatment over time. The day of the meal was day 0, and the provision of the hom.s to the replicates (r=5) started at day 14, after the complete digestion of the worm. Data (behaviors and TC/MM) were collected at day 14, 18, 22, 26 and 30. The reactions of hom. and control treatments (Fig. 5) were significantly different on days 14 and 18 (both $p<0.005$), on day 22 ($p=0.0105$) and 26 (0.0216) and not significantly different on day 30 ($p=0.84$). The FM behavior decreased consistently with TC/MM, as showed in the following graphs (Fig. 6a). At day 30, polyps reacted very weakly to the Shom. (Fig. 6b), which was significantly different from the reactions of the polyps at day 14 ($p<0.005$) and 18 ($p<0.005$). Results showed that polyps gradually lose their ability to remember the predatory behavior during time.





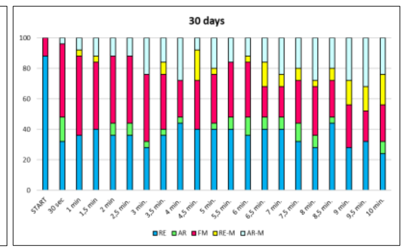


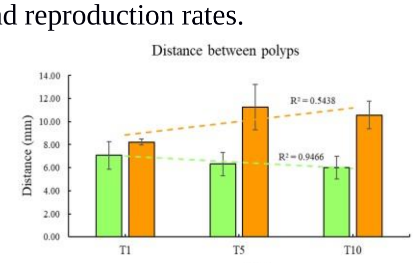
Fig. 5 Fig. 6 a b

WP 2_Large prey as a driver for polyps to form aggregations

Objective: The aim of this experiment is to test the formation of aggregations in *A. coerulea* polyps (n=8) under three different diets: i) small planktonic prey (*A. salina*), ii) large prey (*S. prolifera*) and iii) Mix, 3 replicates each diet. I hypothesized that the polyps are encouraged to form aggregations when a large prey is provided as food resource.

Methods: The amount of food of each diet was standardized on the dry weight of the prey (0.22 mg ±0.147). The experiment lasted 36 days, water was renewed three times per week, food was provided once a week, photos were taken twice a week. The response variables were: i) distance between polyps; ii) individual somatic growth by measuring the mouth disc diamenter (MDD) and iii) n° of newborns at the end of the experiment. Software ImageJ was used for the photo-analyses.

Results: Polyps selected randomly for the experiment were homogeneous in size ($p > 0.05$). Both time, diet and their interactions had significant effects both on MDD ($p=0.0069$) and on distance ($p=0.0375$). The planned comparisons highlighted significant differences in MDD of small prey *vs.* large prey diets ($p=0.0059$) and large prey *vs.* mix diets ($p=0.0056$), while for the distances between polyps, the significant differences can be found between the diets small prey and large prey ($p=0.0176$). To summarize, three patterns are evident: polyps that eat the polychaete are on average 5.026 mm closer (Fig. 7a) and 0.353 mm larger (Fig 7b) than those that did not eat the large prey; they also reproduce more with higher rates (from +25% to + 130%). These results confirm our initial hypothesis for which polyps are encouraged to decrease their inter-individual distances when they feed upon the large prey: the vicinity allow them to have a energetic surplus (the large prey) that, even in the presence of the small zooplankton, lead to higher somatic growth and reproduction rates.



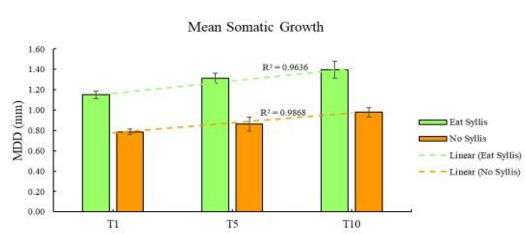


Fig.7 a b

WP 3_Predation success in relation to polyps' inter-individual distances

Objective: The aim is to test whether the predation of a large prey is dependent on the aggregation between polyps, hence, on their distances.

i) **Methods:** Three aggregation levels (AL) were designed, 7 replicates each with 8 *A. coerulea* polyps per replicate (n=8), based on the diameter (d) of the capture area (CA = the surface covered by the extended tentacles of a polyp, on average). The distance between polyps is **d/2** in the maximum LA, **d** in the intermediate LA and **2d** in the minimum LA. One individual of *S. prolifera* (ca. 1-1,5 mm long) is provided to each replicate. The response variables are success and failure of predation (%).

Results: the lowest AL (**2d**) lead to 28.6% of success, the intermediate AL to 71.4% and the maximum AL (d/2) to 57.14%. These percentages highlighted that a close net of conspecifics could increase the success in large prey predation, also by and that tentacles of polyps do not need to be overlapped but simply in contact. On the other hand, the experiment showed some technical issues, underlined by the high rate of solitary captures (38.1% of all the 21 replicates): the solitary capture is due to the rolled shape of the worm when it touches the water, or if the capture start from the head portion. In these two cases, one single polyp is able to capture the worm. Thus, further experiments are needed with a different administration method.

ii) **Methods:** I am curerntly carrying out the same kind of experiment with different species: the size ratio is the same (10:1 prey:predators), where prey are jellyfish of the species *A. coerulea* and predators are *A. pallida* sea anemones. After sampling, jellyfish are transferred in a circular tank, (room temperature 22°C ±2.0; 12h:12h light:dark; 38 PSU) where they swim until the beginning of the experiment. Three AL are designed: Maximum AL (12 equidistant polyps, ca. 10 cm far); Intermediate AL (3 groups of 4 close polyps); Minimum AL (group of 12 close polyps). At the beginning of the experimetns, groups or single polyps are attached on the walls of the circular tank and from 1 to 5 jellyfish are released inside the tank.

Expected results: To note a higher predation success in the maximum LA, decreasing in parallel with the increase of distance between polyps.

WP 4_Differences in the predation rate of zooxanthellate vs. azooxanthellate polyps

Objective: to test whether the predation success could be influenced by the presence or absence of photosynthetic symbionts.

Methods: I will use the same species of the previous experiment. Part of the *Aiptasia pallida* (a zooxanthellate anemone) will be reared under appropriate light regimes to ensure the proliferation of zooxanthellae. The other part will be reared in complete darkness, fed with *A. salina* newly hatched nauplii. When the polyps are completely bleached (zooxanthellae will be counted in a Fuchs-Rosenthal chamber for cell-counting), they will be attached on the walls of the circular tank and the same method as the previous experiment will be followed.

Expected results: If zooxanthellate polyps are less willing in engaging cooperation while azooxanthellate species, that do not receive photosynthesis products from microalgae, are more encouraged, a step forward will be done for the understanding of this kind of feeding strategy.

WP 5_ Field observations

Objective: to perfomr ethological studies on the predatory protocooperation in the natural environment.

Methods: observations are made by SCUBA diving in three locations (Passetto, Ancona; "Sant'Anna" and "Le Formiche" diving spots in Ischia Island, NA). Jellyfish are collected during outbreaks from the boat with a bucket or a zooplankton net, then transferred in 5 liters bottles with the lid and brought to the diving site. Jellyfish (from 1 to 10) are released in the vicinity of the corals' walls and some time is left for the contact to happen. If it finally happens, a metric reference is placed close to the predation scene, videorecord and photographed to be further analyzed in the laboratory (Photoshop, ImageJ, Microsoft Video Editor). Before to leave the site, the point is tagged with a surface buoy to be easily found for the next dives.

Variables are: i) size ratio of prey and predators; ii) n° of polyps and colonies involved; iii) duration of the capture; iv) duration of the prey partitioning phase; v) fate of the large prey. I tested 4 species: *Parazonathus axinellae* (Schmidt, 1862) (Fig. 8a); *Astroides calycularis* (Pallas, 1766) (Fig. 8b), *Cladocora caespitosa* (Linnaeus, 1767) (Fig. 8c), *Anemonia viridis* (Forsskål, 1775) (Fig. 8d), and observed a fifth one [9]. Prey were *Pelagia noctiluca* (Forsskål, 1775) for the first three species and *Rhizostoma pulmo* (Macri, 1778) for *A. viridis*.

Preliminary results: the large prey is from 6 to more than 10 times larger than the single polyps; the number of polyps simultaneously involved in the predation is from 3 to more than 14, often from different colonies (*P. axinellae* (Fig. 8a) and for *A. calycularis* (Fig. 8b)), while for *C. caespitosa* (Fig. 8c) the colony tested was one at a time; hydrodinamic conditions of the site strongly influence the availability of the large prey for corals, because it can be pushed away by currents or waves. Thus, the capture phase takes place within seconds. The duration of the partitioning phase is not known yet: after one hour, the jellyfish is at the same position of the beginning of the experiment and no tissue lost is recorded. After 24 hours the jellyfish is not present and possible ingestion of jellyfish parts cannot be recorded as well. Thus, it is necessary to perform several dives at different time intervals, to study the fate of the large prey.

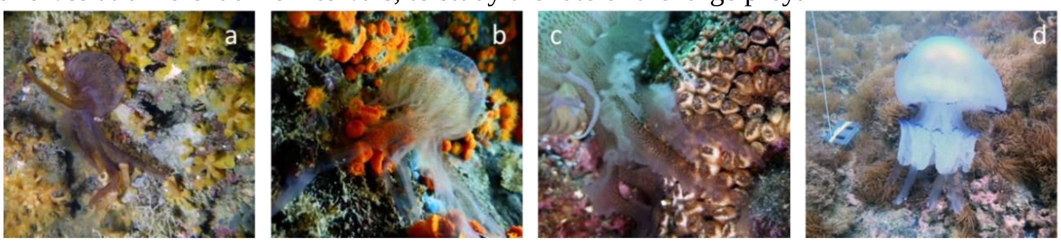
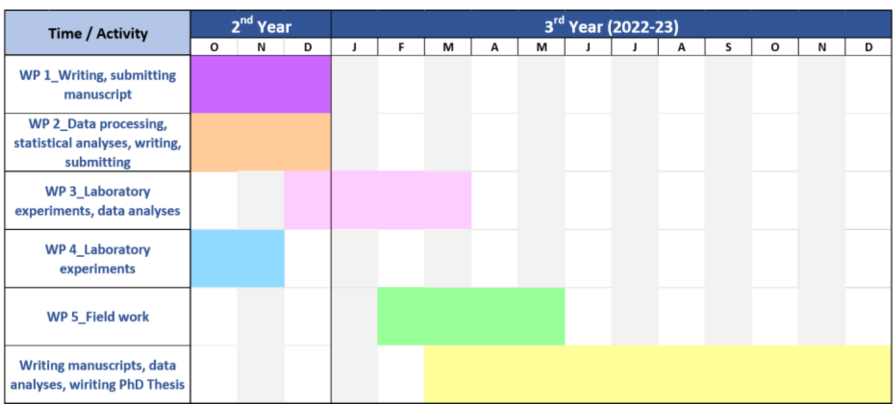


Fig. 8

NEXT PLANNING:



PART 3: Scientific production activity

Lectures 1st Year:

- Introduction to LaTeX for scientific documents (Prof: Francesco Spinozzi, UnivPM)
- Elements of Marine Policy (Prof. Emanuela Fanelli, UnivPM)
- Climate risk (Prof. Pierpaolo Falco)
- Theory and application of complex networks (ongoing – Prof. Maria Grazia Ortore, UnivPM)
- Biological effects of chemicals in sentinel species: from molecular to cellular and organism level (Practical laboratory, ongoing – Prof. Stefania Gorbi, Francesco Regoli, UnivPM)
- Design of Research: European projects (Prof. Nicola Paone)
- Technology transfer and innovation (Prof. Donato Iacobucci)

- Experimental design (Dr. Trevor Willis, Dr. Tomas Vega Fernandez, SZN)
- Phylogeny (Dr. Wiebe Kooistra, Dr. Roberta Piredda)
- Animal Welfare (Dr. Graziano Fiorito, Dr. Claudia Gili, SZN)
- Scientometric Evaluation of Young Researchers (Prof. Roberto Danovaro, SZN)
- Molecular Biology – Advanced (Dr. Marco Borra, Dr. Pasquale De Luca, Dr. Monia Russo)

Lectures 2nd Year:

SZN Internal Seminars:

- "An Eco-Evo-Devo analysis of metamorphosis in clownsfish", Vincent Laudet, Okinawa Institute of Science and Technology.
- "Comparative and transcriptomics and genomics of cone snails", Rafael Zardoya, Museo Nacional de Ciencias Naturales.
- "Prokaryotic life on particles in the surface and deep ocean" Josep M. Gasol, Institut de Ciències del Mar (ICM) – CSIC.
- "Ever since Giesbrecht ..... Form and Function in Pelagic Copepods" J. Rudi Strickler, University of Wisconsin-Milwaukee.
- "The non-gradual nature of adaptive radiation in African cichlid fishes", Walter Salzburger, University of Basel.
- "The genomics of fish invasions in the Mediterranean Sea", Giacomo Bernardi, University of California Santa Cruz.
- "Genetics and Epigenetics of Coral Adaptation", Mikhail V. Matz, University of Texas.
- "Tracing the origins of phyla in the Earliest Cambrian", Jakob Vinther, University of Bristol.

Congresses and Workshops:

- UZI 81st Congress, POSTER presentation (title: "Protocooperation in anthozoans. The collective predation of large prey by small-sized cnidarian polyps"). Trieste, 20-23 September, 2022.
- SIBE Congress 9th Edition, ORAL presentation (title: "Do cnidarian polyps remember their last meal? The role of learning in the protocooperative capture of large prey"). Ancona, 4-7 September, 2022.
- International Scientific Cooperation. Fostering marine biotechnology as best practice example: existing projects and future opportunities_ 15-16 December 2020
- Evolmar 2020 _ Marine Evolution 1st Italian Congress _ 23-25 November 2020

PhD curriculum publications:

- **Gregorin C.**, Musco L., Puce S., 2022. Protocooperation in *Tubastraea* cf. *micranthus* to catch planktonic large prey. *Marine Biodiversity*, 52 (3), 1-2.
- **Gregorin C.**, Roveta C., Pulido Mantas T., Gridelli S., Domenichelli F., Fernandez T.V., Musco L., Puce S. Do cnidarian polyps remember their last meal? The role of learning in the predation of a large prey. – *In preparation*.
- **Gregorin C.**, Spano D., Gridelli S., Domenichelli F., Fernandez T.V., Musco L., Puce S. The predation of large prey induces the formation of aggregates in *Aurelia coerulea* (Cnidaria Scyphozoa) polyps. – *In preparation*.

- **regorin C.,** Roveta C., Musco L., Puce S. Review on the predatory *behavior* in benthic cnidarians. – *In preparation.*

Extra – PhD curriculum publications:

- Di Camillo C.G., Roveta C.; Pulido Mantas T.; Gravili C.; Cerrano C.; Calcinai B.; Coppari M.; **Gregorin C.**; Marrocco T.; Riccardi A.; Puce S. Bivalve-inhabiting hydroids: from guests to pests *Reviews in Aquaculture* – under review.

- **Gregorin C.**, Albarano L., Somma E., Costantini M., Zupo V., 2021. Assessing the Ecotoxicity of Copper and Polycyclic Aromatic Hydrocarbons: Comparison of Effects on *Paracentrotus lividus* and *Botryllus schlosseri*, as Alternative Bioassay Methods. *Water*, 13(5), 711.
- **Gregorin C.**, Musco L., Somma E., Zupo V., 2020. Behavioral Responses of the Colonial Sea Squirt Botrylloides violaceus Oka to Suspended Food Micro-Particles in Laboratory Cultures. *Journal of Marine Science and Engineering*, 8 (12), 1021.
- Roveta C., Annibaldi A., Afghan A., Calcinai B., Di Camillo C.G., **Gregorin C.**, Illuminati S., Pulido Mantas T., Truzzi C., Puce S., 2021. Biomonitoring of Heavy Metals: The Unexplored Role of Marine Sessile Taxa. *Applied Sciences*, 11 (2), 580.
- Roveta C., Annibaldi A., Domenichelli F., **Gregorin C.**, Gridelli S., Pantano V., ... & Puce S., 2022. "Single and combined effects of two trace elements (Cd and Cu) on the asexual reproduction of *Aurelia* sp. polyps". *Aquatic Ecology*, 1-7.

Other:

- Winner of the "UZI Young Researchers" award for the scientific relevance of the presented contribute, UZI Congress, Trieste, September 2022.
- Tutor of Zoology, years 2021-22, 2022-23.
- Supervision of the students in the course trip "Restoration of hard bottoms and tropical reefs – field work and practices" held on Tremiti Islands in collaboration with Marlin Tremiti diving center. 5 – 8 October 2021 and 12 – 16 September 2022.
- Attendance with scholarship to the Coral Reef Ecology Summer Course at Bios – Bermuda Institute of Ocean Sciences, 9 – 27 August 2021.
- Enriched Air Nitrox license for SCUBA diving, 8 October 2021.
- Reef Check Eco Diver license, 26 March 2022.
- "Team-working exercise" by SZN Open University PhD programme (May 19th, 2022).

References:

[1] Houlbrèque, F., & Ferrier-Pagès, C. (2009). Heterotrophy in tropical scleractinian corals. Biological Reviews, 48, 1-17.

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[3] Alamaru, A., Bronstein, O., Dishon, G., Loya, Y. (2009). Opportunistic feeding by the fungiid coral *Fungia scruposa* on the moon jellyfish Aurelia aurita. Coral Reefs, 28, 865.

[4] Hoeksema, B.W., & Waheed, Z. (2012). It pays to have a big mouth: mushroom corals ingesting salps at northwest Borneo. Marine Biodiversity, 42, 297–302.

[5] Mehrotra, R., Scott, C.M., Hoeksema, B.W. (2016). A large gape facilitates predation on salps by *Heteropsammia* corals. Marine Biodiversity, 46: 323-324.

[6] Jarms, G., & Tiemann, H. (2004). *Actinostola callosa* (Verrill, 1882)(Actinostolidae, Anthozoa), a medusivorous sea anemone and its mass occurrence in the Lurefjord, Norway. *Helgoland Marine Research*, *58*(1), 15-17.

[7] Musco, L., Vega Fernández, T., Caroselli, E., Murray Roberts, J., Badalamenti, F. (2018). Protocooperation among small polyps allows the coral *Astroides calycularis* to prey on large jellyfish. Ecology, 99(10), 2400-2401.

[8] Ter Horst, L. J., & Hoeksema, B. W. (2021). Salpivory by colonial reef corals at Curaçao, Southern Caribbean. Diversity, 13(11), 560.

[9] Gregorin, C., Musco, L., & Puce, S. (2022). Protocooperation in *Tubastraea* cf. *micranthus* to catch large planktonic prey. Marine Biodiversity, 52(3), 1-2.

[10] Bavestrello, G., Puce, S., Cerrano, C., & Balduzzi, A. (2000). Life history of *Perarella schneideri* (Hydrozoa, Cytaedidae) in the Ligurian Sea. Scientia Marina, 64(S1), 141-146.

[11] Bateson, M., Martin, P. (2021). Measuring *behavior*: an introductory guide. Cambridge University Press.

[12] Ewer, R. F., & Fox, H. M. (1947). On the functions and mode of action of the nematocysts of *Hydra*. In Proceedings of the Zoological Society of London (Vol. 117, No. 2-3, pp. 365-376). Oxford, UK: Blackwell Publishing Ltd.

Student Signature

Supervisor Signature