Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Compete or cooperate? Protocooperation in catching large prey may be the driver of gregarism in cnidarian polyps

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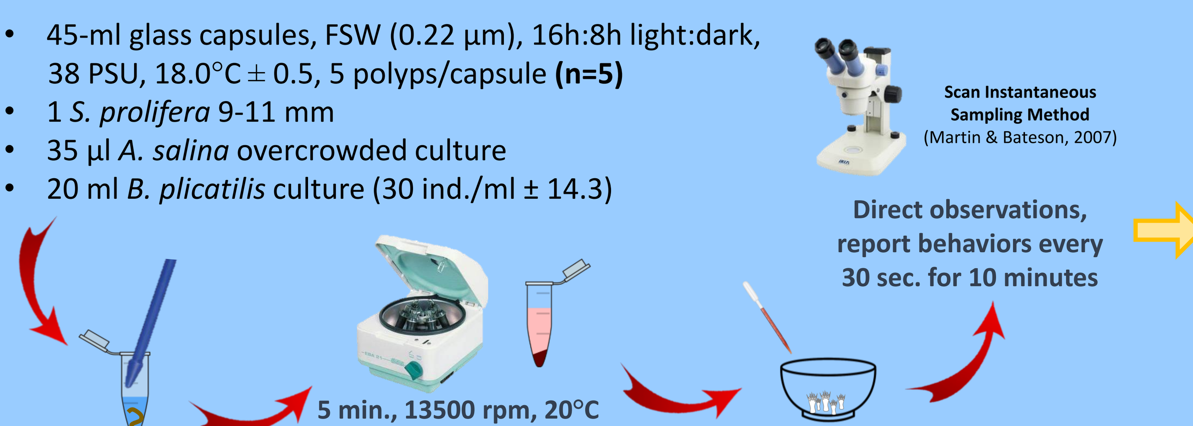
1: Study of the learning abilities in *Aurelia coerulea* polyps, focusing on the protocooperative behaviour *vs.* suspension-feeding

***Hypothesis:** Polyps are able to remember protocooperative behaviour (characterized by strong feeding reactions – FM behaviour) and learn to play it following a smell stimulus ("Large prey juice")*

3 PREY ITEMS: **1. Large prey, *Syllis prolifera* (S); 2. Small prey, *Brachionus plicatilis* (B); 3. Control food, *Artemia salina* (A). Water flow (wf) treatments to test the mechanical injection of the homogenates. 45-ml glass capsules, n=5, FSW (0.22 µm), 16h:8h light:dark, 38 PSU, 18.0°C ± 0.5**

Methods

- 45-ml glass capsules, FSW (0.22 µm), 16h:8h light:dark, 38 PSU, 18.0°C ± 0.5, 5 polyps/capsule **(n=5)**
- 1 *S. prolifera* 9-11 mm
- 35 µl *A. salina* overcrowded culture
- 20 ml *B. plicatilis* culture (30 ind./ml ± 14.3)



Direct observations, report behaviors every 30 sec. for 10 minutes

Response variables:

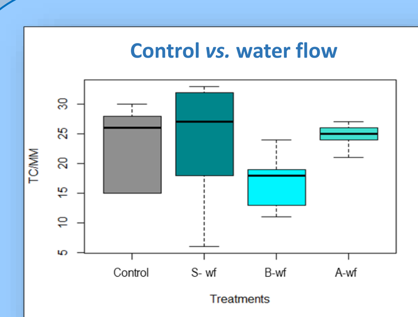
- Reference Code for Behaviors (8 categories)
- Number of Tentacles Contractions and rapid Mouth Movements (TC/MM), proxy for feeding reaction

Experimental design

	Group S (20 Experimental Units)				Group B (20 E.U.)				Group A (20 E.u.)				Control (5 E.U.)
Last Meal	alive ***Syllis prolifera***				alive ***Brachionus plicatilis***				alive ***Artemia salina***				alive ***A. salina***
After complete digestion → **Prey HOMOGENATE**	5 R Water flow (S-wf)	5 R S homog. (S-s)	5 R B homog. (S-b)	5 R A homog. (S-a)	5 R Water flow (S-wf)	5 R S homog. (S-s)	5 R B homog. (S-b)	5 R A homog. (S-a)	5 R Water flow (S-wf)	5 R S homog. (S-s)	5 R B homog. (S-b)	5 R A homog. (S-a)	5 R No Food or Homogenates Control)

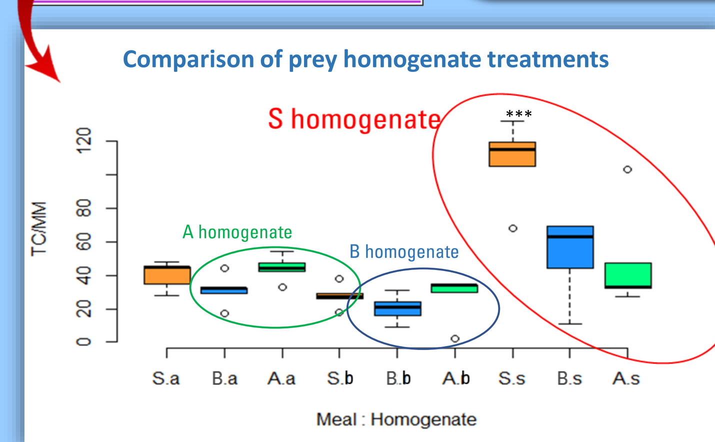
Results

TC/MM

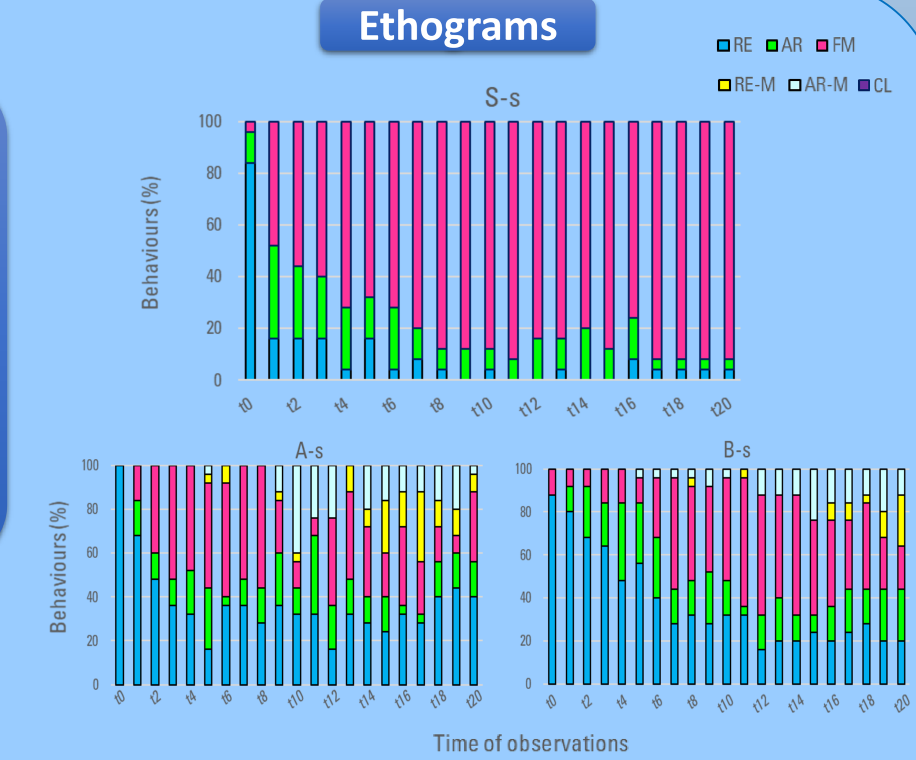


Treatments	P value	Significance level
S-s : S-a	0.0000149	***
S-s : S-b	0.0000005	***
S-s : B-a	0.0000011	***
S-s : B-b	0.0000001	***
S-s : B-s	0.0003039	***
S-s : A-a	0.0000423	***
S-s : A-b	0.0000004	***
S-s : A-s	0.0001417	***

The n° of TC/MM, and thus, the feeding reactions do not vary between control and water flow treatments (p>0.05). ***S. prolifera* homogenate** triggers **significantly different reactions** (see table up) in polyps who received alive ***S. prolifera*** as last meal.



Ethograms



The **FM behaviour** (Feeding, rapid Movements) is the most performed in the **S-s treatment**, confirming the TC/MM results and the stronger feeding reactions. This suggest that the polyps that received the polychaete as last meal, react accordingly to the polychaete's homogenate (the prey "meat juice").

2: Evaluation of benefits of protocooperation in terms of individual fitness of *A. coerulea* polyps, three diets

***Experimental questions:** What is the impact of the large prey predation on the reproduction success, health and growth of cnidarian polyps? Does the "large prey" diet encourage polyps to get closer and to form aggregates?*

DIETS: 1. Large prey, *Syllis prolifera*; 2. Small prey, *Artemia salina* and 3: Mix, n=8, 100 ml glass capsules, FSW (0.22 um), 16h:8h light:dark, 38 PSU, 18.0°C ± 0.5

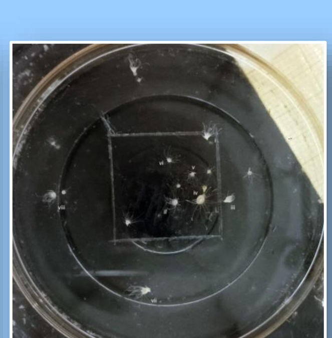
Response variables:

- Mouth Disc Diameter (MDD) → INDIVIDUAL GROWTH
- Distance between polyps → AGGREGATION LEVEL
- N° of buds/newborns → ASEXUAL REPRODUCTION
- Health Scale (0=dead; 0.5=degenerated but possible recovery; 1=healthy) → HEALTH STATUS

Results

Photos Processing:

MIX – R2- t1

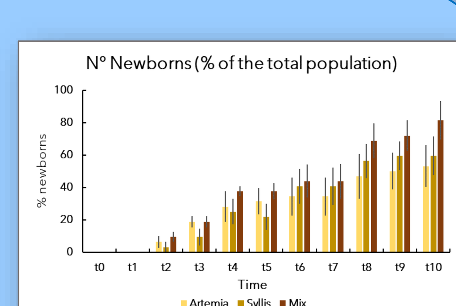


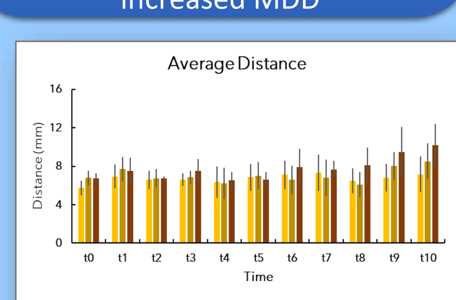
MIX – R2- t10

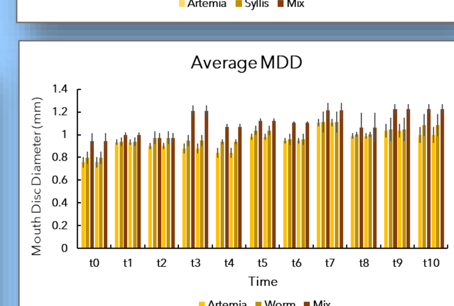
Statistical Analyses: LOADING....

RStudio StatSoft

PRELIMINARY RESULTS: basing on the same amount of food (d.w. – mg), the mixed diet led to increase of asexual reproduction and individual growth. The distance between polyps slightly decreased with the "only worm" diet, that did not lead to increased MDD







3: Predation success in relation to protocooperation: field work and lab experiments

***Experimental questions:** What is the extent of protocooperation? Is there any difference in the predation success of large preys in relation to the population density?*
***Hypothesis:** high population density promotes protocooperation and predation of large food items, thus overcoming spatial competition between conspecifics*

FIELD WORK

Pelagia noctiluca being captured by several *Parazoanthus axinellae* polyps – Ischia, Jan 2022; Detail of polyps holding jellyfish's oral arms

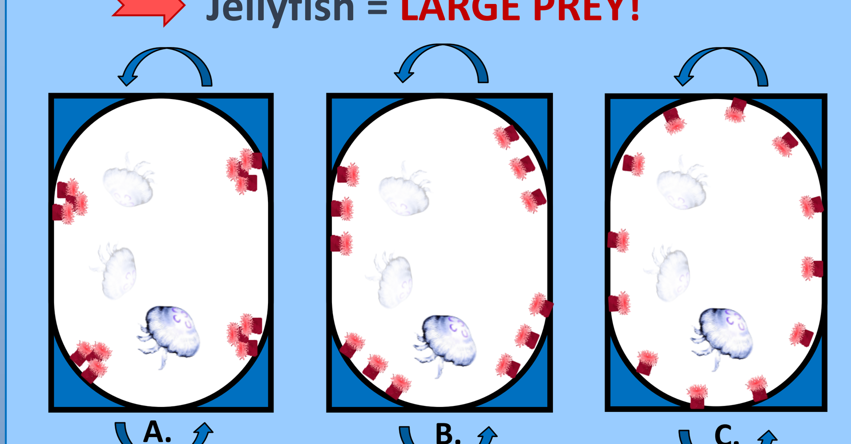
Aequorea sp. being captured by *Tubastraea* cf. *micranthus* polyps – Red Sea, Dec 2021.

Pelagia noctiluca being captured by several *Cladocora caespitosa* polyps – Ischia, Jan 2022;

METHODS: underwater photography and video recording, time-lapse. **DATA ANALYSIS, Imaging softwares:** Evaluation of population density (i.e. distance between polyps), size of polyps (Mouth disc diameter, length of calyx), prey-predator size ratio, time from capture to prey escape/ingestion

LABORATORY EXPERIMENTS

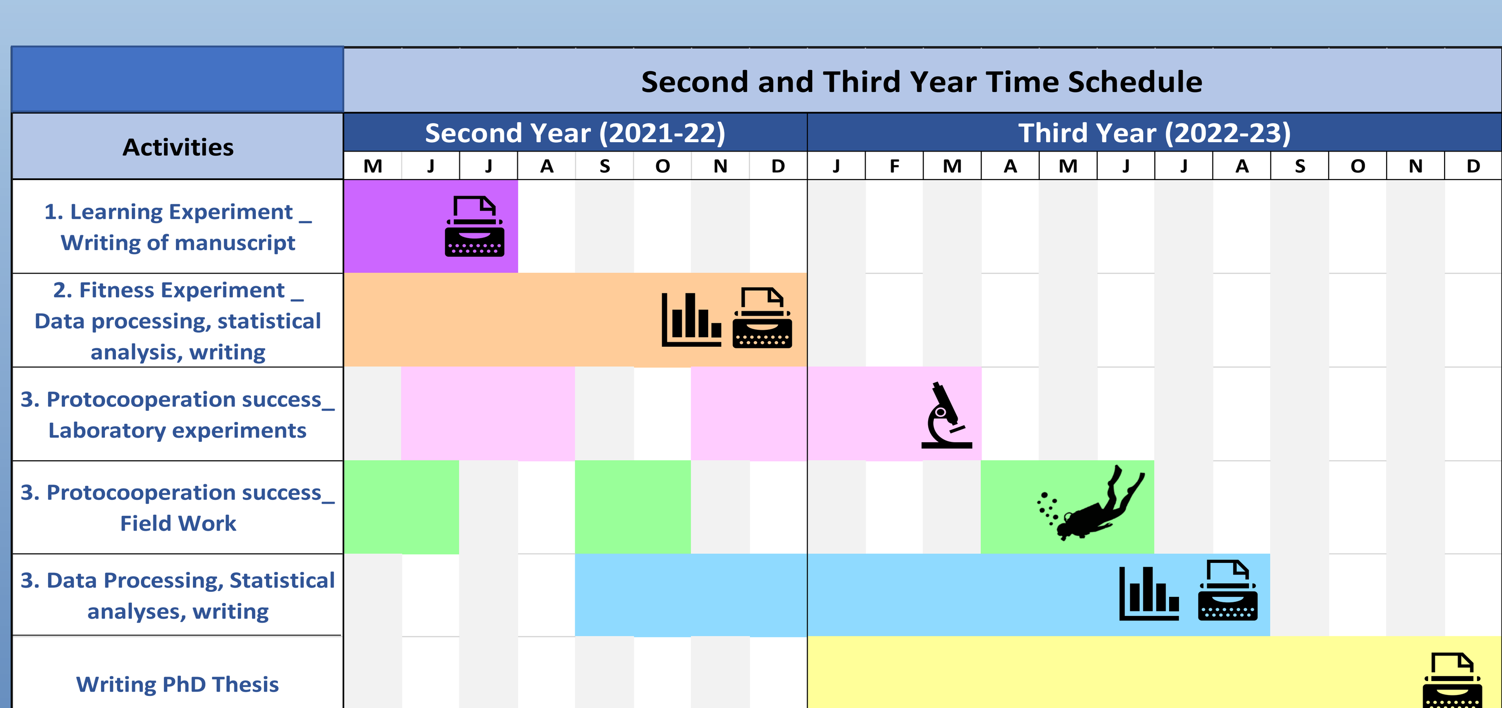
Jellyfish = LARGE PREY!



METHODS: Circular tanks, manipulation of polyps on the walls to vary the population density, evaluate the **predation success** in relation to density

EXP. DESIGN: Three aggregation levels, n=12: **A.** max: 4 groups of 3 polyps closely aggregated; **B.** intermediate: 4 groups of polyps less aggregated than in A.; **C.** no groups, polyps are equally distant to each other

Planning of activities: Laboratory, Field work, Data analysis, Writing



PhD *curriculum* publications:

- **Gregorin C.**, Musco, L., Puce, S., *in press*. Protocooperation in *Tubastraea* cf. *micranthus* to catch planktonic large prey. *Marine Biodiversity*.
- **Gregorin C.**, et al. - *in preparation*. Do cnidarian polyps remember about their last meal? The protocooperative behaviours in response to the large prey homogenate.

Extra - PhD *curriculum* publications

- Roveta C., et al., 2022. Single and combined effects of two trace elements (Cd and Cu) on the asexual reproduction of *Aurelia* sp. polyps. *Aquatic Ecology*, 1-7.
- Roveta C., et al., 2021 Biomonitoring of heavy metals: the unexplored role of marine sessile taxa. *Applied Sciences* 11 (2), 580.
- **Gregorin C.**, et al., 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.**, et al., 2020. Behavioural 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.