



UNIVERSITÀ POLITECNICA DELLE MARCHE
DIPARTIMENTO SCIENZE DELLA VITA E
DELL'AMBIENTE

Corso di Laurea Magistrale in Biologia Marina

**COMPORTAMENTO ALIMENTARE DI POLIPI DI
SCIFZOZI: UN CASO DI PROTOCOOPERAZIONE?**

**FEEDING BEHAVIOUR OF SCYPHOZOAN POLYPS: A
CASE OF PROTOCOOPERATION?**

Tesi di Laurea Magistrale
di: Jessica Vettese

Relatore
Chiar.ma Prof. Stefania Puce

Correlatore: Dott. Luigi Musco

Sessione Straordinaria Febbraio 2020

Anno Accademico 2019/2020

SOMMARIO

Osservazioni recenti hanno evidenziato l'abilità dei polipi di *Astroides calycularis* di nutrirsi in gruppo di prede diverse volte più grandi dei singoli polipi. I ricercatori hanno classificato questo comportamento come proto-cooperazione: gli organismi traggono mutuo beneficio gli uni dagli altri, ma l'associazione non è obbligatoria come nel mutualismo. È stato ipotizzato che questa interazione possa essere una spinta evolutiva, favorendo l'aggregazione in questa madrepora, ed eventualmente in altri cnidari.

Per verificare queste ipotesi in questi animali così semplici, abbiamo nutrito polipi dello scifozoo *Aurelia* sp., i cui individui tendono ad aggregarsi in natura, con esemplari dell'anellide *Syllis prolifera*; inoltre abbiamo confrontato l'efficienza nella predazione da parte dei gruppi e dei singoli polipi. Dopodiché abbiamo analizzato le differenze di comportamento in presenza di una preda più piccola, nauplii di *Artemia salina*, e testato la presenza di composti chimici rilasciate da entrambe le prede che potessero attivare un comportamento di alimentazione da parte dei polipi.

I polipi (da 1.5 a 2.5 mm) sono stati allevati in ciotole di plastica, dove si sono assestati e si sono riprodotti, formando gruppi; altri sono stati isolati. Gli anellidi (da 7.5 a 12.5 mm) sono stati dati sia ai gruppi che agli individui singoli. Abbiamo considerato riuscite le catture in cui i polipi hanno effettivamente ucciso e ingerito il verme.

Dopo 15 test, le percentuali di successo dei gruppi e degli individui singoli sono state del 100% e del 27% rispettivamente. Gli esperimenti su un'eventuale preferenza alimentare non hanno portato risultati chiari; al contrario, i polipi hanno chiaramente reagito ai composti

ottenuti da entrambe le prede, suggerendo che questi possono innescare un comportamento di alimentazione.

Nonostante ricordi comportamenti di competizione intraspecifica, il comportamento protocoperativo osservato è vantaggioso per pressoché ogni polipo che partecipa alla cattura. Ipotizziamo che questo comportamento potrebbe essere presente in altre specie di cnidari, come osservato in studi precedenti, e che possa aver favorito l'aggregazione in questi polipi.

ABSTRACT

Recent observations focused on the ability of polyps of *Astroides calycularis* to feed on preys several times bigger than the size of the single polyps. Researchers defined this behaviour as proto-cooperation: organisms mutually benefit from each other, but association is not obligatory as in mutualism. This interaction was hypothesized to be an evolutionary driver favoring gregarism in this dendrophylliid, and possibly in other cnidarians.

To verify these hypotheses in such simple animals, we fed polyps of the scyphozoan *Aurelia* sp., whose individuals tend to aggregate in the wild, with specimens of the annelid *Syllis prolifera*; we also compared predation rates of the groups and the solitary polyps.

Then we checked for differences of behaviour in presence of a smaller prey, nauplii of *Artemia salina*, and for the presence of chemical cues from both the preys that could trigger the feeding behaviour. Polyps (1.5 to 2.5 mm long) were reared in plastic bowls, where they established and reproduced forming groups; others were taken isolated. Worm preys (7.5 to 12.5 mm long) were given to both the groups and the individuals. We considered successful those catches in which polyps effectively killed and ingested the worm.

After 15 trials, the success rates of the groups and the individual polyps were 100% and 27% respectively. Experiments on food preference did not show clear results; on the contrary, polyps reacted to cues obtained from both the preys, suggesting that they can trigger a feeding behaviour.

Although somehow resembling intraspecific competition rather than cooperation, the observed proto-cooperative behaviour is advantageous for almost every polyp taking part in the hunt. We hypothesize that this behaviour could be present in other species of cnidarians, as observed in other studies, and that it could have promoted aggregation in these polyps.

Index

ABSTRACT	3
INTRODUCTION	6
1.1 Cnidarians	6
1.2 The life cycle of Scyphozoa	7
1.2.1 The polyp morphology	10
1.2.2 The genus <i>Aurelia</i>	11
1.2.3 Feeding behaviour of the genus <i>Aurelia</i>	13
1.3 Interactions among organisms	15
1.3.1 Mutualism and cooperation	18
1.3.2 Protocooperation and group living in populations	24
1.3.3 Group living in cnidarians	26
1.3.4 Cooperation in cnidarians	27
MATERIALS AND METHODS	29
2.1 <i>Aurelia</i> sp. polyps	29
2.2 Behavioural Sampling Methods	30
2.3 Experimental preys	31
2.3.1 The polychaete <i>Syllis prolifera</i>	31
2.3.2 Nauplii of <i>Artemia salina</i>	33
2.4 The experiments	34
2.4.1 Successful catches in group and individuals	34
2.4.2 Food preferences	35
2.4.3 Chemical signals	36
RESULTS	37
3.1 Activity budget	37
3.2 Single polyp success	41
3.2.1 Polyps behaviour	41
3.2.2 A special case: autotomy of the prey	42
3.2.3 Data	43
3.3 Successful catches of groups	45
3.3.1 Polyps behaviour	45
3.3.2 Data	48

<i>3.4 Food preference</i>	54
3.4.1 Polyps behaviour	54
3.4.2 Data.....	56
<i>3.5 Chemical signals</i>	58
3.5.1 Polyps behaviour	58
3.5.2 Data.....	59
DISCUSSION	63
<i>4.1 Observations on single polyps success</i>	63
<i>4.2 Observations on group success</i>	65
<i>4.3 A case of protocoooperation?</i>	68
<i>4.4 Food preference</i>	73
<i>4.5 Chemical signals</i>	75
CONCLUSIONS	77
BIBLIOGRAPHY	78

Chapter I

INTRODUCTION

1.1 Cnidarians

The phylum Cnidaria includes marine invertebrates whose name come from cnidocytes, cells containing characteristic subcellular stinging organelles known as cnidocysts. They represent an ancient group with more than 9000 species of very efficient predators, even if many of them are sessile or with limited capability of movement. The most famous representative organisms are reef forming corals, even if the phylum currently comprises five different classes:

- Anthozoa
- Cubozoa
- Hydrozoa
- Scyphozoa
- Staurozoa

They are abundant in temperate and tropical waters and they present two morphological stages: the polyp and the medusa. These two stages can be part of the same life cycle of a species, as in Scyphozoa for example, or one of them can be absent in some classes: the medusa stage is absent in Anthozoa and many Cubozoa lack the polyp stage. (Hickman et al., 2013)

1.2 The life cycle of Scyphozoa

Scyphozoa are an exclusively marine class of the phylum Cnidaria. Their jellyfish blooms are known worldwide because of their ecological and economical importance, and for implications for human health.

They play important roles as predators: analysis on gut content of *Aurelia aurita* jellyfish revealed the presence of hydromedusae, copepods and fish larvae (Sullivan, 1994). Predation upon fish larvae by jellyfish of *Chrysaora quinquecirrhu* increases when the prey increases and its presence affects other predators (Cowan & Houde, 1992), as well as predation of bivalve and gastropod veligers, barnacle larvae and crab zoeae when these are abundant (Purcell, 2003). Scyphozoan jellyfish can also prey other scyphomedusae, as well as ctenophores (Arai, 2005). The medusa diameter plays a significant role on food choice (Sullivan, 1994; Purcell, 2003).

A wide variety of animals prey upon them. In addition to the well-known sea turtles (Den Hartog & van Nierop, 1984) and many fish species (Milisenda et al., 2014), birds and even humans eat scyphomedusae (Arai, 2005), which could also be eaten by other medusae and corals (Musco et al., 2018). Nudibranchs (Arai, 2005; Hoover et al., 2012; Takao et al. 2014), polyps of other species (Tang et al., 2019) and crustaceans (Takao et al., 2014) can control the benthic stage of scyphozoans.

Scyphomedusae can have major impacts on human health and industry, particularly when they form mass aggregations or ‘blooms’. Blooms of these jellyfish can affect fish populations (Bailey & Batty, 1983). Moreover, they can replace fish as top pelagic predators in overfished ecosystems (Lynam et al., 2006), and cause painful stings (Purcell et al., 2007). Blooms may have multiple causes, such as the absence of predators, climate change with

water becoming warmer and suitable for tropical species (Richardson et al., 2009), the presence of artificial substrates (Duarte & al., 2013) which enhance the settlement of planulae, impacting the number of jellyfish by increasing the polyps number.

It is particularly important, therefore, to pay attention to their life cycle and especially to benthic stages that have been seldom studied and that are still little known.

Two main stages characterize the scyphozoan life cycle:

- 1) An asexual stage, the polyp, also called scyphistoma;
- 2) A sexual stage, the jellyfish.

The polyp (Figure 1) can reproduce asexually, for example with lateral budding, fission, free-swimming particles, production of stolons and cysts (Vagelli, 2007; Adler-Ohde & Jarms, 2009).

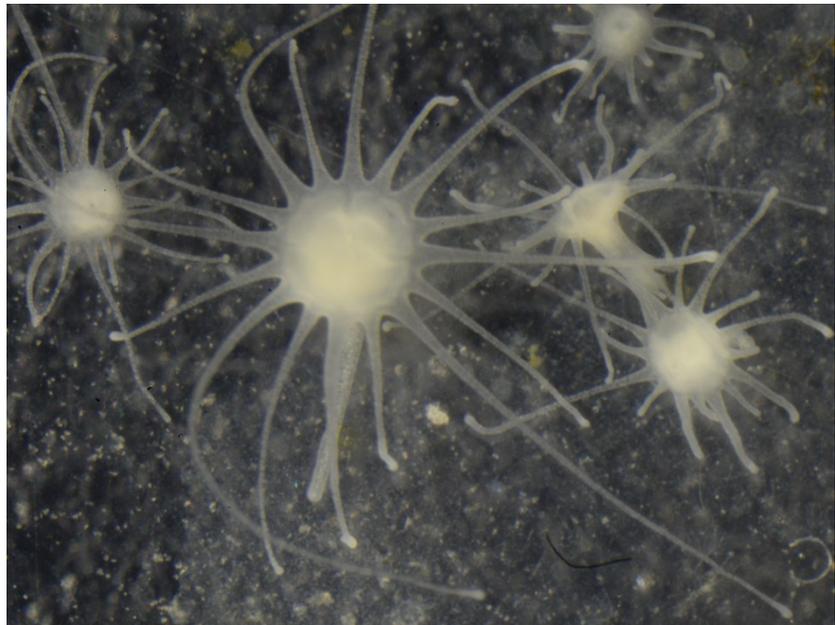


Figure 1 Polyps of *Aurelia* sp., a scyphozoan

However, in particular

conditions, it can undergo a transversal segmentation (simple or multiple) of its superior portion, forming a strobila (Figure 2). One or more free-swimming ephyrae detach from the strobila and develop into jellyfish. Temperature and salinity significantly affect these processes in the genus *Aurelia*, with strobilation occurring only at low temperatures (9-15°C) and high (40) and low (15) salinity reducing the number of buds and ephyrae. (Xing & al.,



Figure 2 Strobila of *Aurelia* sp.

2020). Low salinity also induce mortality in these polyps (Sokołowski & al. 2016).

The jellyfish (Figure 3) releases gametes in the seawater; fertilization leads to a larva, the planula, which settles on the seabed

and matures into a polyp. (Grassé, 1993)

These reproductive modes can show temporal and spatial separation and comprise such drastic transformations of the animals' body plan that researchers originally believed that scyphozoan polyps, jellyfish, strobilae and ephyrae were different and unrelated animals. (Ceh et al., 2015)



Figure 3 Jellyfish of *Aurelia* sp.

This perception changed when

Agassiz documented the discovery of the life cycle of two scyphozoan species, which included the alternation between these two stages (Agassiz, 1860).

1.2.1 The polyp morphology

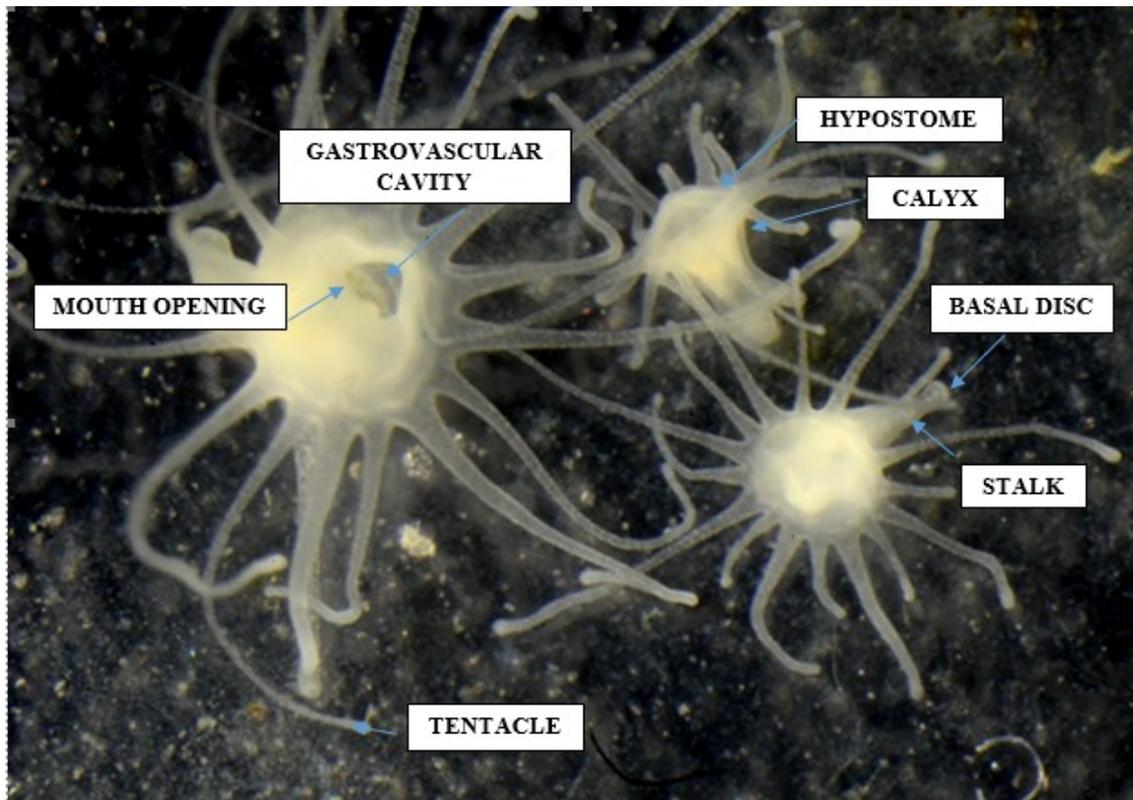


Figure 4 Morphological characteristics of scyphozoan polyps

All scyphozoan polyps have columnar bodies that attach to a hard substrate at the aboral surface with a basal disc (Helm, 2018). A stalk departs from the basal disc, becoming a calyx in the upper part of the organism. The oral surface is composed of a ring of tentacles surrounding a central mouth arranged on a hypostome. (Figure 4) The mouth is the opening that leads to the gastrovascular cavity, in which food is ingested and digested. Tentacles present cnidocytes and help in the catch of the prey, blocking it.

1.2.2 The genus *Aurelia*

Aurelia (Lamarck, 1816) is a widely known genus of scyphozoans, whose jellyfish is cosmopolite and often used both in laboratory research and in aquariums.

Despite this, little is known about its polyp stage and identification can be very difficult, especially without molecular techniques (Dawson & Jacobs, 2001). Recent works, both referring to worldwide species (Gambill & Jarms, 2014) and Mediterranean ones (Scorrano et al, 2016), tried to set parameters for morphological identification. Still, differences among the species are limited: polyps are very similar, and sometimes show identical characteristics (e.g. the number of tentacles). For this reason, the identification requires the knowledge of all of the scyphozoan life stages (polyp, strobila, ephyra and jellyfish), as well as the geographic origin of the studied population.

The benthic phase has gained more interest in the last years in order to better understand the dynamics of jellyfish blooms (Liu, 2008). It is perennial and populations can survive through years; under specific conditions, polyps form ephyrae by strobilation; thus, they have a crucial role in jellyfish blooms. In fact, population density and dynamics of the sessile stage of *Aurelia* has been at the centre of many recent studies (Willcox et al., 2008). In the wild, polyps live densely aggregated on hard substrates, sometimes even on other living organisms (Di Camillo et al. 2010), with some polyps (especially related ones) that can remain in contact for the entire lifetime. (Figure 5)



Figure 5 Polyps living strictly associated in the plastic bowl

Previous studies considered the population density controlled by interspecific competition from other encrusting organisms (Watanabe and Ishii 2001). However, Willcox, (2006), found no relationship between the area covered by scyphistomae and the area covered by other organisms; moreover, a percentage of the substrate in his observations was bare. This indicated that competition for space was not the limiting factor for polyp populations. Furthermore, density and population growth of *Aurelia aurita* scyphistomae can change with different environmental conditions and density dependant effects (Watanabe and Ishii 2001). Environmental factors like temperature and light have showed positive effects on the population size (Liu et al., 2008; Willcox et al., 2007; Purcell, 2007), with increasing temperature and light leading to population growth, but also negative effects as the reduction of budding and strobilation (Xing & al., 2020).

1.2.3 Feeding behaviour of the genus *Aurelia*

The feeding behaviour of the *Aurelia* jellyfish has been widely studied due to the ecological impact of its blooms on food webs and on the fish industry (Ceh, 2015). Jellyfish feed on mesozooplankton (Purcell, 2003), especially copepods and molluscs larvae, but can also eat other organisms. The size of the jellyfish has a role in determining prey selection (Sullivan, 1994). Polyps are sessile suspension feeders that wait for a small prey that reaches their tentacles or mouth: for this reason, a high density of preys or detritus leads to a more efficient feeding, increasing the possibility of the encounter.

As for other scyphozoan polyps (Cargo, 1975), *Aurelia* polyps can be fed with nauplii of *Artemia salina*, but recent studies have shown the possibility of feeding them with planktonic ciliates

(Kamiyama, 2011). A study reports *Aurelia* polyps feeding on planulae, even of their own species.

Feeding on molluscs larvae

and fish larvae was also observed. When a prey passes by the tentacles or the mouth of a polyp, cnidocysts immediately paralyze it (Gröndahl, 1988); the polyp opens its mouth and move the tentacle towards it, ingesting the prey (Arai, 1997), similarly to other cnidarians (Lewis, 1981).

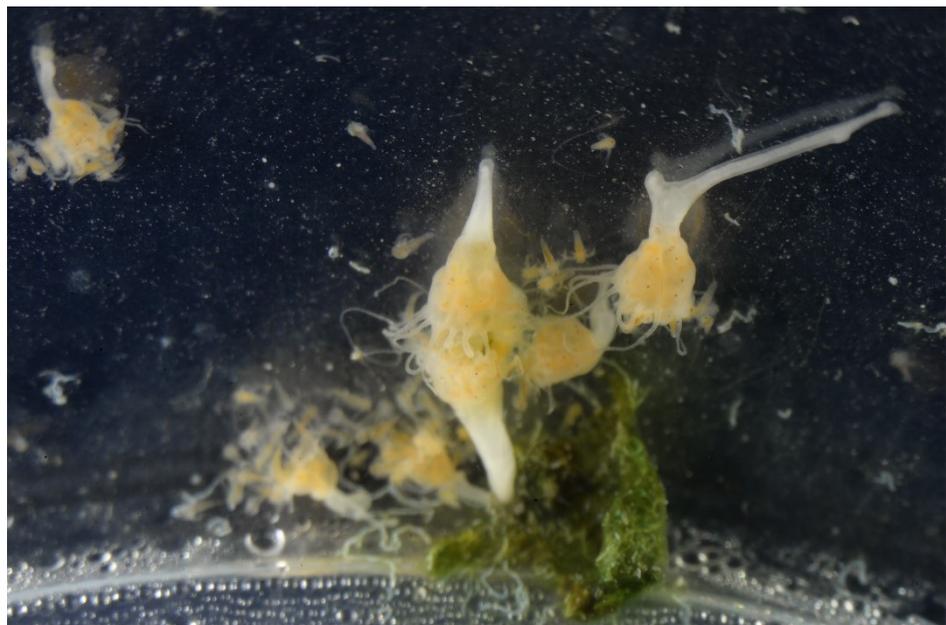


Figure 6 Polyps of *Aurelia* sp. feeding on nauplii of *Artemia salina*

Both the mouth and the gastrovascular cavity can stretch during feeding (Cargo, 1975); one single polyp can ingest a big amount of nauplii of *Artemia* (Figure 6), changing provisionally its size.

1.3 Interactions among organisms

Interactions among species, populations and individuals are at the base of ecosystem functioning (Maestre et al. 2010). Two populations (or organisms) interact when the actions or properties of one of them result in effects on the other. When both cause and receive effects, interactions are considered two-way. There is a wide range of possible effects and each interaction should be treated as unique, even if we need a classification to better understand biological processes (Abrams, 1987).

According to Abrams, there are two main methods to classify interactions:

- considering the actions or the properties of each effector;
- considering the effects produced in each member of the pair.

In the first case, the focus is on mechanisms. This method has several benefits that could be preferred to the effect definition: mechanisms are easier to observe and they eliminate the possibility that different interactions with different mechanisms are included in the same kind of interactions. However, it is still discussed because of some limits and the risk of not taking count of indirect processes that involve organisms. In addition, there are not clear criteria for distinguishing categories.

When we consider the effects, instead, we can distinguish three types:

1. effects on population size;
2. effects on population growth;
3. effects on individual fitness.

However, Odum's classification, based on effects on populations, remains one of the most common used, and states that "theoretically, populations of two species may interact in basic ways that correspond to combinations of 0, +, and -" (Odum, 1983).

The zero means no effects of one population on the other; a plus that one population is benefitted by the second; a minus, that one population is inhibited or adversely affected by the other. An interaction corresponds to a pair of these signs, each representing the effect on the population, resulting in six possible pairs (Table 1):

Table 1 Interactions among organisms

	0	+	-
0	Neutralism	<i>Commensalism</i>	<i>Amensalism</i>
+	<i>Commensalism</i>	Mutualism	Predation/Parasitism
-	<i>Amensalism</i>	Predation/Parasitism	Competition

- (00) Neutralism:

“The situation in which two organisms coexist, with neither population being affected by its association with the other.”

- (+0) Commensalism:

“An interaction between organisms in which one species (the commensal) benefits from the association while the other is not significantly affected.”

- (-0): Amensalism

“An interaction of organisms, in which one population is inhibited while the other (the amensal) is unaffected.”

- (+-): Predation or Parasitism

“In predation, one organism, the predator, kills and eats another organism, its prey.”

“Parasitism is a relationship between species, where one organism, the parasite, lives on or in another organism, the host, causing it some harm, and is adapted structurally to this way of life.”

- (--): Competition

“An interaction between organisms or species, in which the fitness of one is lowered by the presence of another.”

- (++): Mutualism

“Mutualism is an interaction between two or more organisms, where organisms derive a mutual benefit.” Mutualistic interactions include cooperation and protocoooperation.

Even if this system leaves some open questions, it is widely used and often integrated with mechanisms to better understand interactions among organisms. (Abrams, 1987)

1.3.1 Mutualism and cooperation

Due to the lack of common evolutionary mechanisms and to the variety of mutualism forms in nature, there is no consensus on how to define mutualism and cooperation. While mutualism is defined as “an interaction between species that is beneficial to both” (Boucher, 1982), the term cooperation is generally used to describe mutually beneficial interactions between individuals of the same species (Holland, 2008).

Many authors, for example Connor (1995) and West et al. (2007), tried to clarify the conditions under which mutualism and cooperation occur. While West’s study is based on the proposal of a clear definition of cooperation, Connor’s focuses on the three main mechanisms by which organisms transfer benefits between each other. Benefit can either be considered on an individual level, in terms of relative fitness of the organisms that participate in the interaction, or on a population-level effect, with consequence on its growth rate.

Connor divides benefits in three categories:

- 1) By-products: behaviours or attributes of A, designed to benefit A, accidentally benefit B without additional cost to A.
- 2) Invested: benefit invested in another organism because such investments guarantee fitness returns that exceed the initial cost of the investment.
- 3) Purloined: an organism can purloin a benefit from another, e.g. with parasitism or predation.

The combination of these benefits between the two actors of the interaction results then in six different cases that could possibly have originated all the forms of mutualism; these combinations are:

- 1) By-product, by-product: both the actors receive by-product benefits;
- 2) By-product, invested: the actor who receives by-product benefits starts to invest in the other;
- 3) By-product, purloined: a parasite gives by-product benefits to its host;
- 4) Purloined, invested: a parasite begins to invest on its host;
- 5) Invested, invested: each actor invests in the other;
- 6) Purloined, purloined: a host begins to parasitize its parasite.

Even if the debate on the definition is still open, Connor himself in a recent article defines cooperation as “an interaction where both parties receive invested or by-product benefits and one or both parties have an adaptation to increase the receipt of these benefits from the other” (Connor, 2010). In other words, one or both the actors should present adaptive traits to have benefits from the interaction: these could be morphological structures, physiological adaptations or modified behaviours.

Cooperation can be found across taxa, especially in social animals: the swarm behaviours of bees, ants and birds are at the centre of many mathematic models and studies for artificial intelligences (Drias, 2005) and researchers have often used cooperative behaviours in animals as examples for explaining human actions. This is because cooperation is present at all levels of biological organization, from cells (Deisboeck, 2009) to higher metazoans (Carter, 2013) and it is considered a focal point of the studies on human societies.

Despite this, cooperation may appear at odd with the traditional theory of natural selection: the survival of the fittest and competition among organisms have been considered the drivers

of evolution and ecosystem functioning for many years, while cooperation became an “adaptive” phenomenon with a secondary role in the formulation of evolutionary theories (Axelrod & Hamilton, 1981). Yet we can find cooperation in a wide variety of species and even if many ecologists have considered it negligible for many years, its evolutionary role is becoming more and more important, as the understanding of cooperative processes in the development of ecosystems (West et al., 2007).

The main problem of cooperation is that a cooperative action results in an initial cost for the effector, sometimes including extreme acts that lead to a risk for its life. This is the case, for example, of cooperative mobbing against predators in flycatchers (Krams, 2009): when one of these little birds sees a predator (e.g. a hawk), it emits an alarm noise to call the neighbours. These last respond attacking the intruder together and forcing him to retire. The same behaviour is present in meerkats, which also show other mutualistic behaviours like helping conspecifics in the parental care of their progeny (Manser, 2002).

Finding out the benefits that these actions bring to the actors is crucial to understand cooperative behaviours. The simplest way is to look for direct or indirect benefits (Connor, 1995). For example, it is true that taking part in the action enhances the risk for every bird or meerkat of being attacked by the predator, but this choice reduces the possibility that the latter reaches the nest. This leads to an increased direct fitness; in other words, the offspring survives until the reproductive age, permitting the transmission of the genetic heritage, and it is an example of direct benefit.

Another important example of cooperation with direct benefits happens in lions. Even if the possibility of hunting larger prey or to protect cubs more efficiently doesn't seem enough to explain the group patterns of females, many cooperative behaviours such as defence of a common territory and competition with neighbour female groups result in short-term

advantages for them and for their progeny (Packer et al., 1990)

In summary, direct benefits include the possibility of:

- Reaching more or larger prey;
- Being protected from predators;
- A better defence of the territory, of the nests or of the cubs;
- Mating opportunities.

All of these have a clear impact on the individual fitness and can explain quite well the decision of an organism to cooperate.

While in these contexts direct benefits are evident, in other cases it is more difficult to define the costs and the benefits of a cooperative behaviour.

When direct fitness is not involved, we can distinguish two main cases:

- the kinship of the individuals;
- reciprocity (the possibility that the helped individual gives help in return in the future).

Researchers have developed several theories to define the conditions under which cooperation occurs: Hamilton's rule (Hamilton, 1963) for kin selection or the Lehmann & Keller model (Lehmann, 2006), which specifies the requirements to maintain intra-specific cooperation, are perfect examples for the first case. Both, as other studies (Graphen, 1984; Foster, 2006), refer to the preference of individuals to enter in cooperative actions when related organisms are involved. An organism is more willing to cooperate when it can help someone who shares its gene pool, because it can transmit part of its genetic heritage to its progeny; the result is an extended fitness of the helper that justifies the price of the action, called indirect fitness.

Even if relatedness is usually the response behind cooperative actions, cooperation can occur also in non-kin individuals. Researchers have developed several hypotheses to explain this phenomenon, and the debate is still open. Non-kin cooperation is generally associated with reciprocity: the individual offers its help in a first moment, hoping that the other will return the favour in the future. Many theories analyse reciprocity with the aid of game theory and especially with the famous Prisoner's Dilemma, in which two players have a choice between cooperation and defection (Nowak, 2004).

In this model:

- if the first player cooperates and the second one defects, the first one gets a lower payoff or even a damage, while the other takes the highest benefit;
- if both cooperate, they both obtain the highest payoff;
- if both defect, they both take the lowest payoff.

In communities where all individuals are likely to interact with each other and the two players do not meet again, defectors have a higher average payoff than unconditional cooperators (Ohtsuki, 2006). In these communities, cooperators are unable to emerge evolutionarily because defectors have a highest fitness.

Things change when the same two players meet more than once: in this circumstance, cooperators have a huge set of strategies to prevail on defectors. In ecology, the most famous one is the tit-for-tat strategy, in which the player cooperates in the first round and then does whatever the other did previously. The tit-for-tat strategy implies that players can recognize each other; but in those species in which individuals are not able to discriminate the conspecific members this is only possible with continued association and quasi-permanent pairing of individuals, to maintain continuous contact with the same individual and to avoid defection (Axelrod, 1981).

In addition, to the withholding of the rewards of cooperation, researchers thought that punishment by conspecifics could prevent cheaters and that organisms develop systems to isolate or even attack defectors. However, a recent study (Riehl, 2016) demonstrate that cheating and punishment are not so frequent in nature and often occur only in manipulative experiments. In this paper, Riehl proposes that, instead of developing punishment as an evolutionary response, organisms selected conspecifics that showed cooperative behaviours and therefore cheaters are not so common in nature.

All these elements made cooperation an evolutionary stable strategy, widely distributed among species, and an interesting field of ecological research with still open questions.

1.3.2 Protocooperation and group living in populations

Protocooperation is “an interaction between organisms of different species in which both organisms benefit, but neither is dependent on the relationship” (Allaby, 2010); in other words, a form of mutualism in which organisms are not obliged to cooperate.

Recent examples of protocooperation were described in bacteria (Mengjin, 2009), plants (Avishek, 2018) and animals (Maslova, 2016). The term has also been used to describe unconscious cooperation between conspecifics, for instance in sailfish (Herbert-Read, 2016) and in cnidarians (Musco et al., 2018). Many descriptions of protocooperation are present in Allee’s works (Rana, 2005). Allee’s studies were focused on cooperative behaviours and how these could be used in understanding human social dynamics, especially in a comparative psychology perspective. In his work, protocooperation gains an important role in shaping communities, as he was firmly convinced that positive interactions have a fundamental evolutionary role, sometimes more important than negative ones, and that they contribute to the aggregation of organisms. The Allee principle of aggregation states that, even if intraspecific competition for resources (food, space, etc.) may be enhanced, the survival of the group and its organisms is assured by some other kind of benefits, e.g. a better way to defend themselves from predators or abiotic factors, new resources made accessible, or habitat modification (Odum, 1971). The degree of aggregation and the density of the population can be limiting both in conditions of lack of aggregation and overcrowding. The optimum varies among species and even populations.

Early ecological theories supported the importance of competition in the regulation of populations and communities. This resulted in the prediction that positive intraspecific interactions would occur only where competition is weak; nevertheless, gregarious behaviour is also present in communities in which competition is strong (Stachowicz, 2001).

High densities of terrestrial plants (Maestre et al., 2010) or marine sessile invertebrates (Buss, 1981), for example, reduce the exposure of individuals to biotic and abiotic stresses. Buss suggests that this is because benefits of positive interactions can overcome the costs of the negative ones and are normally summarized in three main categories:

- Protection from physical stresses;
- Protection from predators;
- Increased feeding performance.

However, benefits of group living can include the enhancing of the interspecific competitive ability. Since in some ecosystems interspecific competition can be very aggressive, leading to the total replacement of a species, groups can resist in a better way to competitors. In these cases, the reduction of growth rate caused by intraspecific competition is better than mortality induced by the interspecific one (Buss, 1981). Moreover, Leigh suggests that group selection effectiveness is inversely proportional to the population lifetime compared to that of its individuals, and to the number of populations that contribute founders to a new population (Leigh, 1983).

1.3.3 Group living in cnidarians

Group living is common in cnidarians species. Reef forming corals, as well as populations of scyphozoan polyps and colonies of hydrozoans present different forms of polyps, generally related, living together.

Octocorals display a wide range of colonial organization. In this subclass we can find possibilities that go from an extreme to the other, with different levels of complexity. The first one is extremely simple colonies founded by an individual and composed of asexually produced clones with a loose organic connection where individual zooids are essentially independent identical organisms, as in some species of the family Clavulariidae (Bayer, 1973). The order Pennatulacea represents the other one (Bayer, 1973; Achituv & Benayahu, 1990; Soong, 2005), with highly organized colonies and differences in the structure of every zooid, from those with the role of feeding the colony to those with reproduction functions. All the specialized zooids cooperate as a sort of giant individual but cannot function alone (Bayer, 1973). Many halfway conditions are possible, and every species can have a characteristic organization of the colonies. A similar range of possibilities is present in the subclass Hexacorallia (Soong, 1992). The variety of colony morphologies is well-known in hydrozoans too (Cartwright, 2004; Nawrocki & Cartwright, 2010, Nawrocki & Cartwright, 2012) and in some cases colonies can fuse with each other even if they are not closely related (Chang et al., 2018).

Sea anemones can live in aggregating forms composed by clones of the same individual (Francis 1976; Shick & Lamb, 1977). Scyphozoan polyps live on different substrates (Holst & Jarms, 2007; Uriz et al. 1992; Jarms et al., 2002), in many cases forming vast aggregations (Jarms, 1991; Astorga et al., 2012) with polyps living together, but not interconnected.

1.3.4 Cooperation in cnidarians

Little is known about positive interactions in cnidarians.

In colonial species, polyps of the same colony are related; thus, researchers separated polyps of *Lobophyllia corymbosa* from different colonies to see if they are able to recognize non-kin conspecifics (Philipps, 2006); results show that they do recognize unrelated individuals and refuse to fuse and cooperate with them. The presence of symbiosis (Montano et al., 2014; Montano et al., 2015) between some hydrozoan species and reef corals, as well as the possible defensive role of hydroids in this association (Montano et al., 2017) suggest that positive interactions may occur in the phylum, but more research and attention is needed. Hydrozoan colonies show cooperation (Bavestrello et al., 2000): hydroids of *Perarella schneideri* were observed eating a polychaete worm larger than single polyps together. Christensen (Christensen, 1976) observed other hydroid colonies, in which several polyps capture large prey items, such as nematodes. Francis (Francis, 1976) registered a difference in contacts among clonemate and non-clonemate polyps of *Anthopleura elegantissima*. An enhanced feeding success seems to happen in *Paramuricea clavata* (Coma et al., 1995) and *Galaxea fascicularis* (Wijgerde, T. et al., 2012). Shick (Shick & Lamb, 1977) reports the observations of Hausman (Hausman, 1919) of *Orchestia agilis* easily escaping from a single polyp of *Haliplanella luciae*, but being eaten by its gregarious form.

Recent studies showed examples of proto-cooperation between polyps of different colonies (Musco et al., 2018). The observation of polyps of the Mediterranean endemic coral *Astroides calycularis* blocking and eating jellyfish of *Pelagia noctiluca* (Musco et al., 2018), which is ten times bigger than a single polyp, has been considered a case of proto-cooperation. This behaviour was recorded in different areas of the Mediterranean Sea, and it was observed both in polyps of the same colony and of different ones.

Aim of this research project

The research focussed on protocoooperation and used as starting point the observations carried out by Musco et al. (2018).

The research hypotheses are:

- Other than *A. calycularis*, the protocoooperation behaviour is present in other cnidarian taxa such as scyphozoans
- Small scyphozoan polyps in group can catch prey larger than prey they are able to catch individually
- In presence of both small and large prey scyphozoan polyps give priority to small easily accessible prey, thus avoiding cooperation
- Proto-cooperative behaviour in cnidarians is modulated by chemical signals that stimulate scyphozoan polyps to focus towards the prey

Chapter II

MATERIALS AND METHODS

2.1 *Aurelia sp. polyyps*

The Aquarium of Cattolica provided polyyps of *Aurelia* sp. on October 24th, 2018 and on March 7th, 2019. Our specimens correspond to the description of the unidentified species *Aurelia* sp. 1 (Gambill & Jarms, 2014) but we were unable to verify the provenience of the polyyps and their jellyfish stage.

After collecting polyyps in plastic jars filled with natural filtered seawater, we put the jars in a thermal bag and then we transported it in the laboratory within 3 hours.

There we transferred the polyyps in several plastic bowls (15-20 polyyps for each one) covered with a plastic top or plastic wrap in order to rear them; the acclimation and settlement lasted 7-10 days.

Bowls were kept inside a thermostatic chamber (Figure 7), maintaining a constant temperature ($18 \pm 1^\circ\text{C}$) and photoperiod (L:D 16h:8h).

Once a week, polyyps were fed with nauplii of *Artemia salina* (Linnaeus,

1758) and the water was partially changed with natural filtered seawater.



Figure 7 Thermostatic chamber with plastic bowls containing polyyps

2.2 Behavioural Sampling Methods

Using videos and observations in real time, we performed behavioural studies for each group, in order to be aware of the normal behaviour of polyps in the plastic bowl.

We established a Behaviour Code Reference, as follow:

- **Alert/Research (AR):** The polyp moves actively its body and tentacles in the surrounding area
- **Feed/Mouth open (FM):** The polyp opens its mouth, or brings one or more tentacles to it.
- **Contact (CO):** One or more tentacles touch or are in contact with a part of the body of a conspecific.
- **Rest (RE):** The polyp is static.
- **Other (OT):** All the behaviours that are not listed above.

Then we evaluated different methods from “Measuring Behaviour: an Introductory Guide” (Martin & Bateson, 2007) and chose the “Scan Instantaneous Sampling”. It consists in the observation of each organism of the group for a previously established amount of time and in the recording of its behaviour at fixed time intervals.

In our behavioural tests, we observed the polyps for five minutes and the behaviour of each polyp of the group was recorded at time intervals of 15 seconds during the period of observation. Time was later split in two periods, each of two minutes and half.

2.3 Experimental preys

2.3.1 The polychaete *Syllis prolifera*



Figure 8 Female of *Syllis prolifera* (Courtesy of Luigi Musco)

Syllis prolifera (Krohn, 1852) is a polychaete species of the Syllidae family and it is very common in temperate-warm waters (Musco & Giangrande, 2005), easy to find in littoral vegetated rocky reefs and seagrass meadows, although it is omnivorous (Giangrande et al., 2000).

This species is gonochoric and characterized by epitoky reproduction through stolonization (Franke, 1999) (Figure 8). It can be easily cultured in laboratory (Gallucci & Gambi, 2014). We chose this species as “large prey” owing to its size (10-25 mm) (Hayward & Ryland, 2017).

In fact, they appear too long for being captured by a single polyp, and long enough to be caught by pairs or groups of polyps. Moreover, specimens are easy to collect and in preliminary trials they resulted edible for the polyps: they did not show defensive responses and polyps did not reject them.

They were collected from populations sampled in vegetated rocky reefs from the Passetto in Ancona at 30-50 cm depth. After scraping off the substrate using a net with an indented edge, the algal cover were collected within net bags, rapidly transferred into tanks filled with

natural seawater. We transported the samples to the laboratory within 30 minutes after collection and rapidly sorted. Small amount of the collected substrate (rocky substrate + macroalgae thalli) was processed at the time inside the tray covered with enough sea water and, if necessary, shaken vigorously in order to dislodge the macroinvertebrates. Polychaete individuals were sorted by visual identification, collected using pipettes and carefully transferred in a glass bowl until the species identification.

We maintained polychaetes, following a protocol present in literature (Gallucci & Gambi, 2014), in natural seawater in 100 ml glass dishes with sloping sides and flat bottom. In order to avoid excessive evaporation and increase of salinity, we placed the culture dishes in a glass container over paper blotted with distilled water and closed with a perforated plastic wrap, making sure of allowing exchanges between the inner and the outer environment.

2.3.2 Nauplii of *Artemia salina*

The brine shrimp *Artemia salina* (Linnaeus, 1758) is a well-known species and its nauplii are widely used in aquaculture (Asem et al, 2010) as food for many organisms, e.g. foraminifers, coelenterates, flatworms, polychaetes, squids, insects, chaetognaths and a wide variety of both marine and freshwater crustaceans and fishes. It is not exactly known if this is due to their biochemical composition, their very thin carapace, the fact that they are a moving prey (swimming) or a combination of all these factors (Sorgelos et al, 1986)

The capability of forming cysts which are in fact inactive embryos and the fact that cysts are commercially available, can be stored for years and only have to be incubated for 24 hr in seawater to produce a large number of free-swimming larvae make *A. salina* a perfect organism for laboratory cultures.

2.4 The experiments

2.4.1 Successful catches in group and individuals

To test the ability of groups and single polyps to catch a prey bigger than their mouth gape, we performed the following experiments in both circumstances, to make a comparison between their success rates.

Every day before the feeding session with *A. salina*, we measured the length of a specimen of *S. prolifera* with the ruler of a stereoscopic microscope. Then we collected and inserted it in one of the plastic bowls containing *Aurelia* sp. polyps with a plastic pipette. We observed the behaviour of the polyps with the stereoscopic microscope and filmed it with a Nikon D610 camera assembled on the top of it. (Figure 9)



Figure 9. Stereoscopic microscope with Nikon D610 camera

After checking the group who captured the worm, we measured the area where it fell and its density of polyps. The time that polyps spent in blocking and dismembering the prey was also measured.

We followed the same procedure with isolated individuals, in order to compare the success rate of the group and of single polyps, respectively, and we considered successful those catches in which polyps effectively killed and ingested worms.

2.4.2 Food preferences

In order to investigate on whether the polyps lose interest in the bigger prey in presence of a smaller one, we decided to observe their behaviour feeding them with both worms and nauplii.

As for the previous ones, we carried out the experiments with polyps in fasting conditions and we provided them with a polychaete worm. After three minutes we inserted in the plastic bowl 1.5 ml of water filled with nauplii, which is a quantity that allows every polyp to reach a great amount of preys.

Then we observed the behaviour of polyps that had caught the worm when nauplii reached their tentacles.

2.4.3 Chemical signals

Since polyps who could not reach the provided worm seemed interested in and stretched towards the area in which the others were eating, even without touching the prey, we checked the presence of chemical signals in the water that could reach the polyps, activating an alert behaviour.

Twelve polychaete worms of different lengths were collected, filtered with a cell strainer with a 40 μm mesh and put in an Eppendorf tube, where they were chopped with a plastic stick.

We added filtered seawater to reach a 1.5 ml volume, and we blended the mixture, that was successively filtered with the cell strainer in order to collect only the water in an Eppendorf. We collected 0.1 ml of the water obtained with a syringe and we pushed it into each of 15 different plastic bowls containing polyps. Polyps reactions were observed and filmed for 5 minutes.

We repeated the same experiment with nauplii of *Artemia*, collected on the cell strainer, pressed and blended in the same amount of water with the help of a vortex mixer.

In the end, we reproduced the experiments with only filtered seawater, in order to understand if the mechanical signal of the water flow, without any food chemical compound, had effects on the polyp behaviour.

Chapter III

RESULTS

3.1 Activity budget



Figure 10 Polyps of Aurelia sp. showing resting behaviour

static pose, simply waiting. (Figure 10)

Sometimes, however, they showed sporadic feeding behaviour (FM), opening their mouth or leading a tentacle to it, as showed in Figure 11, with a percentage of 19,98% of the observed behaviours.



Figure 11 Polyp of Aurelia sp. showing Feeding-Mouth Open behaviour

On a sample of 53 polyps, the main behaviour resulted from the Scan Instantaneous Sampling is rest (RE), which occupies the 65,46% of the registered behaviours. In control situations (no food or water insertion in the plastic bowls), polyps spent most of their time in a



Figure 12 Polyps of Aurelia sp. showing Alert-Research behaviour

Alert behaviour (AR), with polyps actively extending their tentacles and calyx in different directions, looking for a prey (Figure 12), and Contact (CO), with polyps

touching the conspecific body or tentacles with their tentacles (Figure 13) between polyps, are very limited in control conditions (7,59% and 6,96% respectively). We did not detect other behaviours. (Figure 14)

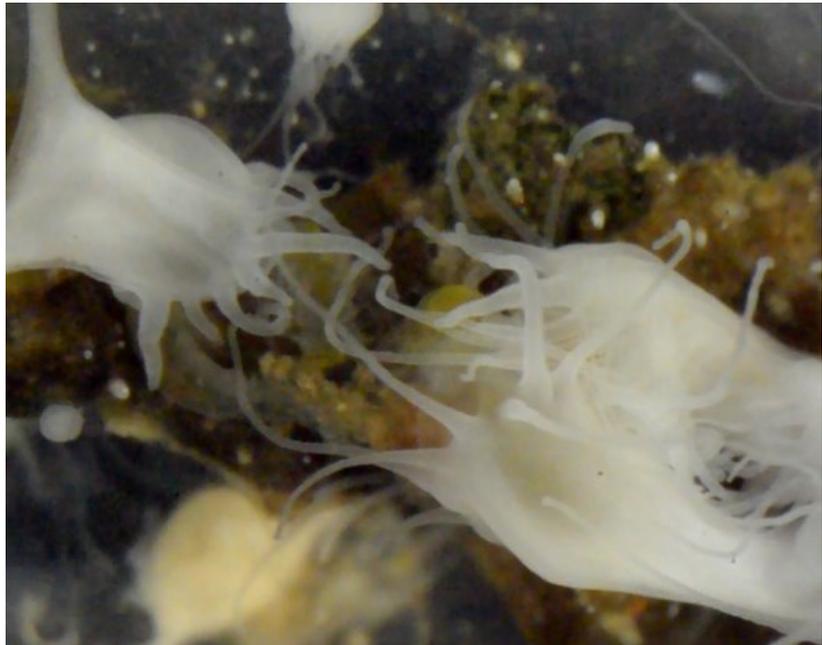


Figure 13 Polyps of Aurelia sp. showing Contact behaviour

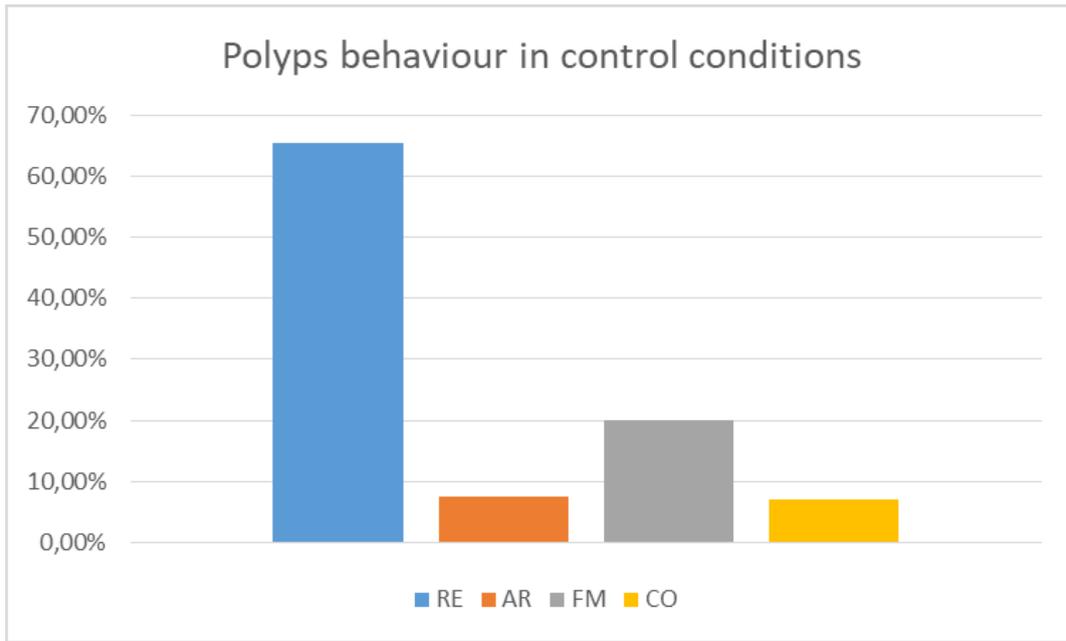


Figure 12 All behaviours showed in control conditions. RE=Rest; AR= Alert; FM= Feeding; CO= Contact.

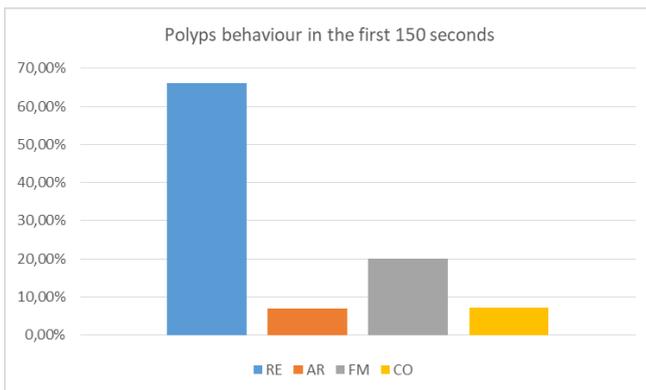


Figure 15 Behaviours recorded in the first 150 seconds in control conditions. RE=Rest; AR= Alert; FM= Feeding; CO= Contact.

We also split the time of observation in two periods of two minutes and half each (150 seconds). In the first one (Figure 15), resting (RE) and feeding (FM) behaviours represented the 66,04% and 20,07% of the registered behaviours, while active research (AR)

and contact between polyps (CO) were limited to 6,86% and 7,03% respectively. In the last 150 seconds (Figure 16), the percentage of resting (RE) and feeding (FM) behaviours represented the 63,45% and 22,55%,

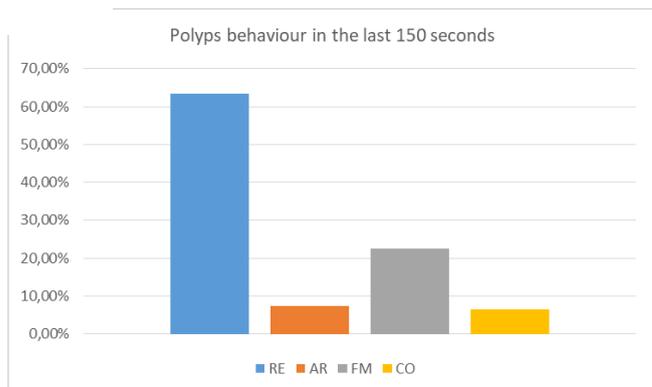


Figure 16 Behaviours recorded in the last 150 seconds in control conditions. RE=Rest; AR= Alert; FM= Feeding; CO= Contact.

while active research (AR) rose to 7,45% and contact between polyps (CO) decreased to 6,55%.

3.2 *Single polyp success*

3.2.1 Polyps behaviour

Single polyps could not catch *Syllis prolifera*, even if they always tried to.



Figure 17 Polyp catching *Syllis prolifera*

In some cases they managed to block the worm, but this

was a very rare case. In these cases, however, the polyps blocked the prey from the middle of its body (Figure 17).



Figure 18 Single polyp eating the polychaete

In one case, the ingestion was too difficult and the worm was rejected. Moreover, these polyps seemed unable to eat the entire worm and left part of it when their

gastrovascular cavities were full (Figure 18).

3.2.2 A special case: autotomy of the prey



Figure 19 Autotomy of Syllis when caught



The terminal parts of *Syllis prolifera* underwent autotomy. In fact, being unable to escape with only its movements, in a very short time (typically seconds) the worm is able to detach the terminal part of its body, which is able to escape (Figure 19). In this case, since the pygidial end keeps moving for a while, the polyp ate it anyway.

The polyp, on the other side, seemed to invest less energy and time in blocking the prey: in many cases, the escape of the worm coincided with the retirement of tentacles by the polyp.

3.2.3 Data

Specimens of *Syllis prolifera* that were given to single polyps ranged from a minimum of 7,5 mm to a maximum of 12,5 mm (Figure 20), with an average length of 10,24 mm.

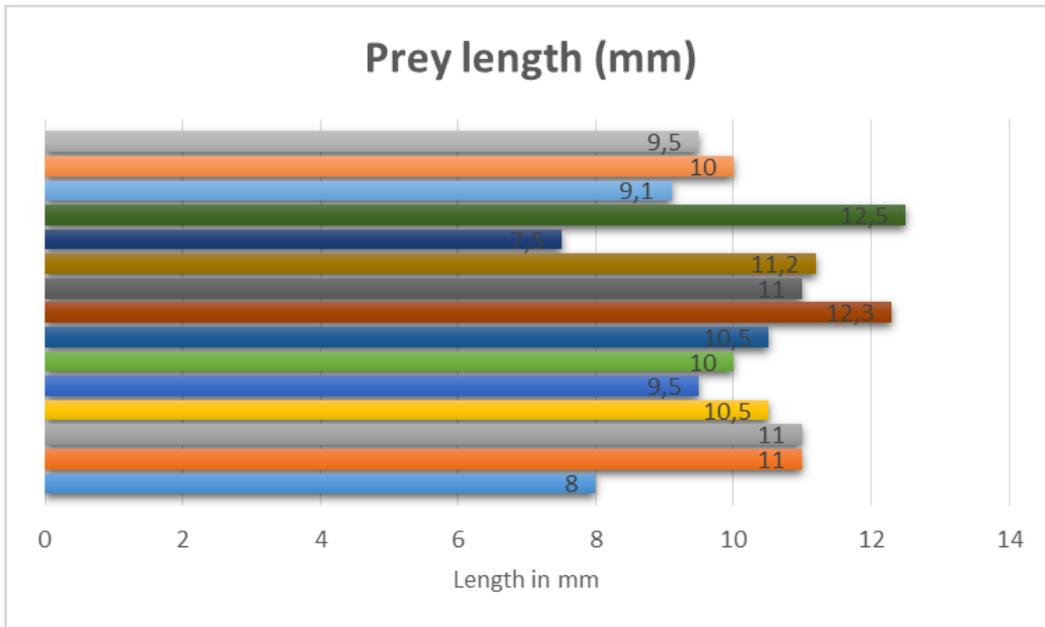


Figure 20 Length of worms given to single polyps

The worm measured in the four cases of successful ingestion had a length ranging from 7,5 mm to 11,2 mm (Figure 21).

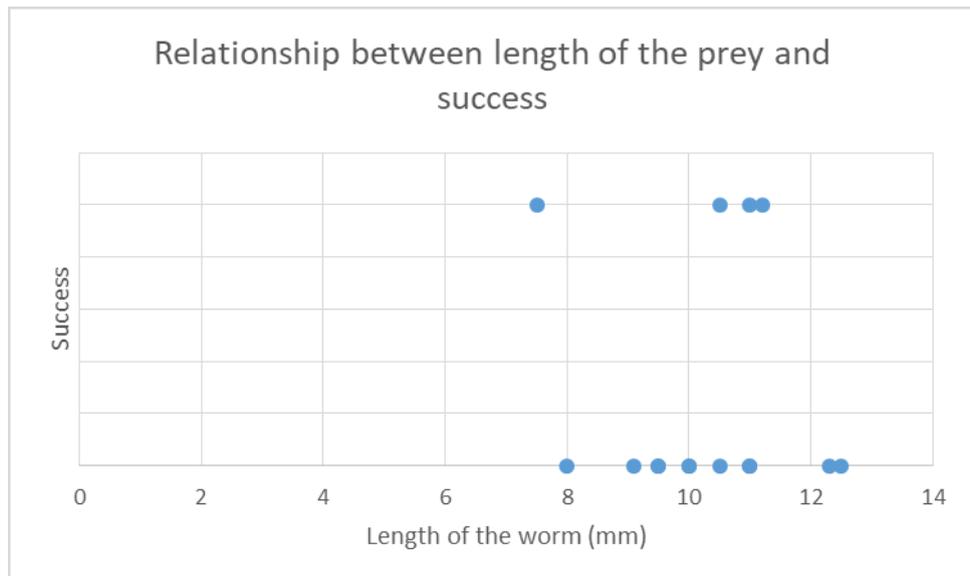


Figure 21. 0= worm not ingested 1= worm successfully ingested

Table 2 Length of polychaete worms given to single polyps and respective success

Test	Length of the worm (mm)	Success
1	8,0	X
2	11,0	X
3	11,0	√
4	10,5	X
5	9,5	X
6	10,0	X
7	10,5	√
8	12,3	X
9	11,0	X
10	11,2	√
11	7,5	√
12	12,5	X
13	9,1	X
14	10,0	X
15	9,5	X
Average	10,24	
Σ	1,35	

On 15 experiments, single polyps were able to catch and ingest the worm only in four cases. This led to a percentage of success of 27% on all trials. (Figure 22)

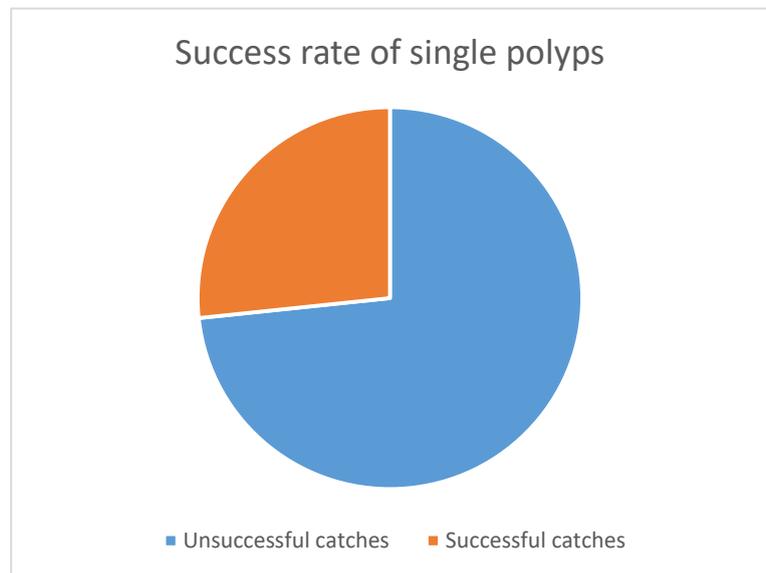


Figure 22 Percentages of successful catches on all attempts

3.3 Successful catches of groups

3.3.1 Polyps behaviour

When the worm reached the mouth or tentacles of more than one polyp, each polyp reacted grasping the head, the middle or the final part of the worm's body, slowly preventing any possible escape. (Figure 23)



Figure 23 Syllis proliferata falling among a group of polyps

The mechanical block caused by tentacles and the action of nematocysts paralyzed the prey while polyps pulled in their own direction, sometimes in addition to a rotating movement of the scyphistoma, which became more evident in the final phases of the catch, when this action helped the distribution of the prey among participants. (Figure 24)



Figure 24 Blocking of the polychaete worm

When the worm ceased to fight, polyps moved closer to each other, sometimes extending (especially when in pairs) their calyx and tentacles to reach tentacles and mouth of the neighbouring polyp, while ingesting its own part of the prey (Figure 25).



Figure 25 Polyps moving towards each other

By a rotatory movement of the scyphistoma, combined with pulling in different directions, polyps divided several parts of the prey and abandoned the remaining part of the worm to other polyps, which took their own part of the prey (Figure 26).



Figure 26 Polyps dividing the prey

This process lasted hours, typically 2-7, depending on the length of the worm, on the number of polyps and on the distance among them.

It happened that some polyps quitted the catch, especially polyps that were excessively far from the prey or in a position that did not allow them to properly hold of the prey.

3.3.2 Data

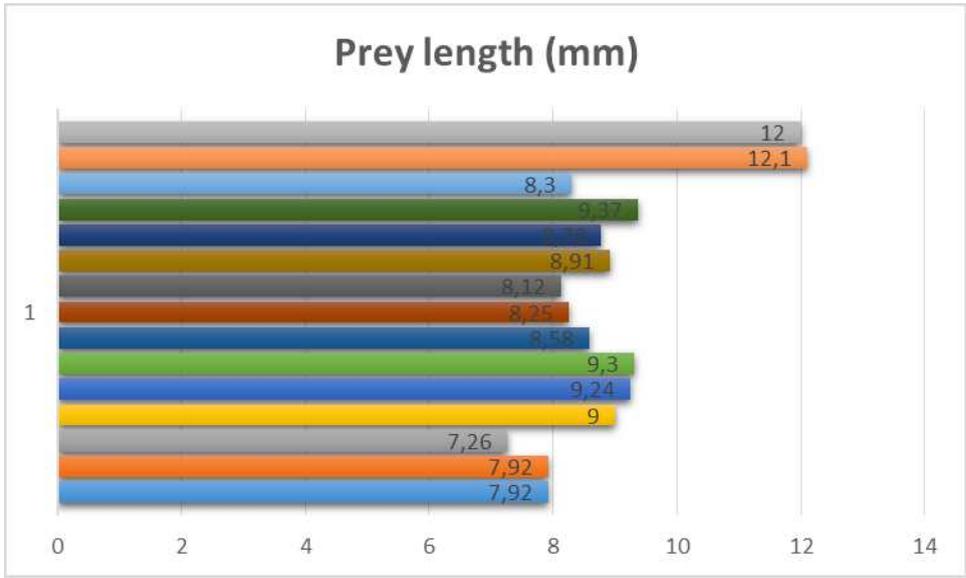


Figure 27 Length of polychaete worms given to groups

Specimens of *Syllis prolifera* that were given to groups of polyps ranged from a minimum of 7,26 mm to a maximum of

12,1 mm (Figure 27), with an average length of 9,0 mm. Worms were successfully ingested in all trials.

The area of catch, measured after the worm was caught, ranged between 43,56 and 192 mm², with an average of 96,21 mm² (Figure 28).

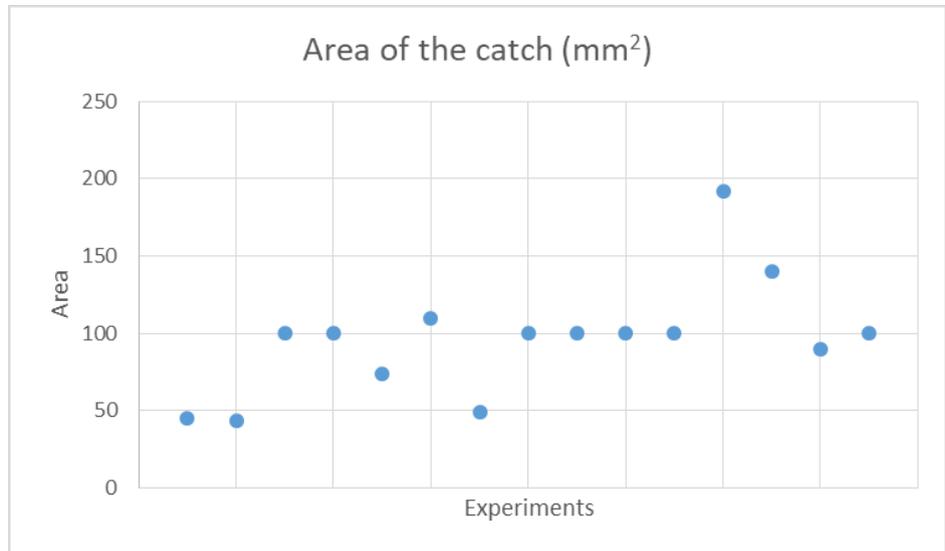
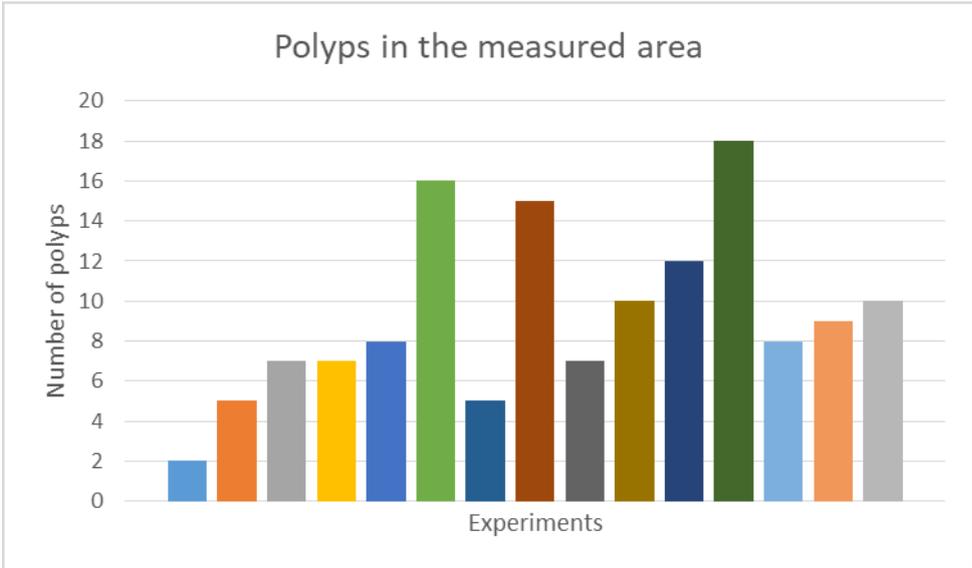


Figure 28 Area of the catch in each experiment



The number of polyps ranged from 2 to 18 (Figure 29), with an average of 9,27 polyps.

Figure 29 Number of polyps in the measured area for each experiment

Density ranged from 0,44 to 1,45 polyps/10 mm² (Figure 30) with an average of 0,96 polyps/10 mm².

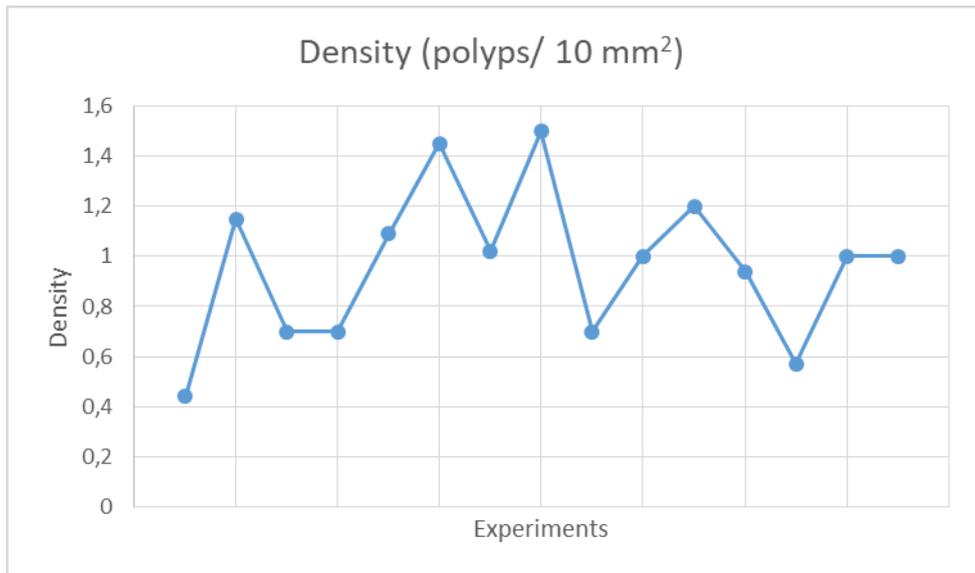


Figure 30 Density of polyps (polyps/ 10 mm²)

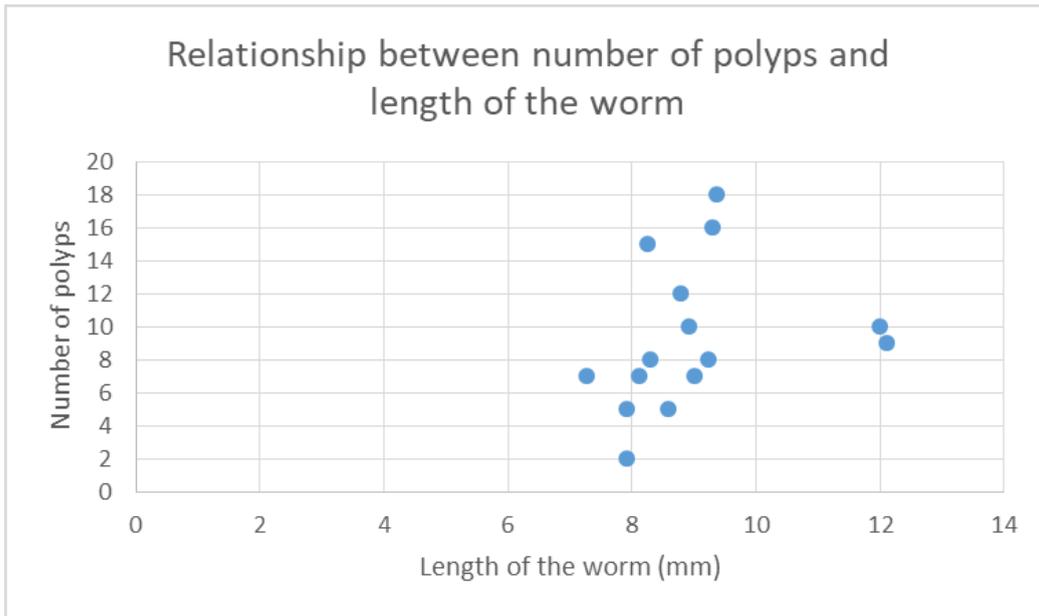


Figure 31 Number of polyps in the measured area and length of the worm

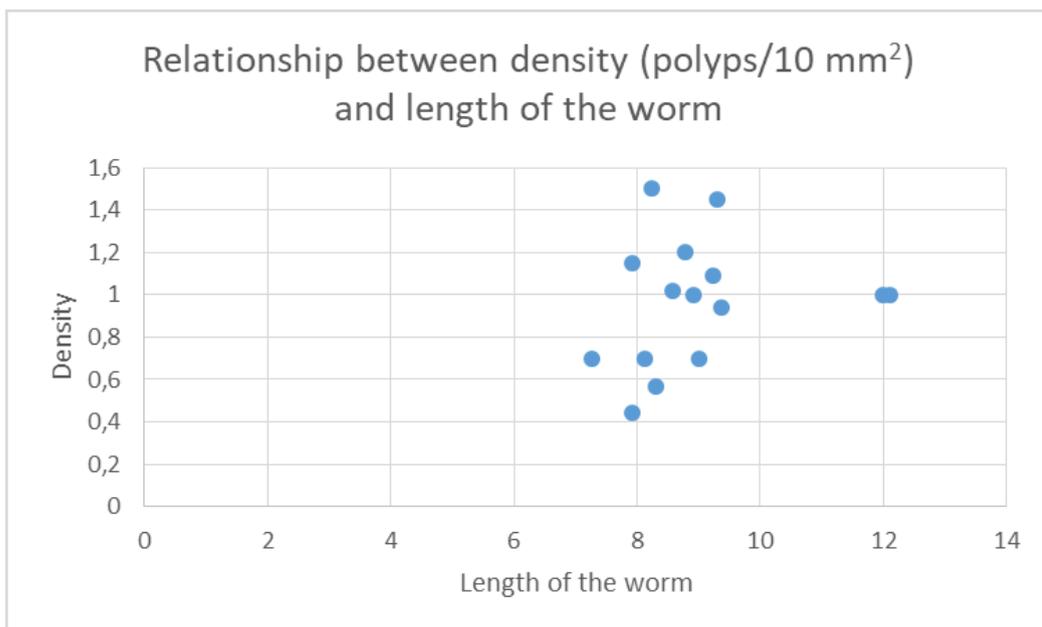


Figure 32 Density and length of the worm

In Figure 33, for each test, the x-axis shows the area of the catch (in mm²), the y-axis shows the number of polyps and the size of the circles represent the density of polyps

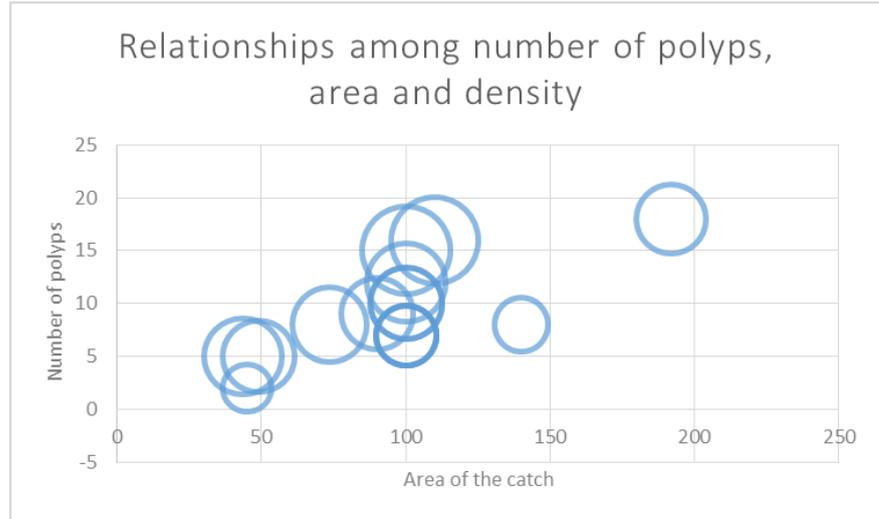


Figure 33 Area, number of polyps and density of each catch

(polyps/10 mm²). All data are reported in Table 3.

Table 3 Group experiments data

Test	Worm lenght (mm)	Area of the catch (mm ²)	Number of polyps/ measured area)	Density (polyps/10mm ²)	Success
1	7,92	45	2	0,44	√
2	7,92	43,56	5	1,15	√
3	7,26	100	7	0,7	√
4	9	100	7	0,7	√
5	9,24	73,62	8	1,09	√
6	9,3	110	16	1,45	√
7	8,58	49	5	1,02	√
8	8,25	100	15	1,5	√
9	8,12	100	7	0,7	√
10	8,91	100	10	1	√
11	8,78	100	12	1,2	√
12	9,37	192	18	0,94	√
13	8,3	140	8	0,57	√
14	12,1	90	9	1	√
15	12	100	10	1	√
Average	9,00	96,21	9,27	0,96	
σ	1,32	36,10	4,25	0,29	

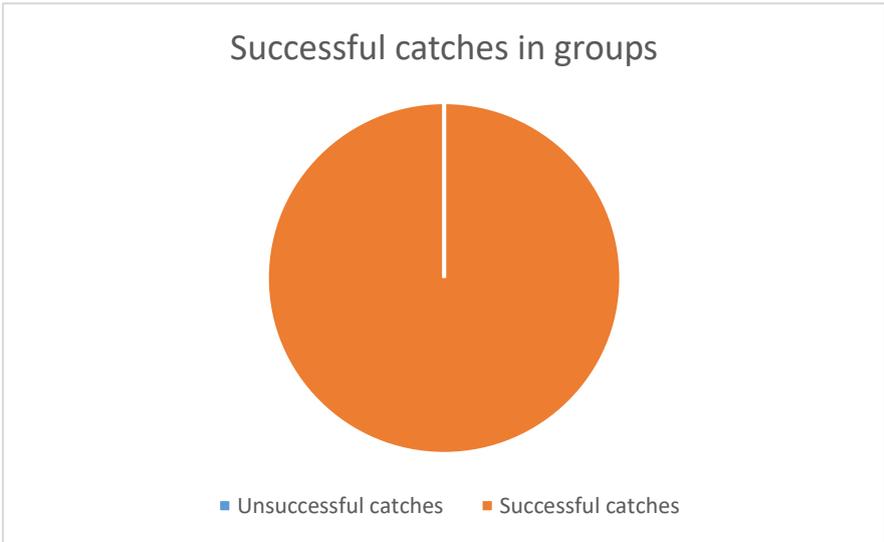


Figure 33 Percentage of successful catches of the group on total catches

On 15 experiments, two or more polyps were always able to catch the polychaete worm, with a success rate of 100% (Figure 33).

In order to evaluate how many polyps

effectively had a benefit for taking part in the catch, we compared the number of initial

polyps that cooperated with the final number of polyps that effectively gained a part of the prey at the end of the action. (Figure 34)

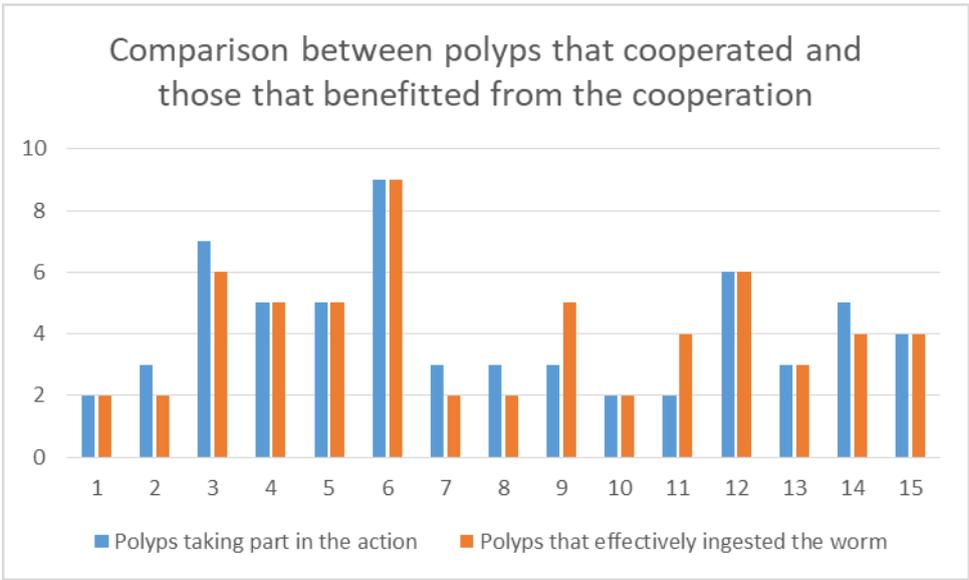


Figure 34 Comparison between first and final polyps

Table 4 Comparison between initial and final polyps

Test	Polyps taking part in the action	Polyps that effectively ingested the prey	Delta
1	2	2	0
2	3	2	-1
3	7	6	-1
4	5	5	0
5	5	5	0
6	9	9	0
7	3	2	-1
8	3	2	-1
9	3	5	+2
10	2	2	0
11	2	4	+2
12	6	6	0
13	3	3	0
14	5	4	-1
15	4	4	0
Average	4,13	4,06	
Σ	1,97	1,95	

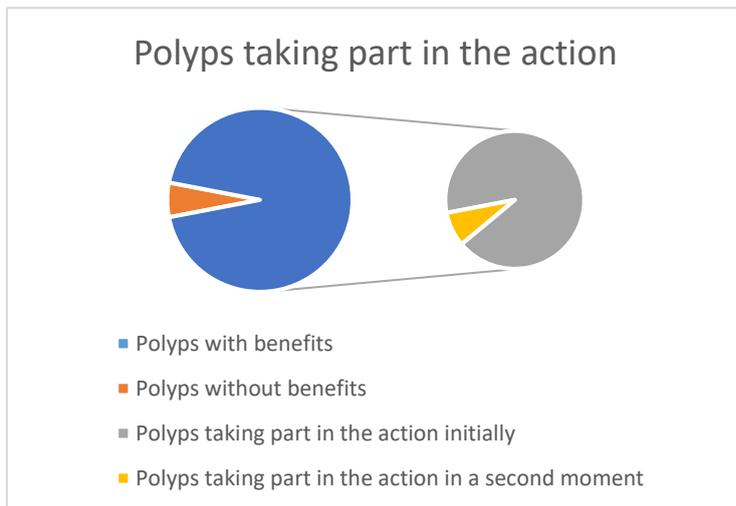


Figure 35 Relationships between polyps that took part in the action and polyps that had a benefit

On an average of 4,13 polyps taking part in the action at the beginning, an average of 4,06 had a benefit from the catch; in some cases, other polyps added themselves to the initial group, increasing the number of polyps that effectively ingested the worm at the end of the

observations (Figure 35).

3.4 Food preference

3.4.1 Polyps behaviour

In this experiment, polyps were provided of both nauplii and worm in the same time. Three different behaviours were observed.

In the first case, nauplii that reached tentacles and mouth of the polyps, or even the worm that was captured, were immediately paralyzed. Polyps slowly collected a certain amount of nauplii while enduring the blocking and division of the bigger prey, and then ingested both preys together (Figure 36). As in the other experiments, polyps taking part in the action cut the bigger prey with rotatory movements and divided it.



Figure 36 Polyps ingesting both the worm and nauplii

In the second case, polyps did not lose or reject the dead polychaete, but they preferred to ingest nauplii, losing interest in the bigger prey, even if they maintained it in their gastrovascular cavities, without dividing it.

In the third case, the arrival of the smaller preys induced the polyps to leave the worms, even if already paralyzed or dead, and to focus on the nauplii. This happened only in one experiment.

Polyps that were in unfavourable positions to reach the worm changed their focus on nauplii in a very short time. However, some of them could come back to the worm even if their gastrovascular cavity was full of *Artemia*.

3.4.2 Data

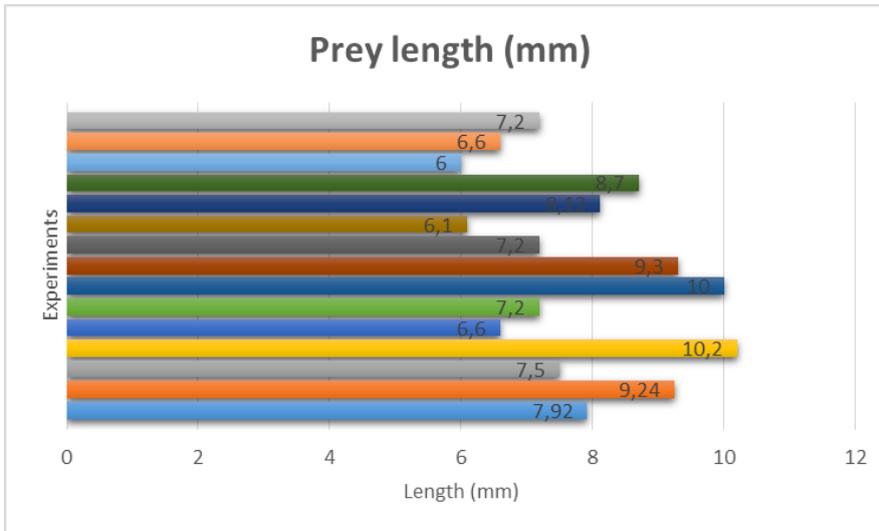


Figure 37 Length of the worm in food preference experiments

The polychaete worms used in these experiments had a range from 6 to 10 mm (Figure 37); only one worm was rejected measuring 6,6 mm.

Trial	Length of the worm (mm)	Reactions
1	7,92	Both nauplii and worm ingested
2	9,24	Both nauplii and worm ingested
3	7,5	Worm not rejected, but not ingested
4	10,2	Worm not rejected, but not ingested
5	6,6	Worm rejected
6	7,2	Worm not rejected, but not ingested
7	10	Worm not rejected, but not ingested
8	9,3	Worm not rejected, but not ingested
9	7,2	Worm not rejected, but not ingested
10	6,1	Both nauplii and worm ingested
11	8,12	Worm not rejected, but not ingested
12	8,7	Worm not rejected, but not ingested
13	6	Worm not rejected, but not ingested
14	6,6	Worm not rejected, but not ingested
15	7,2	Worm not rejected, but not ingested
Average	7,86	
Σ	1,32	

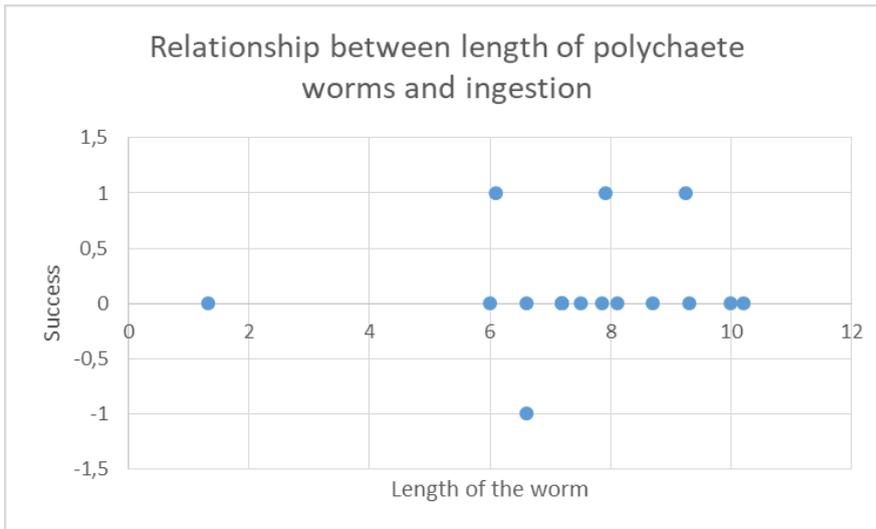


Figure 38. -1: worm rejected; 0: worm neither rejected nor ingested; 1: both nauplii and worm ingested

Experiments with the combination of polychaete worms and nauplii of *A. salina* resulted in three different scenarios (Figure 38):

1. Both nauplii and the worm were ingested together, with polyps cutting the worm and coming back to a resting behaviour without remaining in contact with the others.

2. The worm remained in the gastrovascular cavity of polyps, but nauplii were ingested; polyps did not cut the worm and they maintained contact with the others.

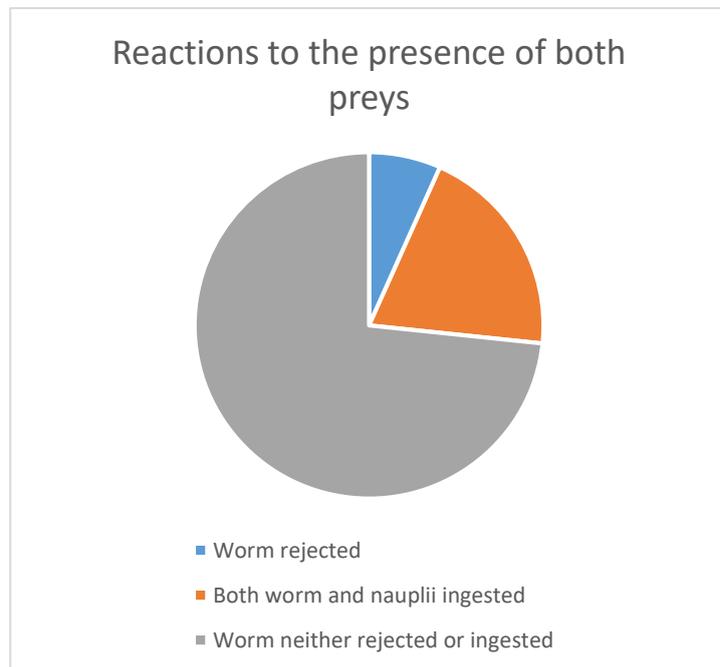


Figure 39 Percentage of behaviours recorded in food preference experiments

3. The worm was rejected by the polyps, which normally fed on *Artemia* (Figure 39)

3.5 Chemical signals

3.5.1 Polyps behaviour

We provided chemical stimuli to the polyps by adding water containing homogenated prey. Even if the amount of water inserted in each bowl was small, almost all polyps reacted immediately showing their normal feeding behaviour (Figure 40): some opened their mouth or approached their tentacles to the mouth, while almost all of them adopted an alert behaviour with tentacles exploring the surrounding environment.



Figure 40 Feeding behaviour

This happened with the water filtered with both nauplii and polychaete worms.

On the other side, tests with only the seawater flow did not trigger the same reactions, and most polyps kept resting in a static position most of the time, or rearranged their tentacles, sometimes showing alert

behaviours.

3.5.2 Data

Observations of a sample of 53 polyps with the Scan Instantaneous Sampling revealed a difference between polyps that were exposed to the water flow alone and those who received water obtained from the dead preys. Our time of observation was five minutes for each experiment, as for previous experiments.

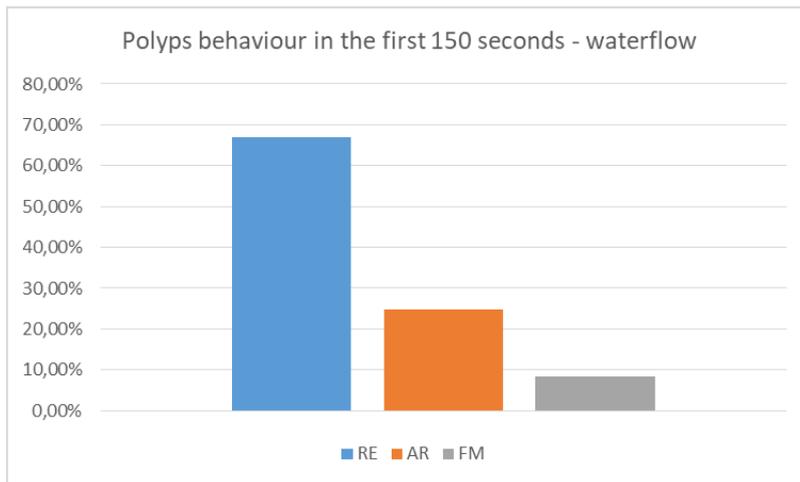


Figure 41 Activity budget of the first 150 seconds in water flow conditions. RE=Rest; AR= Alert; FM= Feeding.

After the water flow, polyps came back to behaviours similar to those already registered in previous observations, with Resting behaviour prevailing on other behaviours (67,07%),

followed by the Alert-Research (24,7%) and Feeding-Mouth open (8,43%) in the first 150 seconds (Figure 41).

In the last 150 seconds, resting occupied 75,08% of all behaviours, Alert-Research 15,25% and feeding behaviour represented 9,67% (Figure 42).

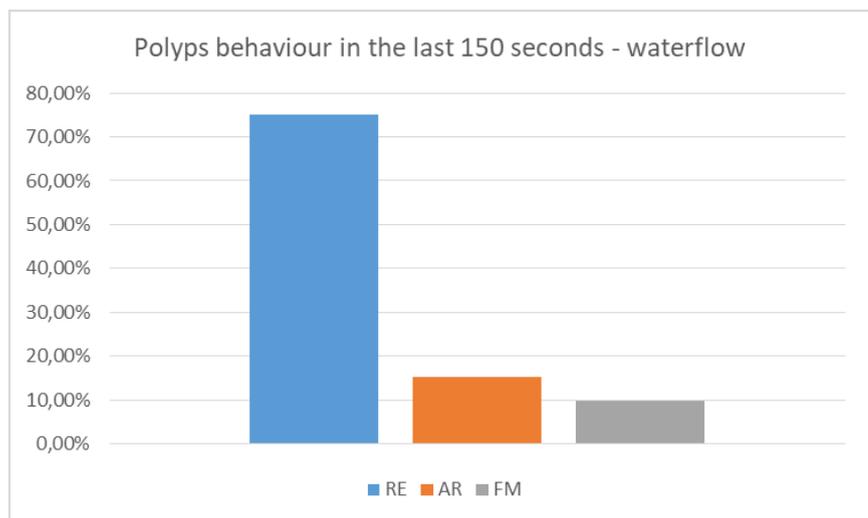


Figure 14 Activity budget of the last 150 seconds in water flow conditions. RE=Rest; AR= Alert; FM= Feeding.

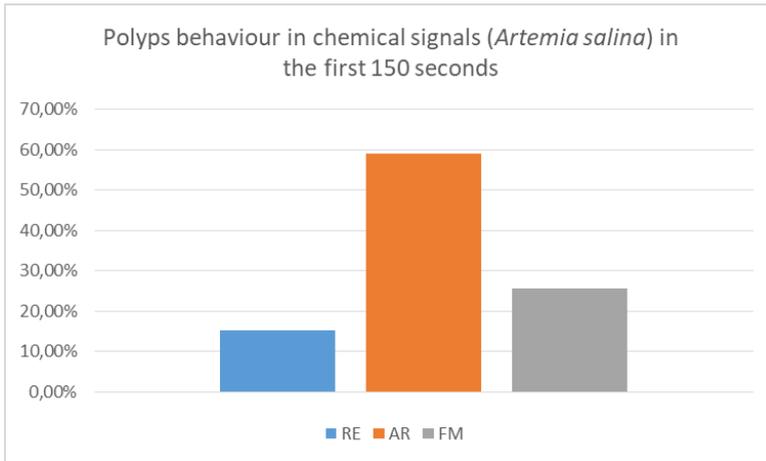


Figure 42 Activity budget of the last 150 seconds with Artemia salina.

RE=Rest; AR= Alert; FM= Feeding.

In experiments with water in which nauplii of *A. salina* were filtered, the Resting behaviour went down to 15,27%, while Alert-Research behaviour rose to 59,18% and feeding behaviour to 25,56% in the

first 150 seconds of observation (Figure 42).

In the last 150 seconds, resting occupied 37,74% of all behaviours, while research and feeding behaviours were 48,11% and 14,15% respectively (Figure 43).

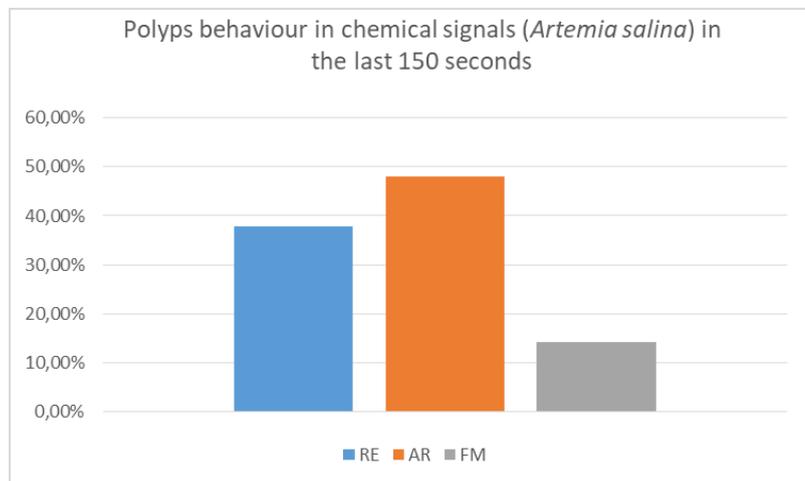


Figure 43 Activity budget of the last 150 seconds with Artemia salina.

RE=Rest; AR= Alert; FM= Feeding.

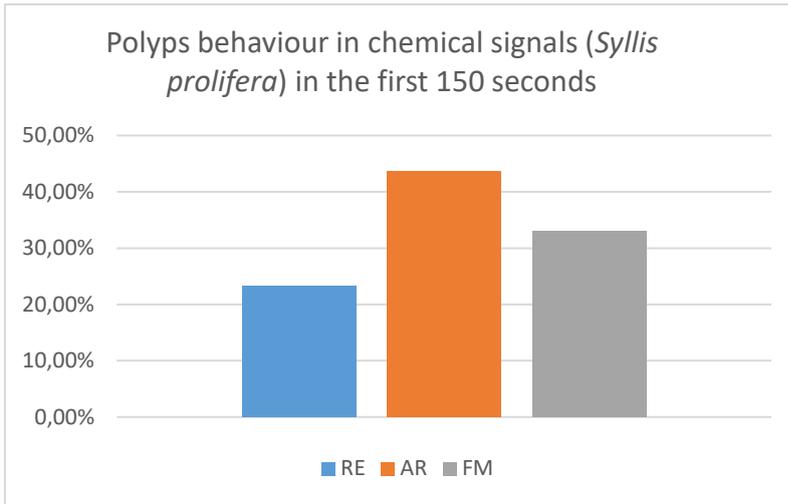


Figure 44 Activity budget of the first 150 seconds with *Syllis prolifera*.

RE=Rest; AR= Alert; FM= Feeding

In experiments with water in which stimuli were obtained from specimens of *Syllis prolifera*, Alert-Research behaviours were 43,57% of all registered behaviours, while Feeding-Mouth Open behaviours and Resting behaviours

represented 33,10% and 23,23% respectively (Figure 44)

In the last two minutes and half, polyps seemed to come back to Resting behaviour (41,51%) and Feeding-Mouth Open behaviours decreased (22,08%), while Alert-Research behaviour remained high (36,42%) (Figure 45).

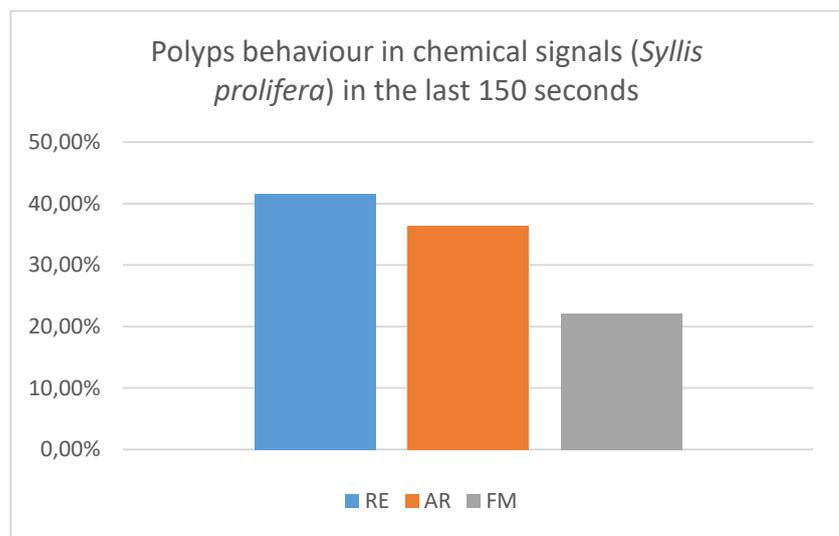


Figure 45 Activity budget of the last 150 seconds with *Syllis prolifera*.

RE=Rest; AR= Alert; FM= Feeding

Figure 46 and Figure 47 show a comparison among the three different situations, in the first 150 seconds and in the final 150 ones.

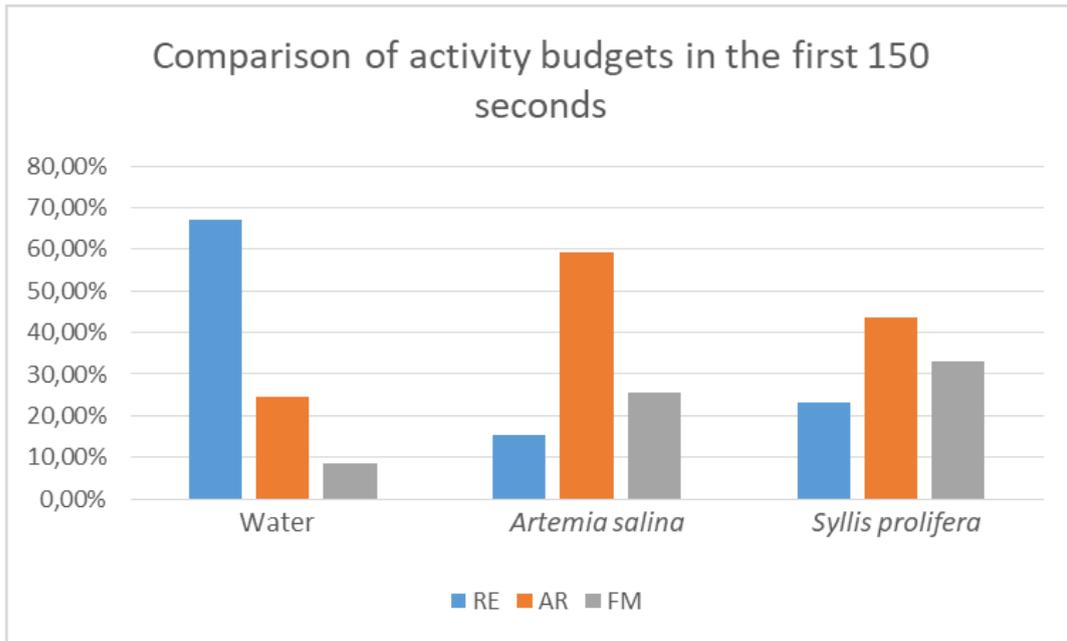


Figure 46 Comparison among different conditions of chemical signals experiments in the first 150 seconds.

RE=Rest; AR=Alert; FM= Feeding

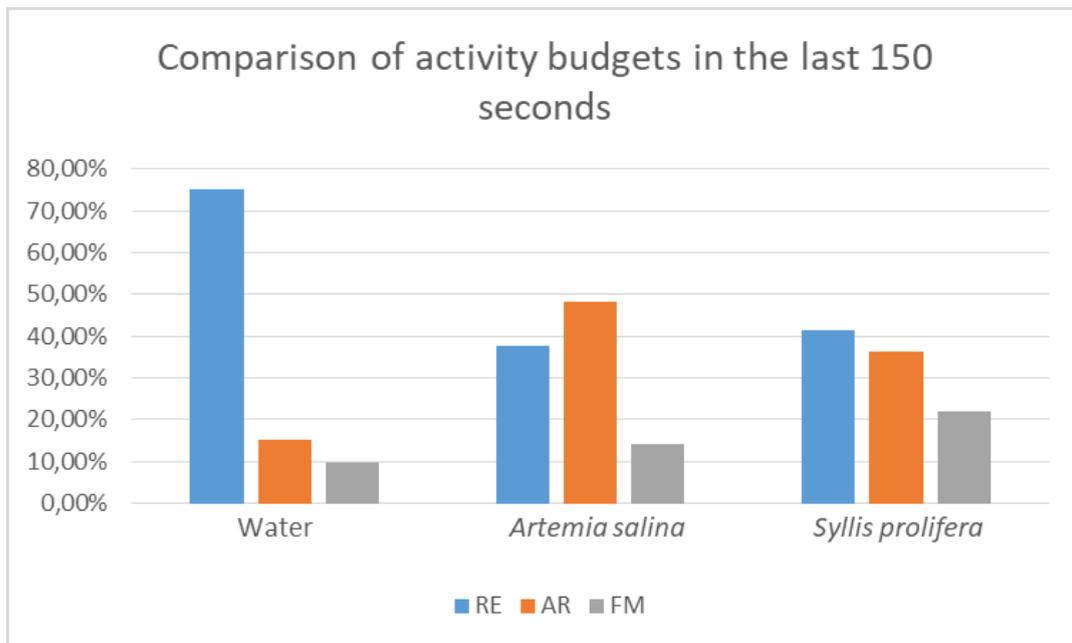


Figure 47 Comparison among different conditions of chemical signals experiments in the last 150 seconds.

RE=Rest; AR=Alert; FM= Feeding

Chapter IV

DISCUSSION

4.1 Observations on single polyps success

Although rare, successful catches from isolated polyps occurred. Size seems to not affect the success: in our tests, capture is not related with the length of the worm (Figure 17) because even the shortest worms were not eaten, and those successfully ingested are near the average length.

Successful catches appeared to have some specific features in common:

- the position of the polychaete worm when it got in touch to the mouth of the polyp;
- the reduction of the worm's motion that this position caused;
- the inability of the prey to reach the substrate;
- a very long time needed for the polyp to find a way to ingest the prey.

These observations allow to hypothesize that polyps successfully reach bigger preys only in particular and casual circumstances while in the experiments were groups of polyps were involved they were always successful. This difference could be due to the large surface areas that groups cover, facilitating the process of catching food, as observed in other cnidarians (Hausman, 1919; Francis, 1976; Hoeksema & Waheed, 2012). The ability of the prey to escape is a key factor determining the foraging success in suspension feeders, with some prey being evasive and accelerating in order to escape catch, or having defensive structures able to prevent capture (Hausman, 1919; Christensen, 1976; Trager et al. 1994). The length and the continuous movement of the prey made the catch very difficult for a single polyp, which was able to win the fight only when the polychaete fell rolled on itself. In these cases,

the polyp could grasp and block the prey in the middle part of its body. This position avoided the worm's escape, but the polyp needs a lot of time to ingest the prey. When caught from the middle, the polychaete could not reach the substrate (i.e. the bottom of the bowl), being thus unable to escape and the polyp was clearly advantaged in the fight. When the polyp reached the prey on its terminal parts, the worm appeared advantaged by the possibility to stay in touch with the substrate and moving using parapods and chaete, and consequently resorting to autotomy. Other worms, such as Nematoda, may free themselves with their rapid movements even from a colony of hydroids (Christensen, 1976). The size of the polyp as well as their mouth gape may play a role in the catch, as in sea anemones (Kenneth, 1997; Hoeksema & Waheed, 2012).

4.2 Observations on group success

Researchers already observed polyps of *Aurelia* sp. eating small preys like ciliates and other organisms from microzooplankton (Kamiyama, 2011). These data, and our results with isolated polyps, suggest that feeding on preys smaller than their mouth gape should be the more efficient way of accessing food resources. However, observations on other cnidarians (Christensen, 1976; Coma et al., 1995; Bavestrello et al., 2000; Musco et al., 2018; Mehrotra et al. 2019) suggest that an oversized prey is not always inaccessible for polyps, especially when they live associated in dense populations or in colonies. Our results showed that the ingestion of bigger preys is indeed possible and probably normal even in polyps with a very small mouth gape as *Aurelia* polyps, when they form groups of two or more specimens.

The surface area covered by polyps seems to be more important than the polyp size even in other species (Palardy et al., 2005; Hoeksema & Waheed, 2012) and the presence of conspecifics can play an important role in feeding efficiency (Wijgerde, T. et al., 2012).

It was not possible to give an exact measure of the distance among all polyps, because the fall of the polychaete worm was casual and because of the fact that polyps could move the calyx. Furthermore, in some tests, some polyps detached their basal disc from the substrate and changed position in order to not losing the prey; for this reason, we were not able to assess if the proximity among polyps had a real effect on the time spent in capturing the worm and on the efficacy of the catch.

Polyps close to each other or that formed groups with individuals in strict contact were more efficient in dividing the prey, maybe because they did not have to stretch to reach a point where they could cut the worm. An interesting behaviour that we observed during this phase is the continuous rotatory movement of the calyx (of either one or all the polyps that took

part in the action), sometimes associated with an enlargement and restriction of the mouth, that appeared to help the scyphistomae with cutting the prey, together with pulling in different directions. Unfortunately, the experimental time did not allow the formation of very dense populations of polyps in our plastic bowls. Even if some test groups contained more than ten polyps in strict contact, our densities did not reach the normal ones observed in nature (Gröndahl, 1989; Willcox, 2006; Di Camillo et al., 2010). However, we can hypothesize that group catching could become more effective in nature in respect to the experimental conditions.

Almost every polyp got its part of the prey. However, due to the pulling in different directions, in some cases bigger polyps or a pair of polyps pulling in the same direction excluded another polyp from the catch. In our tests, this condition was very rare, whilst the opposite (polyps abandoning spontaneously the catch) was more frequent, even if sometimes they try again in a second moment. It is also possible that polyps that were not interested in the prey in a first time take part in the catch in a second time, sometimes because the actions of both the conspecifics and the prey pushed the worm toward other polyps. Physical and chemical signals could trigger this behaviour, since polyps react when something touches them, as cnidarian polyps have sensorial structures, sometimes located on tentacles (Holtmann et Thurm, 2001; Jacobs et al., 2007). Moreover, we in our experiments on chemical signals and in experiments on other cnidarians (Mariscal & Lenhoff, 1968; Lenhoff et al., 1976 Lawn & McFarlane, 1991; Carter, et al., 2016) it was observed that they change their behaviour also when they perceive the chemical presence of the prey. It is well known, that some polychaete worms produce damage-released alarm signals that can be detected by conspecifics (Watson et al., 2005). Thus, we can hypothesize that distant conspecifics could

get interested in the prey by perceiving chemical signals deriving from the specimens of *S. prolifera* during struggle, and/or caused by the other polyps while breaking the worm.

As shown in Table 3, the average number of polyps taking part immediately in the action and the average number of polyps that effectively benefit from it were very similar. Either polyps that abandoned the catch could come back to the prey or sometimes polyps that were excluded could be counterbalanced with polyps that entered in a second moment.

As a whole, the number of polyps that benefitted from taking part in the catch was higher than the number of polyps that had no benefits; for this reason, cooperating appeared the best solution when dealing with otherwise inaccessible preys. The factors influencing on the polyp's evaluation in whether taking part in the group catch or not are not clear.

4.3 A case of proto-cooperation?

In combination with other benefits, the group living strategy offers the possibility to access food resources that are closed off to single polyps, which, in isolation conditions, can only profit of preys that are small enough to be ingested without spending too much energy and time. In addition, accessing more food resources is one of the direct benefits of cooperation (Odum, 1971) and can counteract the effects of the intraspecific competition (Buss, 1981). Polyps of *Aurelia* sp. are perfectly capable of an independent life; moreover, as we observed during the experiments, each polyp was focused on its own actions, pulling in its own direction. Our observations cannot highlight the presence of some form of communication among polyps, even if further studies are needed on this topic. As observed, this behaviour fits in Connor's basal I mutualism (by-product; by-product), since his definition of by-product benefit is "Behaviours or other attributes of A designed to benefit A incidentally benefit B. The benefit to B comes at no additional cost to A". Since single polyps try to catch the prey in any case, it is possible that this mutualism emerge from the sum of single polyps' action that result in a success in predation, even if some behaviours (such as pulling and rotating) seem to be not casual and could be the result of an evolutionary adaptation to group living.

Intraspecific competition could have led to the spatial separation of polyps in a first time. The repression of competition sometimes is one of the major factors leading to cooperation (Frank, 2003) and in optimal conditions, gathering together is a strategy also adopted by simple organisms as bacteria (Rainey & Rainey, 2003). Casual falls of big preys on polyps that were near enough to catch them simultaneously and the casual benefit of having one or more conspecifics to prevent the prey's escape, as in other species (Hausman, 1919; Coma

et al., 1995), could have favoured a gradual aggregation. Polyps that gather have an enhanced probability that other conspecifics block a big prey, and the more they are close, the more this probability increases. The synergy of these actions results in a benefit for everyone that overcome the risk of intraspecific competition. Hausman (1919) wondered if this behaviour, that he observed in the sea anemone *Haliplanella luciae*, could evolutionarily lead to gregarious forms replacing single anemones. We hypothesize that there could have been an evolutionary shift from competition to a facultative cooperation and that both these behaviours are possible in the moment that the prey reaches the group. However, cooperation has an important role in other organisms when they face environmental challenges (Buss, 1981; Korb & Foster, 2010; Carter & Wilkinson, 2013) and several factors could lead to its development. For example: kin selection; direct, indirect and network reciprocity; group selection (Nowak, 2006). All of these could be taken into account and considering that the number of polyps that obtain a benefit is much higher than the number of polyps excluded, cooperation could be a more frequent scenario.

Talking about indirect benefits, instead, both kin selection and reciprocity theories are equally possible. In fact, all our polyps come from the same tank of the Aquarium of Cattolica and they reproduce asexually (Vagelli, 2007; Adler-Ohde & Jarms, 2009). On the basis of results of previous studies on asexual reproduction in cnidarians, for example in sea anemones (Francis, 1976; Shick & Lamb, 1977), we can suppose that they are all more or less related, so kin selection could probably be another important factor in evolving this behaviour, as in other organisms (Francis, 1976; Bastiaans, E. et al., 2016). In particular, polyps that settled and reproduced in the plastic bowls are surely related and some reproductive strategies like lateral budding or fission (Adler-Ohde L. & Jarms G., 2009) present limited dispersion or lead to identical polyps that live in strict contact with the

original ones. Those ones share a very similar gene pool and for this reason, cooperating in the catch of preys is surely a way to increase the indirect fitness (Hamilton, 1963; Francis, 1976; Graphen, 1984; Foster, 2006; Lehmann, 2006; Nowak, 2006). We do not know if cooperation occurs in unrelated *Aurelia* polyps too. This could be tested using polyps that have been separated temporally and spatially for a very long time, in order to be sure that they are not related and therefore excluding the pressure of kin selection on their cooperative behaviour, if present. It is indeed possible that this case of cooperation could be explained using the Hamilton's rule (Hamilton, 1963) and in general, kin selection (Graphen, 1984; Foster, 2006; Lehmann, 2006); however, without genetic information on the original polyps, we could not verify this hypothesis. We can suppose, though, that related individuals tend to settle near their progenitors, while unrelated ones are more distant and less willingly to cooperate; this could explain also the cooperation of other cnidarians that occurs between different colonies (Musco et al., 2018).

Hypotheses on reciprocity are more difficult to be considered. It is true that an organism does not need a brain to develop a strategy (Axelrod & Hamilton, 1981) because we can simply define a strategy as a reaction to a situation that includes choices: in this case, competition or cooperation, a very similar case to the Prisoner's Dilemma (Nowak, 2004). At the same time, however, we have no evidence that cooperative behaviour could have evolved from similar mechanisms, and that competitors have been excluded from populations as it happens to defectors in the repeated game. In fact, every catch is a single event that involve a different number of polyps, at different distances, with different reactions of the prey and polyps seem independent in their choice of whether participate or not in the action. Moreover, testing this hypothesis needs repeated experiments with the same polyps, noticing if something change in their individual behaviour. Our tests, on the contrary,

were carried out on different groups of polyps and therefore could not give information about reciprocity, even if we have little basis to define if a polyp is able to recognize the surrounding conspecifics.

Despite these uncertainties, direct benefits and the strong possibility of increasing the indirect fitness make us think that this is an evident case of mutualism, according to most definitions of this interaction (Abrams, 1987; Boucher, 1982; Connor, 1995; West, 2007; Holland, 2008). Specifically, according to Holland (2008) a case of cooperation, if we identify cooperation as a mutualistic behaviour between conspecifics. In particular, we consider it a case of proto-cooperation because, according to Allaby's definition (Allaby, 2010), proto-cooperation is "an interaction between organisms of different species in which both organisms benefit, but neither is dependent on the relationship". As polyps of *Anthopleura elegantissima* (Francis, 1976), polyps of *Aurelia* live in dense aggregations but every polyp has an independent life; moreover, the cooperation does not occur for every polyp, making us think that this is a rapid choice of the individual in the moment that the prey falls upon it. This interaction is not limited to the genus *Aurelia*, since a similar hypothesis was done for *Anthopleura elegantissima* (Francis, 1976) and the interaction was observed in other cnidarian classes (Hausman, 1919; Bavestrello et al., 2000; Musco et al., 2018;). Thus, we can suppose that it could be frequent also in polyps of other species of Scyphozoa and probably of other cnidarians.

Interspecific mutualism seems less possible to happen. Nevertheless, studying forms of interactions in species that share the same habitat thus living close to each other, as tropical corals, whose interactions have always been described as competitive (Chornesky, 1989; Dai, 1990; Connell, 2004; Chadwick, 2010) could lead to interesting results.

In general, studies on mechanisms behind this kind of interaction in these ancient organisms could help to understand how interactions evolved in response to environmental factors, as it happens in other organisms (Thorne et al., 2003). Gregariousness and cooperation could have developed in different times of natural history, as a result of the Allee's principle of aggregation (Odum, 1971) and ecological pressure. Some hypotheses can be applied to any taxonomic group (Majolo & Huang, 2018; Hamilton, 1963), thus, evidence that cooperation could play an important role among Cnidaria suggests that this kind of interaction may play a role in shaping populations, in a way comparable to competition. Understanding the role of cooperation in cnidarians may contribute to the understanding of the marine community dynamics and to conservation of marine ecosystems (Berger-Tal et al., 2011; Brooker et al., 2016)

4.4 Food preference

Different factors can influence prey selection in organisms (Hausman, 1919; Christensen, 1976; Trager et al. 1994). Prey used in laboratory experiments could not represent the real composition of those available in nature (Trager et al. 1994). Several studies investigated prey in scyphomedusae (Sullivan et al.; 1994; Sullivan et al., 1997; Graham & Kroutil, 2001), but not for scyphopolyps. We need more experiments and different times of observation to better answer the question of whether polyps prefer many small preys or a single big prey. The explicit rejection of the worm is rare; in those cases where polyps neither ingested nor rejected the worm, we do not know if they maintained it for ingesting and digesting it in a second moment. This because the digestion process can be long and slow, especially when a great amount of preys are captured by the polyp, and differs among different prey (González-Valdovinos et al., 2018); therefore we were not able to see what happened after many hours.

Another question concerns the time of capture: we gave *A. salina* after three minutes, but after this time, *S. prolifera* is easily blocked or even already dead. This could induce polyps to postpone its ingestion in order to take advantage of the presence of other food.

Other experiments should be carried out with a minimal difference of time between the two preys; this could clarify if polyps show a real preference, because the worm could have the chance of taking advantage from the distraction caused by the presence of nauplii when it is still capable of escaping. The polyps' reactions in this case are probably more reliable.

Lastly, more attention should be given to the amount of nauplii, which could lead to a change in the polyps reactions.

Studies show that prey density and composition have a correlation with feeding rates of other polyps (Barange & Gili, 1988; Purcell et al., 1999). Even if measuring the real density of nauplii is very difficult due to their size and to the randomness of catching them with a pipette, a minor probability of encounter could change polyps behaviour.

4.5 Chemical signals

In our experiments on feeding behaviour, polyps that could not reach the prey oriented themselves in its direction anyway; moreover, this seemed to be more evident when a conspecific attacked and damaged the prey. Water flow has a particular importance in providing food to suspension feeders as polyps (Sebens, 1998) and absence of a physical contact among polyps made us think that they could detect a signal in the water, its provenience and therefore direct themselves towards the source. Studies carried out on corals show that with food juices, contracted polyps open their mouths widely and express a feeding behaviour (Mariscal & Lenhoff, 1968; Lehnoff et al., 1976; Lawn & McFarlane, 1991). Similar reactions are present in *Hydra* (Carter et al., 2016).

We were not sure if the provided signal was a chemical compound deriving from the prey or from the digestion by other polyps; sampling water containing possible digestion compounds in an exact way, however, was rather difficult to do, and it could lead to ambiguous results. For this reason, our preference went to establish if the prey alone has a role in polyps' behaviour. The immediate reaction of polyps to the water containing traces of the preys and the comparison with only seawater suggest that our hypotheses were right. The activity budget evidences an instantaneous increase of Alert-Research (AR) and Feeding-Mouth Open (FM) behaviour. The comparison with our first sampling behaviour tests, especially in the first two minutes, show that feeding behaviour considerably increases in the experiments, as for other cnidarians (Mariscal & Lenhoff, 1968; Lehnoff et al., 1976; Lawn & McFarlane, 1991; Carter et al., 2016) and it happens in more of one polyp at the same time. Even if this behaviour is evident, the Alert-Research is more frequent, suggesting that polyps may use chemical signals to detect a near prey, and their active movements of the calyx and of the

tentacles could improve the possibility of reaching it. The same does not happen in control situations and in experiments with water flow alone, in which the rare feeding behaviour that occurs after minutes from the water flow can be considered as casual. The situation is similar to the first observations, in the context in which new chemical, biological or physical factors are not present in the plastic bowls and polyps are in steady conditions.

We need more studies to define the nature of chemical compounds that trigger a reaction in polyps, as well as the mechanisms behind their receptors. The reactions to chemical compounds from the prey do not exclude that polyps can communicate in other ways. Even if their detection presents many technical problems, it is possible that particular substances, (e.g. digestive compounds produced by conspecifics) could trigger reactions in the surrounding polyps. The synergy of these results could shape a different view of polyps as active predators and could help to better define their role in marine ecosystems.

Chapter V

CONCLUSIONS

Our study shows that protocoooperation previously observed in other cnidarians in natural habitats is evident also in scyphozoan polyps, with repeated laboratory experiments. While single polyps are limited to accessible resources, the range of size of potential preys increases when they live in groups that have the possibility of cooperating in the catch. Moreover, cooperation seems to be favoured compared to intraspecific competition.

From our experiments, there is no evidence of preference between small and large preys. Nevertheless, more studies are needed to clarify the dynamics behind the food choice, if it exists.

The polyps clearly perceive chemical signals from the prey; they are able to recognize signals and to orient toward the prey. Moreover, these compounds trigger feeding and research behaviour. However, we could not evidence the presence of communication signals among polyps and further studies could improve our knowledge on the group catching mechanisms. Moreover, studies on different group-living cnidarians would be of paramount importance to understand ecosystem functioning and the evolutionary role of cooperation in shaping cnidarian communities.

BIBLIOGRAPHY

- Abrams, P.A. (1987). On classifying interactions between populations, *Oecologia*, 73:272.
- Achituv, Y. & Benayahu, Y. (1990). Polyp dimorphism and functional, sequential hermaphroditism in the soft coral *Heteroxenia fuscescens* (Octocorallia). *Marine Ecology Progress Series*, 64(3), 263-269.
- Adler-Ohde, L. & Jarms, G. (2009). New insights into reproductive traits of scyphozoans: Special methods of propagation in *Sanderia malayensis* GOETTE, 1886 (Pelagiidae, Semaestomeae) enable establishing a new classification of asexual reproduction in the class Scyphozoa. *Marine Biology*, 156, 1411-1420.
- Agassiz, L. (1860). *Contribution to the Natural History of the United States of America*. Boston, USA: Little, Brown & Co.
- Allaby, M. (2009). "Commensalism" in *A Dictionary of Zoology* (3 ed.). Oxford, UK: Oxford University Press.
- Allaby, M. (2009). "Neutralism" in *A Dictionary of Zoology* (3 ed.). Oxford, UK: Oxford University Press.
- Allaby, M. (2010). "Protocooperation." In *A Dictionary of Ecology*. Oxford, UK: Oxford University Press.
- Arai, M. N. (1997). *A functional biology of Scyphozoa*. Berlin, Germany: Springer Science & Business Media.
- Arai, M. N. (2005). Predation on pelagic coelenterates: A review. *Journal of the Marine Biological Association of the United Kingdom*, 85(3), 523-536.

- Asem, A. et al. (2010). The genus *Artemia* Leach, 1819 (Crustacea: Branchiopoda). I. True and false taxonomical descriptions. *Latin American Journal of Aquatic Research*, 38(3), 501-506.
- Astorga, D. et al. (2012). Ecological aspects of early life stages of *Cotylorhiza tuberculata* (Scyphozoa: Rhizostomae) affecting its pelagic population success. *Hydrobiologia*. 690(1).
- Avishek, B. et al. (2018). Characterization of halotolerant, pigmented, plant growth promoting bacteria of groundnut rhizosphere and its in-vitro evaluation of plant-microbe proto-cooperation to withstand salinity and metal stress. *Science of The Total Environment*, 630, 231-242.
- Axelrod, R. & Hamilton, W. D. (1981). The evolution of cooperation. *Science*, 211, 1390-1396.
- Bailey, K. M. & Batty, R. S. (1983). A laboratory study of predation by *Aurelia aurita* on larval herring (*Clupea harengus*): Experimental observations compared with model predictions. *Marine Biology*, 72, 295-301.
- Barange M., Gili J. M., (1988) Feeding cycles and prey capture in *Eudendrium racemosum* (Cavolini, 1785), *Journal of Experimental Marine Biology and Ecology*, 115, Issue 3, 281-293.
- Bastiaans, E. et al. (2016). Experimental evolution reveals that high relatedness protects multicellular cooperation from cheaters. *Nat Commun* 7, 11435.
- Bavestrello, G. et al. (2000). Life history of *Perarella schneideri* (Hydrozoa, Cytaedidae) in the Ligurian Sea. *Scientia Marina*, 64, 141–146.
- Bayer, F. M. (1973). Colonial organization in octocorals. In *Animal colonies*. Stroudsburg, Pa, USA: Dowden, Hutchinson & Koss, Inc

- Boucher D. H. B. et al. (1982). The Ecology of Mutualism. *Annual Review of Ecology and Systematics* 13(1), 315-347.
- Brooker, R. M., et al. (2016). Using insights from animal behaviour and behavioural ecology to inform marine conservation initiatives. *Animal behaviour*, 120
- Buss L. W. (1981). Group Living, Competition, and the Evolution of Cooperation in a Sessile Invertebrate, *Science*, 28, 1012-1014.
- Cargo D.G. (1975). Comments on the Laboratory Culture of Scyphozoa. In: Smith W.L., Chanley M.H. (eds) *Culture of Marine Invertebrate Animals*. Boston, MA: Springer.
- Carter, G. & Wilkinson, G. (2013). Cooperation and Conflict in the Social Lives of Bats, In: *Bat Evolution, Ecology and Conservation*. Springer Science Press.
- Carter, J. A. et al. (2016). Dynamics of Mouth Opening in *Hydra*. *Biophysical journal*, 110(5), 1191–1201.
- Cartwright, P. (2004). The development and evolution of hydrozoan polyp and colony form. *Hydrobiologia*, 530 (1-3), 309-317.
- Cartwright, P. & Nawrocki, A. M. (2010). Character Evolution in Hydrozoa (phylum Cnidaria). *Integrative and Comparative Biology*, 50 (3), 456–472.
- Ceh, J. et al. (2015). The elusive life cycle of scyphozoan jellyfish – metagenesis revisited. *Scientific Reports*, 5:12037.
- Chadwick N.E., Morrow K.M. (2011) Competition among Sessile Organisms on Coral. In *Coral Reefs: An Ecosystem in Transition*. Dordrecht, Netherlands: Springer
- Chang, E. S. et al. (2018). Nonclonal coloniality: Genetically chimeric colonies through fusion of sexually produced polyps in the hydrozoan *Ectopleura larynx*. *Evolution Letters*, 2, 442-455.

- Chornesky, E.A. (1989). Repeated Reversals During Spatial Competition Between Corals. *Ecology*, 70: 843-855.
- Christensen H. E. (1967) Ecology of *Hydractinia echinata* (Fleming) (Hydroidea, Athecata) I. *Feeding biology*, *Ophelia*, 4:2, 245-275
- Coma, R. et al. (1994). Feeding and prey capture cycles in the aposymbiotic gorgonian *Paramuhcea clavata*. *Marine Ecology Progress Series*. 115. 257-270.
- Connell, J.H. et al. (2004). A long-term study of competition and diversity of corals. *Ecological Monographs*, 74: 179-210.
- Connor R. C. (2010). Cooperation beyond the dyad: on simple models and a complex society. *Phil. Trans. R. Soc. B* 365.
- Connor, R. C. (1995). The Benefits of Mutualism: A Conceptual Framework. *Biological Reviews*, 70, 427-457.
- Cowan, J. H. & Houde, E. D. (1992). Size-dependent predation on marine fish larvae by Ctenophores, Scyphomedusae, and Planktivorous fish. *Fisheries Oceanography*, 1, 113-126.
- Dai, C. (1990). Interspecific competition in Taiwanese corals with special reference to interactions between alcyonaceans and scleractinians. *Marine Ecology-progress Series* 60. 291-297.
- Dawson, M. & Jacobs, D. (2001). Molecular Evidence for Cryptic Species of *Aurelia aurita* (Cnidaria, Scyphozoa). *The Biological bulletin*, 200, 92-6.
- Deisboeck, T. S. & Couzin, I. D. (2009). Collective behavior in cancer cell populations. *Bioessays*, 31, 190-197.
- den Hartog, J. C. & Nierop, M. M. van (1984). A study on the gut contents of six leathery turtles *Dermochelys coriacea* (Linnaeus) (Reptilia: Testudines:

- Dermochelyidae) from British waters and from the Netherlands. *Zoologische Verhandelingen*, 209, 3–36.
- Di Camillo, C., et al. (2010). Contribution to the understanding of seasonal cycle of *Aurelia aurita* (Cnidaria: Scyphozoa) scyphopolyps in the northern Adriatic Sea. *Journal of the Marine Biological Association of the United Kingdom*, 90(6), 1105-1110.
 - Drias, H. et al. (2005). Cooperative Bees Swarm for Solving the Maximum Weighted Satisfiability Problem. *Computational Intelligence and Bioinspired Systems*. Lecture Notes in *Computer Science*, vol 3512. Berlin, Heidelberg: Springer
 - Duarte, C.M et al. (2013). Is global ocean sprawl a cause of jellyfish blooms?. *Frontiers in Ecology and the Environment*, 11(2), 91-97.
 - Francis, L. (1976). Social organization within clones of the sea anemone *Anthopleura elegantissima*. *The Biological bulletin*, 150 3, 361-376 .
 - Frank, S.A. (2003), Repression of competition and the evolution of cooperation. *Evolution*, 57: 693-705.
 - Franke, H. D. (1999). Reproduction of the Syllidae (Annelida: Polychaeta). *Hydrobiologia*, 402, 39-55.
 - Gallucci, A. M. & Gambi, M. C. (2014). *Syllis prolifera* Krohn, 1852 (Annelida, Sillidae): A standardised protocol for laboratory culturing of a model species.
 - Gambill, M. & Jarms, G. (2014). Can *Aurelia* (Cnidaria, Scyphozoa) species be differentiated by comparing their scyphistomae and ephyrae?. *European Journal of Taxonomy*, 107 (107): 1-23.
 - Giangrande, A. et al. (2000). The diversity of diets in Syllidae (Annelida: Polychaeta). *Cahiers de Biologie Marine*, 41(1), 55-65.

- González-Valdovinos, M. et al., (2018). Evaluation of digestive capacity in the polyp, ephyrae, and medusae stages of the cannonball jellyfish *Stomolophus meleagris*. *Hydrobiologia*. 10.
- Grafen, A. (1984). Natural selection, kin selection and group selection. *Behavioural Ecology. An Evolutionary Approach*, pp. 62–84. Oxford, UK: Blackwell Scientific Publications.
- Graham W. M. & Kroutil R. M. (2001), Size-based Prey Selectivity and Dietary Shifts in the Jellyfish, *Aurelia aurita*, *Journal of Plankton Research*, Volume 23, Issue 1, 67–74,
- Grasse, P. P. (1993). *Traité de Zoologie — Tome III, fascicule 2: Cnidaires – hydrozoaires, scyphozoaires, cubozoaires; cténaires*. Paris, France: Masson.
- Gröndahl, F. (1988). Interactions between polyps of *Aurelia aurita* and planktonic larvae of scyphozoans: An experimental study. *Marine ecology progress series. Oldendorf*, 45(1), 87-93.
- Gröndahl, F. (1989). Evidence of gregarious settlement of planula larvae of the scyphozoan *Aurelia aurita*: An experimental study. *Marine ecology progress series. Oldendorf*, 56, 119-125.
- Hamilton, W. D. (1963). The evolution of altruistic behavior. *The American Naturalist*, 97 (896), 354-356.
- Hausman, L. (1919). The Orange Striped Anemone (*Sagartia Luciae*, Verrill). An Ecological Study. *Biological Bulletin*, 37(6), 363-370.
- Hayward, P. J. & Ryland, J. S. (2017). *Handbook of the Marine Fauna of North-West Europe*. Oxford, UK: Oxford University Press. pp. 188–194.

- Helm, R. R. (2018). Evolution and development of scyphozoan jellyfish. *Biol Rev*, 93, 1228-1250.
- Herbert-Read J. E. et al. (2016). Proto-cooperation: group hunting sailfish improve hunting success by alternating attacks on grouping prey. *Pro. R. Soc. B*, 283 (1842).
- Hickman C. et al. (2013). *Integrated Principles of Zoology*, (16th ed.). New York, USA: McGraw-Hill Companies.
- Hoeksema, B.W. & Waheed, Z. (2012). It pays to have a big mouth: mushroom corals ingesting salps at northwest Borneo. *Marine Biodiversity*, 42, Issue 2, 297–302
- Holland, J. N. & Bronstein, J. L. (2008). “Mutualism”, *Encyclopedia of Ecology*, 2485-2491, Academic Press.
- Holst, S. & Jarms, G. (2007). Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. *Marine Biology*, 151, 863.
- Holtmann, M. & Thurm, U. (2001). Variations of concentric hair cells in a Cnidarian sensory epithelium (*Coryne tubulosa*). *The Journal of comparative neurology*. 432. 550-63.
- Hoover R.A. et al. (2012) Nudibranch predation and dietary preference for the polyps of *Aurelia labiata* (Cnidaria: Scyphozoa). In: Purcell J., Mianzan H., Frost J.R. (eds) *Jellyfish Blooms IV. Developments in Hydrobiology*, vol 220. Springer, Dordrecht
- Jacobs D.K. et al. (2007). Evolution of sensory structures in basal metazoa, *Integrative and Comparative Biology*, 47, 5, 712–723.
- Jarms, G. (1991). Taxonomic characters from the polyp tubes of coronate medusae (Scyphozoa, Coronatae). *Hydrobiologia*, 216-217(1), 463–470.

- Jarms, G. et al. (2002). Cultivation of polyps and medusae of Coronatae (Cnidaria, Scyphozoa) with a brief review of important characters. *Helgoland Marine Research*, 56, 203-210.
- Kamiyama, T. (2011). Planktonic ciliates as a food source for the scyphozoan *Aurelia aurita* (s.l.): Feeding activity and assimilation of the polyp stage. *Journal of Experimental Marine Biology and Ecology*, 407(2), 207-215.
- Kenneth R. N. A. (1997). Prey Capture by the Sea Anemone *Metridium senile* (L.): Effects of Body Size, Flow Regime, and Upstream Neighbors. *Biological Bulletin*, 192(1), 73-86
- Korb, J., & Foster, K. R. (2010). Ecological competition favours cooperation in termite societies. *Ecology Letters*, 13(6), 754–760.
- Krams I. et al. (2009). The increased risk of predation enhances cooperation. *Proceedings of the Royal Society*, 277: 1681.
- Lawn, I. D., & McFarlane, I. D. (1991). The physiological control of feeding in corals: a review. *Coelenterate Biology: Recent Research on Cnidaria and Ctenophora*, 595–598
- Lehmann, L. & Keller, L. (2006). The evolution of cooperation and altruism, a general framework and a classification of models. *J. Evol. Biol*, 19, 1365–1376.
- Lenhoff H.M. et al. (1976) A View of the Evolution of Chemoreceptors Based on Research with Cnidarians. In: *Mackie G.O. (eds) Coelenterate Ecology and Behavior. Springer, Boston, MA*
- Lewis, J.B. (1982), Feeding behaviour and feeding ecology of the Octocorallia (Coelenterata: Anthozoa). *Journal of Zoology*, 196

- Liu, M. et al. (2009). In Silico Prediction of Horizontal Gene Transfer Events in *Lactobacillus bulgaricus* and *Streptococcus thermophilus* Reveals Protocooperation in Yogurt Manufacturing. *Applied and Environmental Microbiology*, 75 (12), 4120-4129.
- Liu, W. C. et al. (2008). Effects of temperature and light intensity on asexual reproduction of the scyphozoan, *Aurelia aurita* (L.) in Taiwan. *Jellyfish Blooms: Causes, Consequences, and Recent Advances. Developments in Hydrobiology*, 206. Springer, Dordrecht.
- Lynam, C. P. et al. (2006). Jellyfish overtake fish in a heavily fished ecosystem. *Current Biology*, 16, R492–R493.
- Majolo B., Huang P. (2018) Group Living. In: Vonk J., Shackelford T. (eds) *Encyclopedia of Animal Cognition and Behavior*. Springer, Cham
- Manser, M. B. et al. (2002). Suricate alarm calls signal predator class and urgency. *Trends in Cognitive Sciences*, 6 (2), 55-57.
- Maslova, V. & Dochevoy, Y. E. (2016). A case of protocooperation between the pitviper *Gloydius ussuriensis* and the ground beetle *Carabus granulatus telluris*. *Russian Journal of Herpetology*, 23(3), 231-234.
- Maestre, F. T. et al. (2010). Do biotic interactions modulate ecosystem functioning along stress gradients? Insights from semi-arid plant and biological soil crust communities. *Phil. Trans. R. Soc. B*, 365.
- Mariscal, R. N. & Lenhoff H. M., (1968). The chemical control of feeding behaviour in *Cyphastrea ocellina* and in some other Hawaiian corals. 1. expo Bio!. 49: 689-699.

- Martin P. & Bateson P. (2007). *Measuring Behaviour: An Introductory Guide*. Cambridge University Press
- Mehrotra, R. et al. (2019). Selective consumption of sacoglossan sea slugs (Mollusca: Gastropoda) by scleractinian corals (Cnidaria: Anthozoa). *PLoS ONE*, 14(4).
- Milisenda, G. et al. (2014). Jellyfish as prey: frequency of predation and selective foraging of *Boops boops* (Vertebrata, Actinopterygii) on the mauve stinger *Pelagia noctiluca* (Cnidaria, Scyphozoa). *PLoS ONE*, 9.
- Montano, S. et al. (2014). First record of coral-associated *Zanclaea* (Hydrozoa, Zanclidae) from the Red Sea. *Marine Biodiversity*, 44, 581.
- Montano, S. et al. (2015). New insights into the symbiosis between *Zanclaea* (Cnidaria, Hydrozoa) and scleractinians. *Zoologica Scripta*, 44, 92–105.
- Montano, S. et al. (2017). Corals hosting symbiotic hydrozoans are less susceptible to predation and disease. *Proceedings. Biological sciences*, 284.
- Musco, L. & Giangrande, A. (2005). Mediterranean Syllidae (Annelida: Polychaeta) revisited: biogeography, diversity and species fidelity to environmental features. *Marine Ecology Progress Series*, 304, 143-53.
- Musco, L. et al. (2018). Protocooperation among small polyps allows the coral *Astroides calycularis* to prey on large jellyfish. *Ecology*, 99, 2400-2401.

- Nawrocki A. M., Cartwright P. (2012). A Novel Mode of Colony Formation in a Hydrozoan through Fusion of Sexually Generated Individuals. *Current Biology*, 22 (9), 825-829.
- Nowak, M. A. et al. (2004). Emergence of cooperation and evolutionary stability in finite populations. *Nature*, 428, 646–650.
- Nowak M. A. (2006). Five rules for the evolution of cooperation. *Science* (New York, N.Y.), 314(5805), 1560–1563.
- Berger-Tal O. et al. (2011). Integrating animal behavior and conservation biology: a conceptual framework, *Behavioral Ecology*, Volume 22, Issue 2, 236–239
- Odum E. P. (1983). *Basic ecology*. Philadelphia, Pennsylvania: W. B. Saunders Co.
- Odum, E. P. (1971). *Fundamentals of ecology*. Philadelphia, Pennsylvania: W. B. Saunders Co.
- Ohtsuki, H. et al. (2006). A simple rule for the evolution of cooperation on graphs and social networks. *Nature*, 441, 502–505.
- Packer, C. et al. (1990). Why Lions Form Groups: Food is Not Enough. *The American Naturalist*, 136(1), 1-19.
- Palardy, J. et al. (2005). Effects of upwelling, depth, morphology and polyp size on feeding in three species of Panamanian corals. *Marine Ecology-Progress Series*. 300. 79-89.
- Philipps, K. (2006). Divided corals stay in touch. *Journal of Experimental Biology*, 209, i-ii.
- Purcell, J. E. et al. (1999). Temperature, salinity and food effects on asexual reproduction and abundance of the scyphozoan *Chrysaora quinquecirrha*. *Marine Ecology Progress Series*, 180, 187–196.

- Purcell, J. E. et al. (1999). Prey, feeding rates, and asexual reproduction rates of the introduced oligohaline hydrozoan *Moerisia lyonsi*. *Marine Biology*, 134(2), 317–325.
- Purcell, J. (2003). Predation on zooplankton by large jellyfish, *Aurelia*, *Cyanea* and *Aequorea*, in Prince William Sound, Alaska. *Marine Ecology Progress Series*, 246, 137-152.
- Purcell, J. E. (2007). Environmental effects on asexual reproduction rates of the scyphozoan *Aurelia labiata*. *Marine Ecology Progress Series*, 348, 183-196.
- Rainey, P. B., & Rainey, K. (2003). Evolution of cooperation and conflict in experimental bacterial populations. *Nature*, 425(6953), 72–74
- Rana, S. V. S. (2005). *Essentials of Ecology and Environmental Science* (2nd ed). New Delhi, India: Prentice-Hall of India Private Ltd..
- Richardson, A. J. et al. (2009). The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology & Evolution*, 24(6), 312-322.
- Riehl, C. & Frederickson M. E. (2016). Cheating and punishment in cooperative animal societies. *Phil. Trans. R. Soc. B*, 371, 20150090.
- Sebens, K. P. et al (1998). Water flow and prey capture by three scleractinian corals, *Madracis mirabilis*, *Montastrea cavernosa* and *Porites porites*, in a field enclosure. *Marine Biology*, 131(2), 347–360.
- Scorrano, S. et al. (2017). Unmasking *Aurelia* species in the Mediterranean Sea: an integrative morphometric and molecular approach. *Zool J Linn Soc.*, 180(2), 243-267.

- Shick, J. & Lamb, A. (1977). Asexual Reproduction and Genetic Population Structure in the Colonizing Sea Anemone *Haliplanella luciae*. *Biological Bulletin*, 153.
- Sokołowski, A. et al. (2016). Does temperature and salinity limit asexual reproduction of *Aurelia aurita* polyps (Cnidaria: Scyphozoa) in the Gulf of Gdańsk (southern Baltic Sea)? An experimental study. *Hydrobiologia*, 773.
- Soong, K. (2005). Reproduction and colony integration of the sea pen *Virgularia juncea*. *Marine Biology*, 146(6), 1103-1109
- Soong K. & Lang J. C. (1992), Reproductive Integration in Reef Corals. *The Biological Bulletin* 183(3), 418-431.
- Sorgeloos, P. et al. (1986). *Manual for the culture and use of brine shrimp Artemia in aquaculture* (p. 319). Ghent, Belgium: State University of Ghent, Faculty of Agriculture.
- Stachowicz J. J. (2001). Mutualism, Facilitation, and the Structure of Ecological Communities: Positive interactions play a critical, but underappreciated, role in ecological communities by reducing physical or biotic stresses in existing habitats and by creating new habitats on which many species depend. *BioScience*, 51(3), 235-246.
- Sullivan, B. K. et al. (1994). Prey selection by the scyphomedusan predator *Aurelia aurita*. *Marine Biology*, 121, 335–341.
- Sullivan, B. K. et al. (1997). Mechanics of prey selection by ephyrae of the scyphomedusa *Aurelia aurita*, *Marine Biology*, 130, 213–222.

- Takao, M. et al. (2014). Natural predators of polyps of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa: Semaestomeae) and their predation rates. *Plankton and Benthos Research*, 9, 105-113.
- Tang, C. et al. (2019). Intraguild predation by polyps of three scyphozoan jellyfish: *Nemopilema nomurai*, *Aurelia coerulea*, and *Rhopilema esculentum*. *Journal of oceanology and limnology*, 1–7
- Thorne, B. L. et al. (2003). Evolution of eusociality and the soldier caste in termites: Influence of intraspecific competition and accelerated inheritance. *Proceedings of the National Academy of Sciences*, 100(22), 12808–12813.
- Trager, G., et al. (1994). Effects of prey escape ability, flow speed, and predator feeding mode on zooplankton capture by barnacles. *Marine Biology*, 120(2), 251–259.
- Uriz, M. et al. (1992). Parasitism, commensalism or mutualism? The case of Scyphozoa (Coronatae) and horny sponges. *Marine Ecology Progress Series*, 81(3), 247-255.
- Vagelli A A. (2007). New observations on the asexual reproduction of *Aurelia aurita* (Cnidaria, Scyphozoa) with comments on its life cycle and adaptive significance. *Invert . Zool .*, 4 (2): 111-127
- Xing, Y. et al. (2020). Effects of temperature and salinity on the asexual reproduction of *Aurelia coerulea* polyps, *Journal of Oceanology and Limnology*, 38(1), 133–142.
- Watanabe, T. & Ishii, H. (2001). An in situ estimation of the number of ephyrae liberated from polyps of *Aurelia aurita* on settling plates in Tokyo Bay. *Hydrobiologia*, 451, 247-258.

- Watson, G. J. et al. (2005). Chemical alarm signalling in the polychaete *Nereis* (Neanthes) *virens* (Sars) (Annelida: Polychaeta). *Animal Behaviour*, 70(5), 1125–1132.
- West, S. A. et al. (2007). Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology*, 20, 415-432.
- Wijgerde, T. et al. (2012). Water Flow Affects Zooplankton Feeding by the Scleractinian Coral *Galaxea fascicularis* on a Polyp and Colony Level. *Journal of Marine Biology*. 2012.
- Willcox, S. et al. (2008). Population dynamics of natural colonies of *Aurelia sp.* scyphistomae in Tasmania, Australia. *Marine Biology*, 154, 661-670.
- Willcox, S. et al. (2007). Asexual reproduction in scyphistomae of *Aurelia sp.*: Effects of temperature and salinity in an experimental study, *Journal of Experimental Marine Biology and Ecology*, 353 (1), 07-114.
- Willcox, S. (2006). Ecology of moon jellyfish *Aurelia Sp.* in *Southern Tasmania in relation to Atlantic salmon farming*. University of Tasmania.