UNIVERSIDADE DO ALGARVE

The effect of freshwater pulse on *Aurelia aurita* (Linnaeus 1758) (Cnidaria: Scyphozoa): an ecohydrological solution for controlling jellyfish blooms

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Benthic phase of the jelly fish A*urelia aurita*

"… Se falo na natureza não é porque saiba o que ela é,
Mas porque a amo, e amo-a por isso,
Porque quem ama nunca sabe o que ama
Nem sabe porque ama, e nem o que é amar…"

Alberto Caeiro, "O guardador de Rebanhos - Poema II"

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Introduction

Chapter I - *Aurelia aurita* scyphistomae and ephyrae response to freshwater pulse

Chapter II - Scyphistoma and ephyra feeding ecology

Abstract:

Jellyfish blooms are increasingly being reported in coastal areas and have been related to both climate changes and anthropogenic impacts. However, several questions underlying such blooms remain understudied, particularly during their benthic phase (polyps). Salinity is one of the environmental variables that has been linked with blooms occurrence. Reports in Guadiana estuary have correlated high precipitation and high freshwater input to low medusa occurrence. Therefore, we hypothesize that using freshwater pulses from a hydrotechnical structure (Dam) could be a promising ecohydrological approach for controlling jellyfish blooms. To explore this idea, the present study aimed to assess different short term salinity concentrations effects on benthic stages and ephyrae larvae of *Aurelia aurita.* It also explored the feeding ecology of such stages. It had two set of experiment designs. The first set of experiments focuses on responses of scyphistomae **(Experiment I, II)** and ephyrae larvae **(Experiment III)** under low salinity treatments (3,10,17 and 35(control)). The analyzed variables were survival, asexual reproduction and ecophysiological response (feeding rate and number of swimming pulses). It was found significant difference on scyphistomae survival between control and salinity 3 treatment (p-value < 0,05); on budding reproduction, number of scyphistoma actively budding and feeding response between control and salinity 10; on ephyrae survival between control and salinities 3 and 10 ; and ephyrae swimming pulses between control and salinity 17. It did not present significant difference on the number of strobilating scyphistoma and produced medusa. The second set of experiments investigated diet and feeding strategy of scyphistomae (**Experiment IV**) and ephyrae (**Experiment V**). It was used Costello plot method. The present study concluded that short term freshwater pulse may control jellyfish blooms by affecting jellyfish early phases survival, their ecophysiological response, and scyphistomae budding reproduction. It was also concluded that ephyrae and scyphistomae feeding strategy are generalists.

Keywords: jellyfish, polyp, ecohydrological solution, freshwater discharge, strobilation, estuary.

Resumo:

Rios e ecossistemas costeiros estão constantemente sofrendo pressões antrópicas. Tais pressões têm impactado a biota e alterado a constituição de comunidades causando grandes problemas a nível de perda da biodiversidade e dominância do ecossistema por determinadas espécies. O aumento do número de casos reportados de blooms de medusas têm sido sugerido como um desses problemas. Tais blooms parecem estar correlacionados com pressões antrópicas tais como construções de estruturas portuárias e contenções em áreas costeiras , alterações no caudal dos rios, eutrofização e sobrepesca. Desta forma torna-se necessário a investigação das relações entre esses processos e os blooms de gelatinosos para que sejam tomadas medidas efetivas de gestão.

Tem sido observada a relação entre baixa precipitação e caudal com o blooms de medusas. Experiências laboratoriais evidenciaram efeitos negativos da baixa salinidade na reprodução assexuada da fase bentónica de algumas espécies das medusas (pólipos). Assim, colocou-se a hipotese que a variação da salinidade nos estuários devido alterações do caudal dos rios ou de pluviosidade estejam relacionados a sobrevivência, viabilidade e reprodução dessa fase bentónica. Desta forma, o presente trabalho propõe o controle de pólipos e suas larvas (éfiras) por meio de variações de salinidade causadas pela liberação de pulsos d'água por barragens na montante de rios. No entanto, o conhecimento sobre estas fases iniciais bentónicos e planctónicas e sua relação com a dinâmica da população de medusas adultas, causadores de blooms, é ainda escasso. Assim, o objetivo do presente trabalho foi testar experimentalmente os efeitos de alterações da salinidade, produzidos por por pulsos de água doce, na sobrevivência, viabilidade, reprodução desses organismos (experiências I, II e III) e o papel deles na teia alimentar (experiências IV e V) dentro do cenário descrito anteriormente. Assim, tais resultados tornam possível o desenvolvimento de ferramentas de gestão e previsão das possíveis conseqüências da ausência/presença desses organismos na teia trófica.

Dentre as espécies que compõem o plâncton gelatinoso, *Aurelia aurita* é a espécie mais relatada nos blooms. *Aurelia aurita* é uma espécie do Filo Cnidaria e Classe Scyphozoa. Como a maioria dos cnidarios responsáveis pelos blooms gelatinosos, ela

possui um ciclo de vida com fase planctónica e bentónica. A fase plánctonica (medusa) é a responsável pela reprodução sexuada e origem da larva plânula, que então se desenvolverá em um scyphistoma ("pólipo" dos Scyphozoa). O scyphistoma é responsável pela reprodução assexuada e origem de novos scyphistomas e/ou éfiras. Em geral, os pólipos e as éfiras são considerados as fases mais importantes para o sucesso do recrutamento das medusas adultas. Por sua vez, o recrutamento é considerado o processo mais importante para a determinação do tamanho das populações de medusas. Assim, o presente trabalho realizou testes experimentais com scyphistomas e éfiras de *Aurélia aurita*.

As experiências tiveram como tratamento dois pulsos de água doce com duração de 3 horas cada um e um intervalo de 9 horas entre os pulsos. Esse delineamento teve como objetivo simular um pulso de água doce atingindo o estuário com variação de maré semidiurnal. Os pulsos foram aplicados em scyphistomas (fase bentónica da *Aurelia aurita*) e éfiras antes aclimatizadas em água salgada (salinidade 35). A experiência I foi constituído de 3 replicas contendo 3 indivíduos scyphistomas para cada tratamento. Foram aplicados três diferentes tratamentos de salinidade (3,10 e 17) e o controle (35). Após os pulsos de salinidade foram medidas as taxas de sobrevivência e monitorizada a reprodução assexuada durante 5 dias. Conclui-se que a sobrevivência na salinidade 3 é significativamente menor do que a sobrevivência no controle ($p \leq 0,05$). Além disso, a salinidade 10 é a salinidade mais alta com efeito significativo na reprodução assexuada por brotamento. Finalmente, concluiu-se que não houve diferença significativa entre as replicas do controle e dos tratados com relação ao número de scyphistomas que estrobilaram (p-value: 0.824) e ao número de medusas produzidas (p-value: 0.3285).

A *experiência II* foi uma repetição mais detalhada do tratamento salinidade 10 do *experiência I*. A *experiência II* possuía maior número de replicas, replicas individuais de scyphistomas com disponibilidade de substrato natural (conchas de ostra) e análise da resposta alimentar. Relativamente à resposta alimentar houve uma diminuição significativa na ingestão de alimento pelas replicas tratadas. Também existiram diferenças significativas na reprodução por brotamento, entretanto, com grande variabilidade. Este resultado levantou a hipótese da existência de scyphistomas com reprodução por brotamento ativa ou inativa (brotamento nulo). Assim, comparou-se número de scyphistomas que se reproduziram por brotamento e o número de scyphistomas que não se reproduziram, então foi encontrada diferença significativa entre controle e tratamento. Entretanto, mais uma vez não foi encontrada diferença significativa no número de scyphistomas que estobilaram e no número de éfiras produzidas entre tratamento e controle.

A *experiência III* foi constituído pelos mesmos tratamentos da *experiência I* aplicados em replicas individuais de éfiras. Foram analisadas a sobrevivência e o número de pulsações natatórias após a realização dos tratamentos. A sobrevivência foi significativamente maior no controle do que nos tratamentos de salinidade 3 e 10. O número de pulsações natatórias foi significativamente reduzido nas éfiras do tratamento de pulsos de salinidade 17, nas outras salinidades mais baixas a mortalidade foi elevada e não se pode testar este parâmetro. Este resultado demonstra a diminuição da viabilidade da éfira, uma vez que a natação desses organismos é essencial para a alimentação e migração vertical durante o recrutamento.

Também foram realizadas experiências com relação a ecologia alimentar de scyphistomas (experiência VI) e éfiras (experiência V). Foi oferecido plâncton natural recolhido no estuário do Rio Guadiana para 6 réplicas individuais em cada experiência. Foram determinados os grupos taxonômicos e a quantidade de indivíduos ingeridos pelo scyphistoma. Posteriormente, calculou-se a abundância e ocorrência dos taxons ingeridos. Os resultados de abundância e ocorrência foram colocados no gráfico de Costelo e determinou-se que a estratégia de alimentação do scyphistoma. Concluiu-se que a estratégia alimentar é generalista, mas com dominância de Copepoda. A mesma experiência de ecologia alimentar foi repetida com replicas individuais de éfiras. então determinou-se que a estratégia de alimentação da éfira é generalista.

Finalmente, conclui-se que os pulsos de água doce podem ser usados como ferramenta de gestão em sistemas estuarinos com regulação do caudal por uma barragem, para controlar os blooms de medusas. O controle faz-se a nível do recrutamento de medusas através da redução da sobrevivência e da viabilidade de éfiras e scyphistomas, e através da redução da reprodução dos scyphistomas por brotamento. Por outro lado, as condições adversas causadas pelo pulso não desencadeariam uma resposta estratégica de dispersão, já que os tratamentos não tiveram qualquer diferença significativa na estrobila e produção de medusas pelos scyphistomas. Com relação a ecologia alimentar, concluí-se que as efiras e os scyphistomas são generalistas apesar de por vezes os resultados indicarem seletividade para Copepoda.

Introduction:

General Context of jellyfish blooms:

About 60% of world's population inhabits coastal areas and watershed, therefore such ecosystems are constantly suffering anthropogenic pressures (Alongi, 1998; see Chapter 8.01). The human pressures may thread the ecosystems self organization process posing risks to ecosystem integrity and ecosystem services provided by these areas (Nielsen & Muller, 2000). Some of the changes on ecosystem state caused by such pressures include biological invasions and changes in key species abundance, what alters the ecosystem structures and then the ecosystem functions. This increase in pressures on these critical transition zones are accelerating the efforts to manage and restore such areas (Duarte *et al.,* 2008).

Native and invasive jellyfish blooms are increasingly reported in mediterranean and european coastal areas (Chícharo *et al.,* 2009; Boero, 2013), being related to both climate changes and anthropogenic impacts. Intensive blooms have been recently reported in neritic ecosystems, nurseries such as estuaries (Faria *et al.*, 2006). Although these events are a tourist attraction in some areas (e.g., Jellyfish Lake, Palau; Dawson *et al.,* 2001) and some jellyfish species are relevant in medusa-fish mutualistic associations (Boero, 2013), most jellyfish blooms negatively affect coastal ecosystem services. The negative impacts include beach interdiction (Ghermandi *et al.,* 2015), clogging of pipes and fishing nets, indirect effects on fishing resources by increase in medusa competition with fish and predation of early stages of fish (Pereira *et al.,* 2014).

Regarding the ecosystem integrity, jellyfish blooms may change key species abundances what may change the structure and function of the ecosystem and possibly, its ability on self organization. Yet, several processes underlying the occurrence of jellyfish blooms remain unstudied, specially those related to jellyfish benthic life stages (polyps) and its relation with medusa stages. The causes for jellyfish blooms increase are not known, in part because of the lack on knowledge about its benthic stage, the "producers"of medusa. (Purcell, 2005)

Jellyfish Biology and Ecology

Jellyfish is the non formal name given for what marine biologists call gelatinous Macrozooplankton (Boero, 2013). Such group of Macrozooplankton is characterized by a gelatinous body that is mostly constituted by extracellular matrix (Boero, 2013). The jellyfish is represented by three phyla: Chordata, Ctenophora and Cnidaria (Boero *et al.,* 2008). Most of the jellyfish are represented by Hydromedusae, followed by Scyphozoa and Cubozoa, then Tunicata, and Ctenophora (Boero *et al.,* 2008) . The first three most important groups belong to the Cnidaria Phylum. For the present study it will be focused on the most representative groups of jellyfish with polyp stage, such as Hydrozoa and Scyphozoa.

The Cnidaria Phylum representers occupy variety of ecological niches. They occur in the world wide oceans and estuaries, living from the surface to the greatest depths, their size can vary from few millimeters to few meters, they can be solitary or colonial and they can hold either holoplanktonic or meroplaktonic life cycles (Boero *et al.,* 2008). Most of coastal jellyfish holds bipartite life cycles where the medusa (haploid organisms) reproduce sexually giving originating embryos which develop into a planula larvae which settle on hard substrate and suffers metamorphoses into polyps (Boero *et al.,* 2008) (Fig.1) .

Such polyps can give rise to motile or not motile buds which can rise into new polyps (see description in Adler & Jarms, 2009). The jellyfish polyp is a benthic, feeding and modular stage in the lifecycle of most jellyfish representers. Just a single fertilization event is necessary for the formation of a whole polyp colony which will produce numerous ephyrae many times for over the following years, but also such colonies can be active and grow for many years without producing any ephyra, or even regress to a stolon during adverse seasons (Boero, 2008). Such variety on life stages makes jellyfish an adaptable organism on facing adverse situation since it has different manners to continue existing during unexpected environmental changes. However, this complex life style remarks an inherent difficulty to study their biology and therefore these animals has been poorly investigated (Boero, 2008).

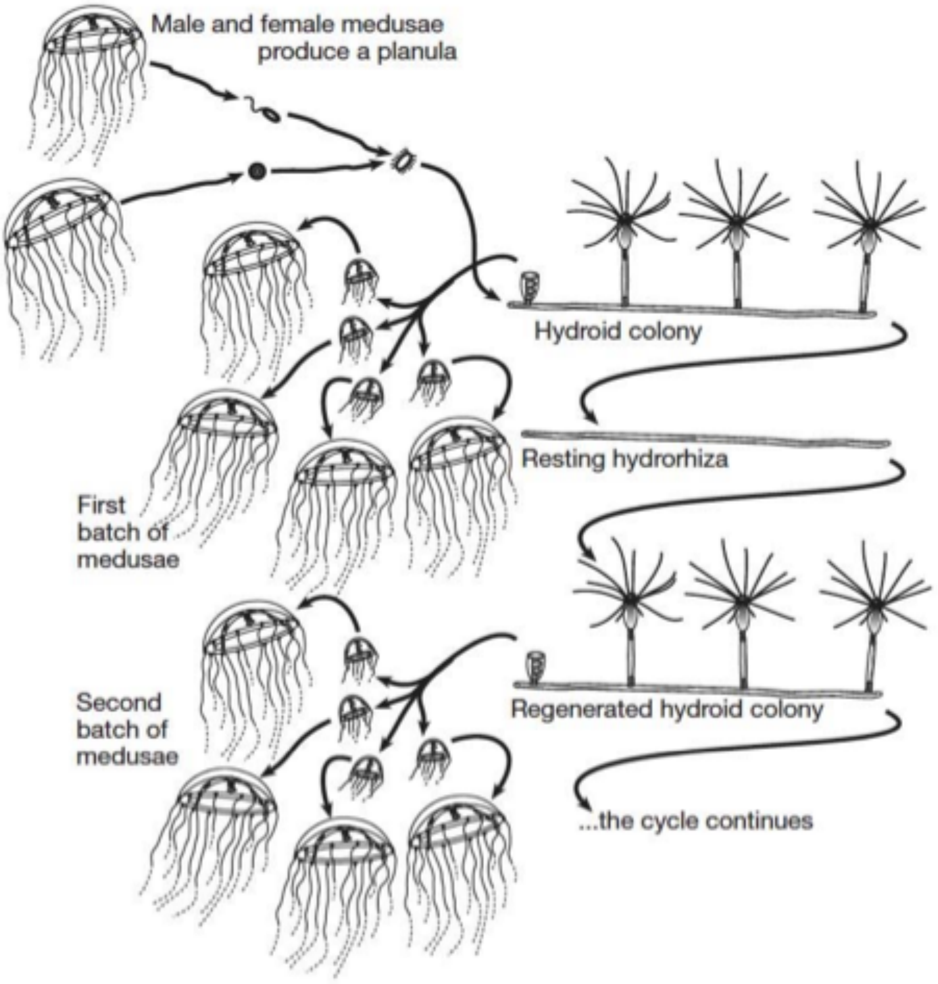

Figure 1. Life cycle of a pelago-benthic jellyfish (after Boero *et al.*, 2008).

In their pelagic state, medusae, they represent abundant guild of top predators in marine ecosystem together with fishes (Purcell and Arai, 2001). They are considered opportunistic predators since they have tentacles equipped with special cells called cnidocystis or colloblasts that enable such organisms to predate almost anything (Boero, 2013). The group affect the food web from microzooplankton (Colin *et al.,* 2005) to bowhead whales (Purcell *et al.,* 2010). Therefore, jellyfish directly interacts with numerous organisms in the marine food web and occupy numerous niches playing an important role in the ecosystem function.

The ecological importance of jellyfish is also hi lighted when it comes to its population dynamics features. Just like most of representatives of marine system, jellyfish population dynamic occurs in pulses of blooms followed by crashes (Boero, 2013; Purcell, 2005). The group has high fecundities rates and life cycles with renowned reproductive variety (Boero *et al.*, 2002).

Thus , when in good conditions, such as a matching of energy availability mismatched with jellyfish predators or competitors pulses, high are the chances of the jellyfish population pulse be successful and monopolize the system. In this way, carnivores jellyfish can be considered keystone predators, since they occur in blooms which can deplete the low levels of the food web (Boero, 2013). Also, non native jelly fish may be prone to suffer blooms, since they can find easier such conditions in the new environment which usually does not hold their natural competitors and predators.

Increase on frequency of Jellyfish blooms

Jellyfish has been abundantly present in the Earth since the Pre Cambrian Age and fossil data have shown its incredible prevalence in the ocean in the past . However, the evolution has brought animals apparently more efficient that posed limits to jellyfish prevalence, such as fish (Boero, 2013). In this way, in the past the ocean suffered a shift of main trophic pathway from **phytoplankton → herbivorous crustaceous zooplankton → carnivorous gelatinous zooplankton** to **phytoplankton → herbivorous crustaceous zooplankton → fishes** pathway (Boero, 2013) (Fig.2). However, scientists have speculated that the first pathway is becoming again the main

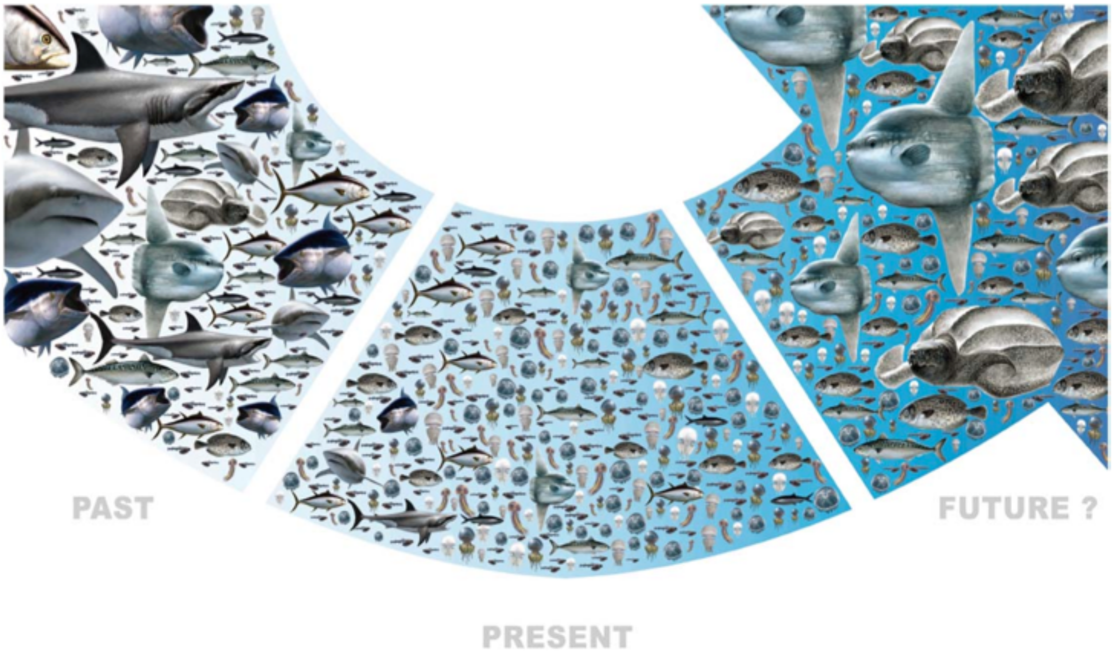

Fig. 2 - A decrease of large fish in the ocean and present apparent higher abundance of jellyfish. Potential future scenario maybe holds high abundance of medusivorours species. Source: Boero (2013).

pathway in the ocean as long as it has been observed an increase of jellyfish outbreaks and decrease of fish in the ocean.

Numerous jellyfish global population reviews have shown that blooms have been more extreme and frequent (Mills, 2001; Purcell, 2007; Chícharo *et al.,* 2009; Boero, 2013). In fact, the Jellyfish population oscillations are natural in global scale (Condon *et al.,* 2012), nevertheless, it has been proposed that such increase in jellyfish abundance is caused by global human interaction with oceans (Mills, 2001; Purcell *et al.,* 2007). It has been suggested that such jellyfish blooms trend is favored by global phenomenas such as Global warming, Global overfishing, eutrophication, transport of non indigenous species, widening natural area of non indigenous species, and habitat modification such as increased space for polyps and stability of freshwater flow in estuaries (Boero, 2013; Mills, 2001; Purcell *et al.,* 2007; Purcell, 2012).

Jellyfish blooms: Threats to ecosystem integrity and ecosystem services

One of the biggest concerns about such increase is regarding to the shift of trophic pathways prevalence in the marine food web from a “fish ocean” to a “jellyfish ocean” (Mills, 1995) (fig.2). Cnidaria phylum holds numerous negative interaction with fishes population such as predation of ichtioplankton (Purcell, 1985; Purcell, 1997; Arai 1988) , predation on fish eggs and larvae (Purcell, 1985), competition with zooplanktivorous fish, predation by fish on medusa and ctenophores (Arai, 1988) and parasite transmission to fish (Arai, 1988). In Azov sea, Ctenophore blooms depleted food supply and devastated small fish, severely affecting commercial catches (Studenikina *et al.,* 1991). Such context concern us about the potential consequences of jellyfish blooms events for fisheries and ecosystem integrity in the future. It is even more threatening when it comes to increasing of non indigenous “blooms jelly”, which are usually opportunistic and does not have natural competitors or predator to regulate its population.

Jellyfish blooms can also threat the integrity of the ecosystem by impacting on zooplankton assemblages, especially when it comes to non indigenous jelly. Findings in Guadiana estuary have linked the decrease of zooplankton abundance specially with jellyfish blooms of non native species, *Blackfordia virginica (*Muha *et al., 2012)*. Also, in the Caspian and Black seas, the invasion of the american comb jelly has caused a decrease in zooplankton community which has led to a pelagic fish reduction (Hulme, 2007).

In a more direct way, the jellyfish blooms impacts humans by causing beach interdiction (Ghermandi *et al.,* 2015), clogging of pipes and fishing nets, and indirect effects on fishing resources (Pereira *et al.,* 2014). Indeed, efficient alert systems which provide online information on beach interdictions and jellyfish species present (medusa stages only) are already available (e.g., Cataluna, Ghermandi *et al.*, 2015). Nevertheless, this animals blooms should not be considered strictly an ecosystem "deservices" since such events are touristic attraction in some areas (e.g., Jellyfish Lake, Palau; Dawson *et* *al.,* 2001) and some jellyfish species are relevant in medusa-fish mutualistic associations (Boero, 2013).

Possible drivers

Climate change have being claimed as a very important factor related to increase of jellyfish blooms (Purcell, 2005) and the temperature has been received by far the most attention (Lucas *et al.,* 2012) being considered an increase of about 2 degrees on water temperature by the end of the century. Recent studies which have correlated jellyfish predators populations with climate variables present the importance of such variables on their population size (reviewed by Purcell, 2005, Purcell *et al.,* 2007). Also, some experiments have shown positive relation between temperature and acceleration and increase in jellyfish production (Reviewed by Purcell 2007, see Liu *et al.,* 2009). Change in rain precipitation due to climate change can also be an important driver. Some authors have linked rain precipitation with low medusa occurrence (Lo & Chen, 2008; Diego & Alison, 2009). High salinity levels have been correlated with abundance of some jellyfish species (Goy *et al.,* 1989, Purcell *et al.,* 1999, Molinero *et al.,* 2005). Experiments shown significant effects of different salinity levels on asexual jellies reproduction (reviewed by Purcell, 2007). Therefore, climate change may be affecting positively jellyfish blooms due to alterations of temperature and precipitation regime.

Eutrophication phenomenon is also being linked to increase in jellyfish blooms. This phenomenon has simple and direct consequences on nutrient enrichment causing increase on primary production what may rise food availability for jellyfish population (Arai, 2001; Purcell *et al.,* 2007; Purcell, 2012). The phenomenon is characterized by small community composition what may cause inter specific advantages to jellyfish, since they are opportunistic organisms (Boero, 2013). In addiction, eutrophication is often associated to depleted oxygen zones, being the jellyfish considered to be tolerant under low levels of oxygen (Purcell & Arai, 2001; Purcell *et al.*, 2001) and even able to reproduce (Condon *et al.*, 2001). Therefore, the anthropic eutrophication expansion may create more suitable habitats for jellyfish .

Overfishing is another factor being considered to affect positively jellyfish populations. Marine resources as being overexploited in all over the world (Pauly *et al.*, 2002), leading to fish stock collapses (Mullon *et al.*, 2005). Cnidaria phylum holds numerous negative interaction with fishes population such as competition with zooplanktivorous fish and predation by fish on medusa and ctenophores (Arai,1988). Pauly *et al.* (2009) has suggested that removal that fishes may cause a lack of jellyfish predators what leads to a future dominated by jellyfish. In addiction, other authors have been focused on overfishing causing on a possible lack of competitors of jellyfish. Some literature has reported jellyfish outbreaks after local fish stocks collapses (e.g., Daskalov, 2002; Daskalov *et al.*, 2007). In this way, overfishing affect positively jellyfish population since fishes have numerous negative inter specific relations with jellyfish.

Ballast water and aquaculture also can be considered drivers of increase in jellyfish blooms. Ballast water and aquaculture seems to be the most important cause of species introduction in all over the world (Gollasch, 2008). Considering jellyfish characteristics such as rapid growth, asexual propagation, intensive predation, cryptic and morphological plasticity, it possess traits which makes it suitable for invasion of new habitats (Graham & Bayha, 2008). Jellyfish introduction has been intensively reported in several places in the world (see Purcell *et al.*, 2007) being *Aurelia aurita* one of the species which is occurring at a global scale (Dawson, 2003). In this way, jellyfish species, such as *Aurelia aurita,* have traits of successful invaders and ballast water and aquaculture allow it spread .

Talking about anthropogenic modification of habitats, the ocean sprawl 's pointed as major contribution to increase jellyfish outbreaks by providing higher availability of settlement substrates (Duarte *et al.*, 2012; Makabe *et al.,* 2014; Qingdao, 2014). Furthermore, this might allow the persistence of species in certain areas, year after year. In Adriatic Sea, Di Camilo *et al.* (2010) has pointed that in the studied area the polyps of *Aurelia aurita* occurs only in a ship wreck and that the blooms of such species are probably being sustained by the polyps of such wreck. Also Duarte *et al.* (2013) conducted experiments which have shown higher recruitment of polyps in the artificial substrate compared to natural substrata, what could be explained by the opportunistic traces of such animals. Taking into account that the construction of artificial structure in coastal areas are increasing in a rate about 3,7% to 28,3% per year (Duarte *et al.,* 2013), the substrata availability for such polyps have increased and we can speculate that such situation contribute to the apparent increase in global jellyfish blooms.

Finally, another possible factor driver which is also related to anthropic habitat modification, is the change in freshwater flow caused by reservoirs construction upstream estuaries. The freshwater discharge in an estuary has been negatively linked to the medusa occurrence (Chícharo *et al.,* 2009; Chícharo & Barbosa, 2011). This reduction of freshwater input and its variability has caused higher and stable salinity concentration in estuaries. As mentioned before, high salinity levels have been correlated with abundance of some jellyfish species (Goy *et al.,* 1989; Purcell *et al.,* 1999; Molinero *et al.,* 2005). Also, the natural short episodic freshwater pulses (salinity variation) have been linked to increase on biodiversity (Chícharo & Barbosa, 2011), then the control of the dominance of species and invasions (e.g jellyfish blooms). Therefore, salinity levels and its variation can increase biodiversity which can be a determinant for abundance of species such as jellyfish in the estuarine ecosystems.

Short term freshwater pulses: An ecohydrological solution for controlling jellyfish blooms

Ecohydrology is a division of hydrology sciences that focuses on ecological processes occurring within the hydrological cycle. This area of sciences suggests the existence of a mutual control between hydrology and biota (Zalewski *et al.*, 1990; Jorgensen, 1996; Zalewski, 2002). The ecohydrology approach can be used for estuarine ecosystem preservation by improvements on freshwater discharge management on upstream areas (Chícharo *et al.*, 2006). Therefore, it enhances environmental sustainability by using the ecosystems properties as tools for Integrated Water Resource Management (IWRM) (Zalewski, 2010).

In fact, Studies of plankton dynamics in coastal and estuarine barely take into account such mutual control. Freshwater inflow and runoff are often neglected on studies of plankton dynamics (e.g. for temperate estuaries, see Bode *et al.*, 2005;

Marques *et al.*, 2006). However, climate change and human perturbations have affected balance between fresh and marine water worldwide by modifying freshwater volume discharge into estuaries. This modifications have changed the structure and function of the estuarine ecosystem (Sklar & Browder, 1998).

Alterations on freshwater discharge have effects on biological and non biological components of estuaries in short or long term, and the most important effects of freshwater discharge alterations on biological components are: Change in productivity of the various trophic levels (Binet *et al.*, 1995); change in phytoplankton community composition (Alpine & Cloern, 1992; Cloern & Dufford, 2005); changes on distributional region of zooplankton species (Kingsford and Suthers, 1994); invasions and endemism impact (Bunn & Arthington, 2002); decrease of fish stock due to physical barrier for migration and decrease of food availability (Doornbos,1982; Chícharo *et al.*, 2001); and impact on spawning and nursery areas (Costa, 1988; Drake *et al.* 2002).

Water salinity is an important environmental factor that determines distribution and biodiversity of marine species in estuaries. The salinity in those ecosystems can reach 0 in the head of estuary and salinity 35 at the mouth. Lowered salinity as well as its variability causes diversity reduction in estuarine zones doe to low tolerance of coastal zooplankton to less salty water. Salinity decrease can cause physiological stress on organisms causing growth rate and final size reduction in several marine animals that immigrates into brackish water, e.g. polychaetes, starfish and mussels (Groth & Thede, 1989) .

There are suggestion that modifications on freshwater inflow are associated with jellyfish outbreaks (Xian *et al.*, 2005; Chícharo *et al.*, 2009; Chícharo & Barbosa, 2011). After Guadiana dam construction and the stabilization of freshwater discharge, jellyfish are increasing in abundance compared to previews time (Muha *et al.*, 2012) such as *Catosylus tagi*, *A. aurita*, and the invasive *Blackfordia virginica*. Pereira *et al.* (2014) has linked year of negative North Atlantic Oscillation and high river discharge with low occurrence of jellyfish at Guadiana estuary. Chícharo *et al.* (2009) and Chícharo & Barbosa (2011) have suggested that the reason of such situation is that jellyfish polyps may not survive under low salinity conditions. Besides such hypothesis, Purcell (2007) experiments results have shown lower strobilation in *Aurelia labiata* on treatments with reduced salinity (20) and higher ephyrae production in intermediate and high salinity levels (27 and 34). In this way, it is inferred that freshwater input in the estuary may control jellyfish by its survival and asexual reproduction. Therefore, freshwater pulses reaching the estuary could be a good management tool to reduce species dominance in an estuary.

However, many oh these studies have been conducted using relatively narrow salinity ranges and/or gradual salinity changes (e.g. Willcox *et al.*, 2007; Holst & Jarms, 2010). These experiments are likely to correspond to seasonal or long term changes in salinities, what do not correspond to the real situation for estuaries and shallow bays. Such water bodies usually suffers high and rapidly changes over short periods of time caused by rainfall and river run off events. Those changes are likely to persist over the scale of hours to days (Cloern & Nichols, 1985). Therefore, there is a lack of knowledge about survival and physiological response of jellyfish under acute salinity changes. Such understanding would also support studies for proposing fresh water pulse released from a Dam as an ecohydrological solution for controlling increase in jellyfish blooms.

Aurelia aurita: the biological model

The *Aurelia aurita* is one of the most representative groups when it comes to jellyfish blooms in coastal and semi-enclosed seas (Mills, 2001). *A. aurita* inhabits nearshore waters, especially closed basins, such as coastal embayments, fjords and estuaries, where there are suitable substrata for the benthic scyphistoma and occupying a great variety of habitats worldwide (Lucas, 2001).

Aurelia aurita can reach 30 cm (Schneider & Behrends, 1994) in diameter and form dense blooms. The fluctuations of population size of adult medusa indicate large fluctuations in recruitment, which in turn is determined by survival of polyps and ephyrae and their growth success (Hernroth & Grondahl, 1985). In addition, the research performed in reproduction of benthic invertebrates states that in a given habitat, the distribution and abundance of marine invertebrates is maintained by the following factors: recruitment through sexual reproduction, migration, mortality and asexual reproduction (Chia, 1990). Therefore, scyphistoma asexual reproduction, ephyrae survival, polyps survival, and their good development are determinants of medusa adult population dynamics.

When *A. aurita* blooms occurs it can significantly impact on coastal plankton communities. In many temperate coastal systems, it has been linked the spring-summer decrease in zooplankton biomass is caused by *A. aurita* predation (Schneider & Behrends, 1994; Schneider & Behrends, 1998). In the case of jellyfish predatory pressure on herbivores is high , nutrients and light not limiting, top-down regulation of planktonic communities may occur, then phytoplankton blooms occurrence resulting in changes on species composition (Lindahl & Hernroth, 1983). Therefore, *A. aurita* blooms are quite important to be studied in order to understand the impacts of it into the food web and community shifts.

A. aurita is the most studied jellyfish group, however the processes involved in its population increase are still poorly understood. Most of *A. aurita* present a benthic stage (scyphistoma) and a pelagic stage (medusa). The current knowledge of *A. aurita* scyphistoma population and environmental factors role on its dynamics are restricted to colony level (e.g. Willcox *et al.*, 2007, Purcell *et al.*, 1999; Watanabe & Ishii, 2001) . Indeed , the process involved on population dynamics on individual level are poorly understood (Lucas, 2001) but extremely important to understand the scyphistoma colony dynamics (Garrabou, 1999).

Aurelia aurita life cycle

Aurelia aurita has a complex life cycle composed by both asexually reproducing polyp and sexually reproducing medusa. The different phases are egg, planula, polyp (scyphistoma and strobila), ephyra and medusa (Fig.3). Medusa is considered dioecious and it releases sperm in the water column. The sperm fertilize ovocytes within the female medusa, then the eggs are released in the water. The egg gives rise to planula larva, which settles on hard substrate after 7 to 10 days after released. Afterwards, planula develop into a polyp (scyphistoma) that will reproduce asexually through a process called strobilation. This process consists on segmentation of polyps into several disks, and each one of the disks will originate an ephyra. The ephyrae will be released in the water column and develop into adult medusa, that will start the life cycle again. (Fig.3)

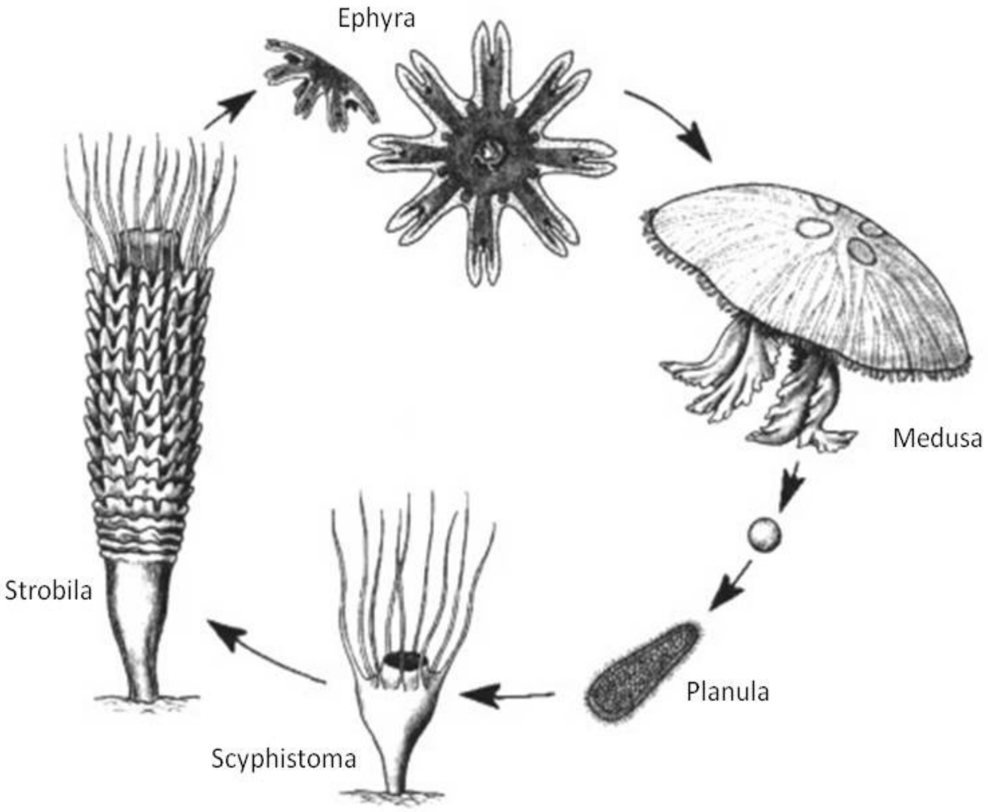

Fig. 3: *Aurelia aurita* life cycle adapted from Ruppert *et al.* (2005). (Arai,1997)

Aims

In the present study, we suggest that the release of freshwater pulses from a hydrotechnical structure (Dam) can be an ecohydrological solution for controlling jellyfish blooms. For supporting this suggestion, the present project aimed to assess benthic stages and medusa larvae (ephyra) survival, asexual reproduction (budding, strobilation and ephyrae production) and ecophysiological response (feeding and swimming rate) under low salinity conditions **(Experiments I, II and III).** This low salinity conditions were delineated in the way that it simulates an estuary, with semidiurnal tide variation, after a short term freshwater pulse released from a dam. The present work also investigated the feeding ecology of polyps and ephyra for supporting predictions of trophic web impacts of jellyfish blooms/control **(Experiments IV and V)**. Previous research studies regarding freshwater pulses effect on several estuarine organisms have been conducted by the Ecoreach laboratory, CCMAR, Faro, Portugal (Chícharo *et al.,* 2006).

The questions of the project are:

(i) Does episodic freshwater pulses affect scyphistoma survival?
(ii) Does episodic freshwater pulses affect scyphistoma asexual reproduction?
(iii) Does the episodic freshwater pulses affect scyphistoma feeding rate?
(iv) Does episodic freshwater pulses affect ephyra survival?
(v) Does episodic freshwater pulses affect ephyra swimming pulsation?
(vi) Is scyphistoma feeding strategy generalists?
(vii) Is ephyra feeding strategy generalists?

Chapter I - *Aurelia aurita* scyphistomae and ephyrae response to freshwater pulse

Introduction

The benthic phase of *Aurelia* sp. reproduces asexually by vegetative budding or by strobilation which produces ephyra, the larvae of pelagic jellyfish (e.g. Spangenberg, 1965). This different types of reproduction are supported by allocation of energy resources in different strategies for perpetuating their existence. Investments on ephyrae production increases the likelihood of successful recruitment to the bloom forming medusa. In the other hand, increases in investments for budding reproduction increases the likelihood of population perpetuation by providing a large asexually reproducing colony that will produce ephyrae in the future (Brewer & Feingold, 1991; Stearns, 1992; Purcell *et al.*, 1999). In this way, both strobilation and budding reproduction are significant for recruitment of pelagic jellyfish.

Asexual reproduction mode, rate and timing are regulated by environmental variables conditions experienced by scyphistoma (Willcox *et al.*, 2007). As previously mentioned, high salinity levels have been correlated with abundance of some jellyfish species (Goy *et al.,* 1989, Purcell *et al.,* 1999, Molinero *et al.,* 2005). However, the current knowledge about the role of environmental conditions and scyphistoma colony dynamics of *Aurelia aurita*. is mainly limited to responses at the colony level , the change of colony size and the total ephyrae produced by it (e.g. Spangenberg, 1965; Purcell *et al.*, 1999; Watanabe & Ishii, 2001). In fact , it is extremely important to study these processes also at the individual level in order to understand the colony dynamics as long as growth of colonies are ultimately driven by individual response (Lucas, 2001) . However, there are only two studies focusing on individual level (e.g. Willcox, 2007; Gong, 2001).

The survival and physiological responses of jellyfish different life stages are not the same. As an example, despite ephyrae and juvenile medusa occurs in the same habitats and relatively close to each other in time, they do not have the same response to short, severe salinity changes (Diego & Alison, 2009) . Therefore, ephyrae responses to salinity are not the same as the other jellyfish stages and should be investigated. It is essential to analyze survival and physiological status of ephyra in order to investigate recruitment and jellyfish blooms.

Some pelagic jellyfish behavioral response to low salinity has been reported. In Roscoe Bay, British Columbia, *Aurelia labiata* medusa were observed to swimming away when facing water with salinity lower than 20 (Albert, 2012), what shows avoidance to possible physiological chocks when in contact with change in salinity. Mayer (1910) found that swimming pulsation of of rhizostome *Cassiopea xamachana* were directly proportional to salinity and the reason proposed was the change in absolute concentration of ions that are involved in pulsation as long as Robertson (1949) found evidences of ionic regulation in field populations of *Aurelia aurita.* Therefore, salinity has effects on jellyfish swimming abilities or behavior.

For predicting and mitigating from jellyfish blooms impacts it is important to understand how environmental factors affect all jellyfish life history stages. The present work focused on salinity variation as the environmental variable affecting it. It has used scyphsitomae and ephyrae stages into account because they are important for recruitment, the most important process for determining adults population. Variables such as survival, asexual reproduction and physiological responses (represented by feeding rate and swimming ability) are important to be measured and analysis because they impact recruitment success.

Hypothesis:

(i) Episodic freshwater pulses reduce scyphistoma survival
(ii) Episodic freshwater pulses reduce scyphistoma asexual reproduction.
(iii) Episodic freshwater pulses reduce scyphistoma feeding rate
(iv) Episodic freshwater pulses reduce ephyra survival
(v) Episodic freshwater pulses reduce ephyra swimming pulsation

Aims:

i) To compare 3 different salinity pulses (3, 10, 17) and the control (35) effects on scyphistomae survival, strobilation and budding.

ii) To compare salinity 10 pulses and control (35) effects on scyphistomae survival, budding, strobilation, ephyrae production, time delay of ephyrae production and feeding response.

i) To compare 3 different salinity pulses (3, 10, 17) and the control (35) effects of ephyrae survival and swimming activity.

Methods:

1) ***Acclimatation and Experiment I - "Four different salinity treatments and scyphistoma response"***

Aurelia aurita scyphistomae were obtained from Oceanario de Lisboa/ ZOOMARINE. For acclimation they were kept into tanks, fed *ad libidum* with newly hatched *Artemia salina* nauplii once weekly (Fig.4). In this way it was intended to provide saturating prey briefly, resulting in equal treatments. For performing

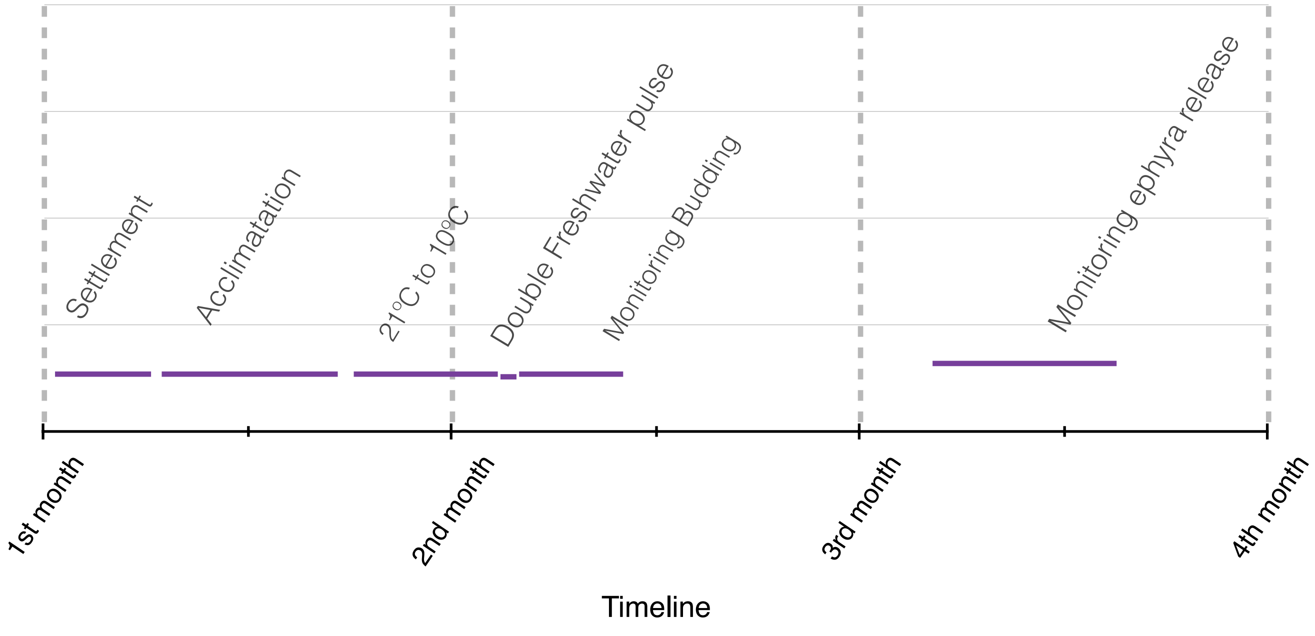

Fig.4 - Timeline of experiments steps

experiments scyphistoma ranging from 1,5 and 2,2 mm were gently detached from substrata, then randomly placed into polystyrene plaques inside 150 mL beckers. The treatments had 3 replicates and each replicate contained 3 individual scyphistoma together in the same plaque. The individuals did not present any sign of strobilation. During the whole experiment it was assure the replicates had only those 3 scyphistomas as producers of buds or ephyra medusa by removing the young buds constantly. Previously, the plaques stood under seawater circulation for 3 days. After then, scyphistoma stood settling for 6 days.

In order to stimulate future strobilation it was decreased the environmental temperature (21ºC) to 10 ºC in 10 days (Kakinuma, 1962; Brewer & Feingold, 1991) (Fig.4) . Afterwards it was applied "**the double freshwater pulse**" of salinities 3, 10 and 17 using 35 as control... The "**the double freshwater pulse**" was a simulation of one freshwater release reaching the estuary and dominating it twice in 24 hours during low tide (semidiurnal tide variation) (Fig.5). The immersion of the animals into

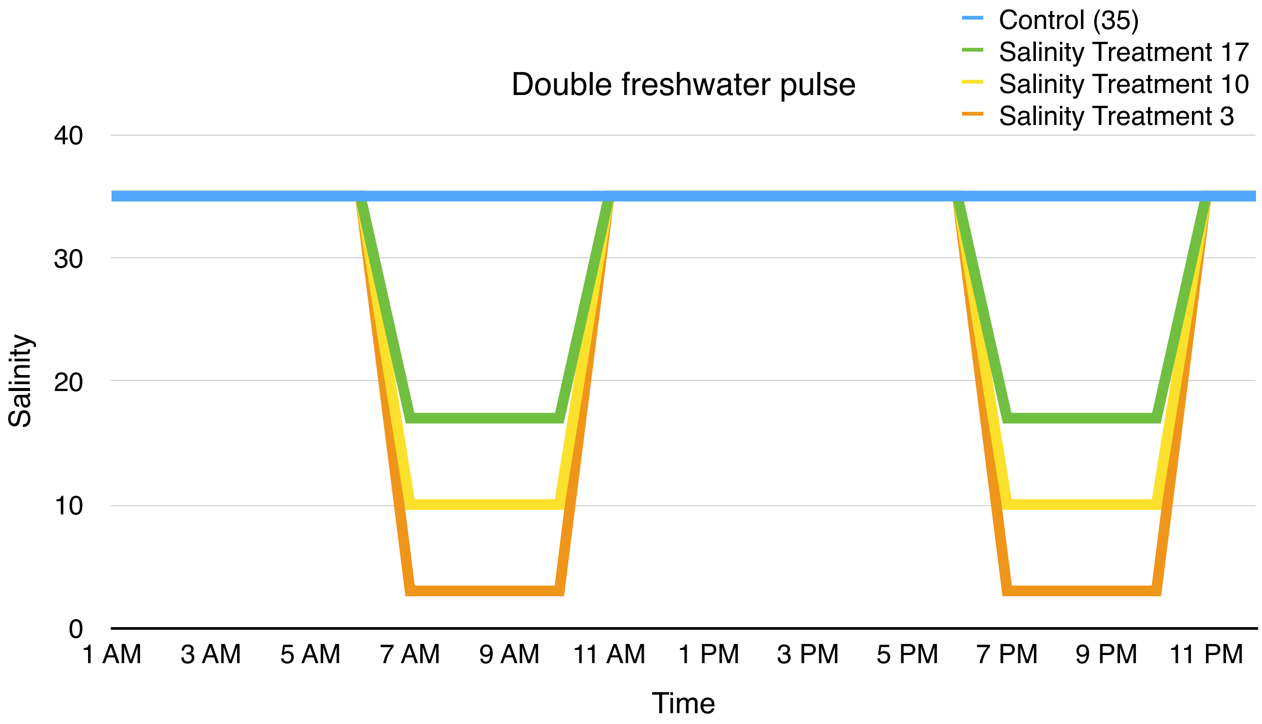

Fig.5 - Graphic Salinity x Time showing how short term salinity pulse was performed. **”The double freshwater pulse”** was done in 3 different treatment (salinities 3,10,17) and control (salinity 35)**.**

treatment water lasted 3 hours each. The pulses were separated by 9 hours interval in which scyphistoma was immersed into salinity 35 filtered sea water conditions.

After treatment, it was checked the survival of the scyphistoma and the dead ones were removed. Also the asexual reproduction (budding, strobilation and ephyrae production) was measured. The remained scyphistoma were monitored regarding budding production for the 5 following days. In the third day it was counted the number of produced buds for each replicate. Every counted bud was removed in order to not allow its reproduction (Purcell *et al.*, 2012) and again on 5th day the number of buds was counted. After 25 days of freshwater pulse, the scyphistoma finally started to strobilate. When the first ephyra was released, the number of ephyrae produced were recorded every 2 days in a total of 12 days (Fig.4).

2) *Experiment II - "Salinity 10 treatment and individual scyphistoma response"*

Based on budding result of the first experiment we found reasonable to perform more detailed experiment with natural substrate (oyster shells), individual replicates, more replicates and feeding rate monitoring under salinity 10 and again monitoring asexual reproduction.

Acclimation procedures followed the same steps of the preview experiment. For performing experiments scyphistoma ranging from 1,5 and 2,2 mm were gently detached then randomly and individually placed into 12 mL polystyrene plaques. The plaques had half of the bottom area occupied by a piece of oyster shell allowing scyphistoma to choose where to settle, shell or polyester substrata. Previously, the plaques stood under seawater circulation with the shells for 3 days (Fig.6). After then , they stood settling for 6 days without food or air in order to not disturb the environment and scyphistoma choice.

Procedures for stimulating strobilation followed the same steps as the preview experiments. Afterwards we applied "**the double freshwater pulse**” of salinity 10 (Fig. 7).

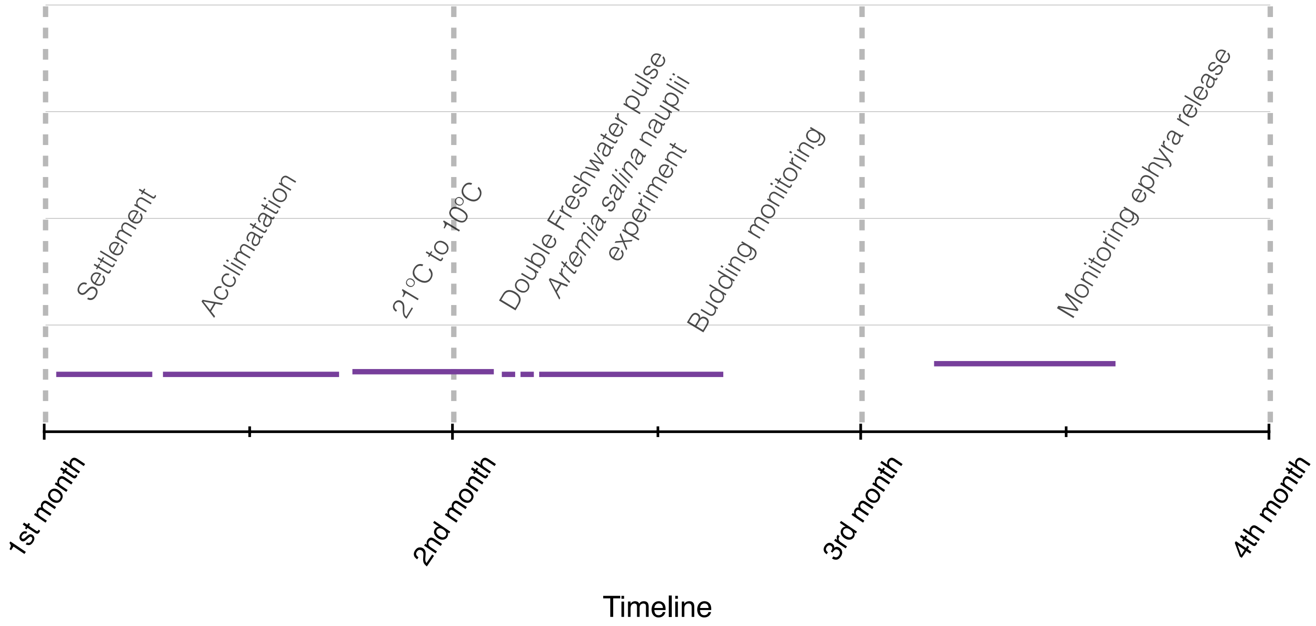

Fig.6 - Timeline of experiments steps

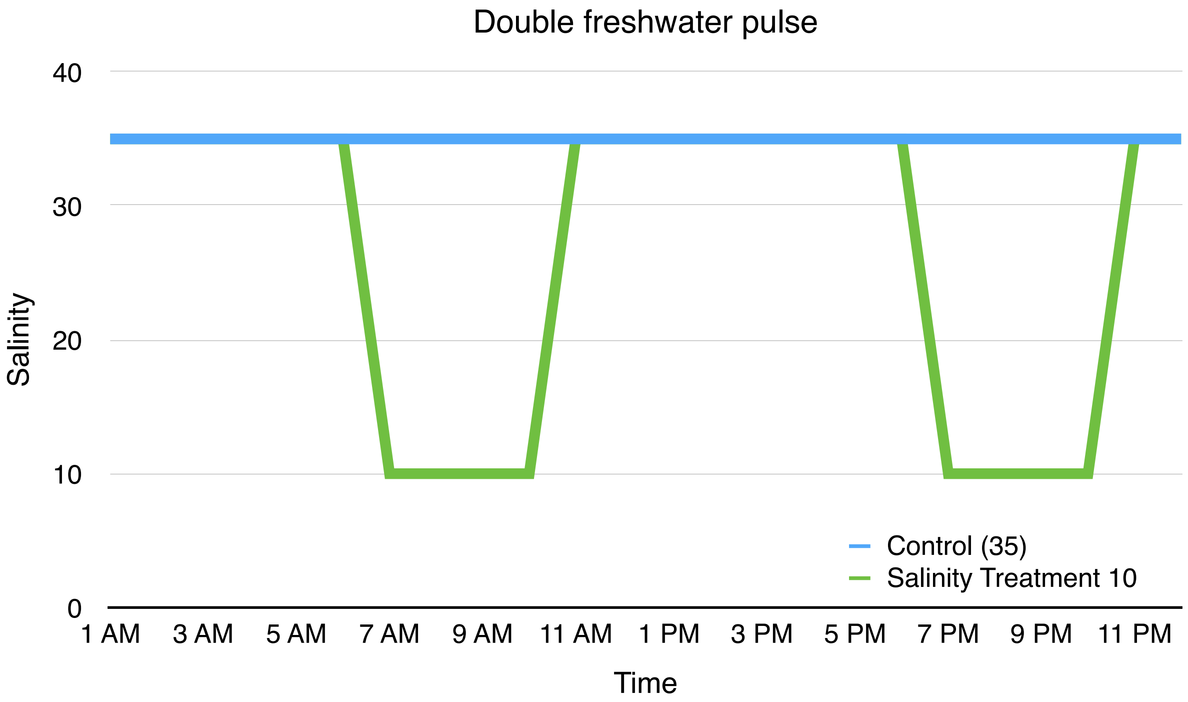

Fig.7 - Graphic Salinity x Time showing how short term salinity pulse was performed the **"the double freshwater pulse"** under salinity 10**.** It aimed to simulate semidiurnal

For measuring ecophysiological response it was tested feeding rate of Scyphistomae after treatment. In about 5 hours after the last pulse, the scyphistoma were fed with newly hatched *Artemia salina* nauplii. A solution of 40 *Artemia salina* nauplii individual per milliliter was prepared. One milliliter of solution was given to each replicate. Each replicate had another representative plaque with the same conditions as the replicate, but without scyphistoma (Fig.8). These plaques were called as “Branco” plaques, they received the same volume of *Artemia salina* nauplii solution. One hour after feeding it was counted the number of remained nauplii in each replicate and each “Branco" plaque. The, the number of eaten nauplii was considered as the average of remained nauplii inside “Branco” replicates subtract by the remained nauplii in each replicate.

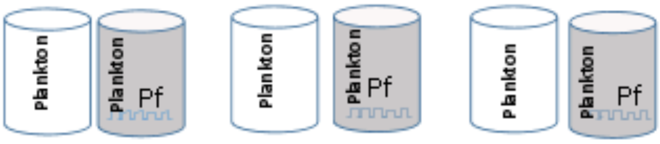

Fig. 8 - Illustration demonstrating Ingestion experiment. White recipients, called as “Brancos”, were the control and contained zooplankton without Scyphistoma, while grey recipient contained Scyphistoma and zooplankton.

Also the asexual reproduction (budding, strobilation and ephyrae production) was measured after treatment. The remained polyps were monitored regarding their budding production for the 10 following days. It was counted the number of produced buds for each replicate with an interval of 2 days. Then the counted buds were removed in order to not allow such buds reproduction (Purcell *et al.*, 2012). After 25 days of freshwater pulse, the scyphistoma finally started to strobilate. When the first ephyra was released, the number of ephyra produced were recorded every 2 days in a total of 12 days

3) *Experiment III - “Four different salinity treatments and individual ephyra response"*

It was picked up 12 ephyrae of 2-3 mm size which were released from our scyphistoma culture. They were distributed individually and randomly in 3 replicates for each treatment into 3 mL plaques (Fig.9). Afterwards, it was applied "**the double freshwater pulse**” of salinities 3, 10, 17 with salinity 35 as control (Fig. 5).

After setting 17 and 35 as salinities suitable for testing swimming abilities of ephyra, it was pick up other 12 ephyrae ranging between 3-2 mm size and distributed randomly in 6 individual replicates for 17 Treatment or control (35). It was applied again the same protocol of "**the double freshwater pulse**”. Afterwards, for measuring ecophysiological response, it was counted the number of swimming pulses of ephyrae just after the treatment.

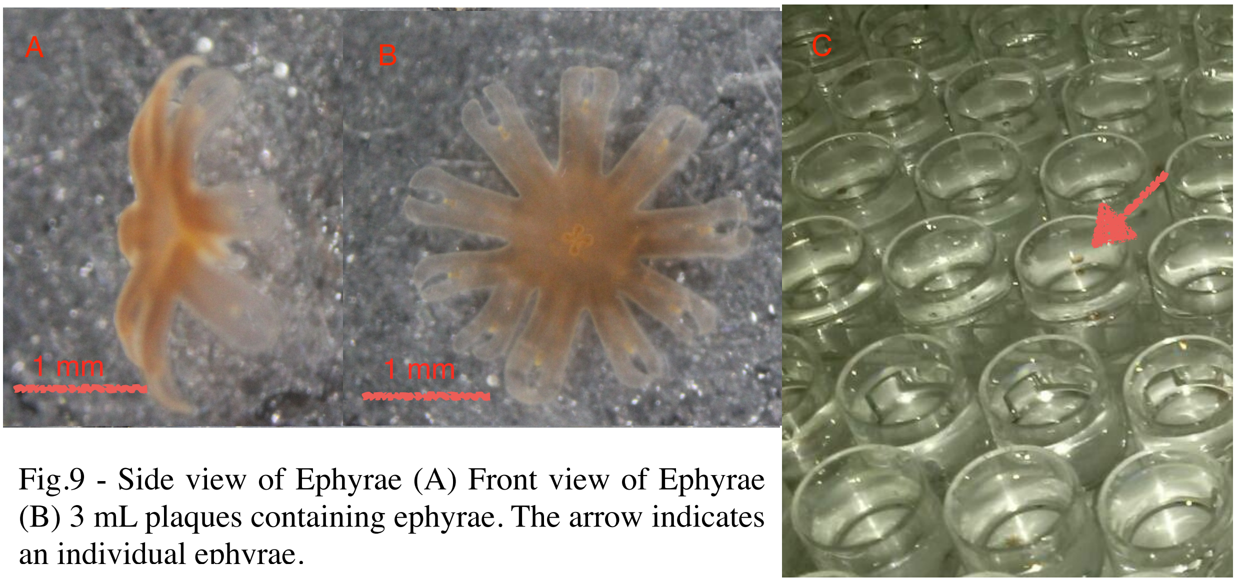

Fig.9 - Side view of Ephyrae (A) Front view of Ephyrae (B) 3 mL plaques containing ephyrae. The arrow indicates an individual ephyrae.

Data analysis:

The percentage of scyphistoma survival, ephyra survival and the number of strobilating scyphistoma were transformed to arcsin. The ANOVA tests were used to test differences in those variables between control and treatment. The number of ingested nauplii, the cumulative number of buds produced (5 days), the cumulative number of produced ephyrae (12 days), the number of swimming pulses of ephyra (1 minute) have adhered to ANOVA assumptions of normality. Again, the ANOVA tests were used to test differences in those variables between control and treatment. Statistical analysis was performed using the software R 3.1.1 (The R Project for Statistical Computing 2014). Number of ephyrae released every 2 days in a total of 12 days were recorded and plotted cumulatively in a graph.

Results:

1) *Experiment I - "Four different salinity treatments and scyphistoma response"*

1.1) *Survival of scyphistomae after treatment:*

Scyphistomae survival was significantly different ***(p-value: < 2.2e-16)*** between salinity 3 treatment and the other treatments (salinities 10, 17 and 35). While all the replicates of salinity 3 presented 100% of mortality, the other treatments have not presented any dead individual (Fig.10).

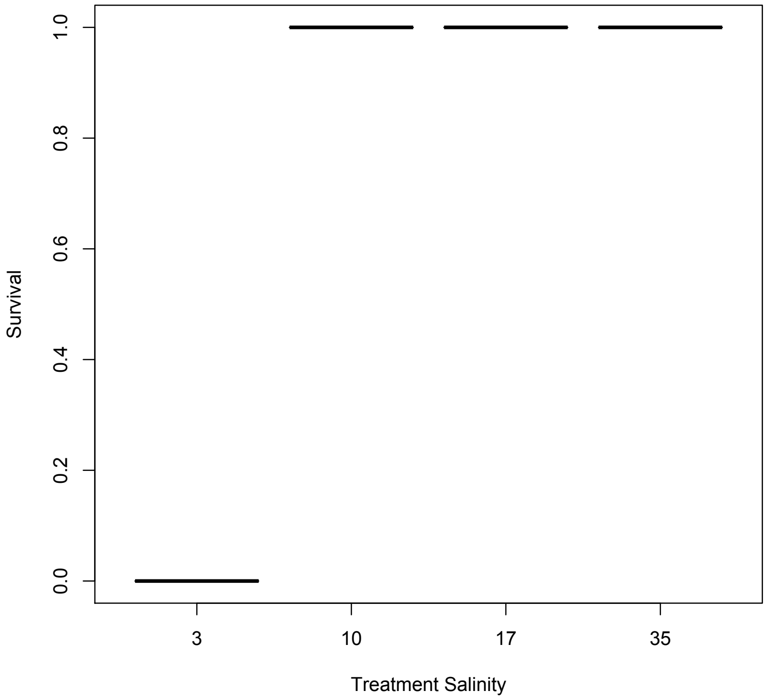

Fig.10 - Graphic of Scyphistoma survival proportion for each treatment (salinities 3,10,17) and control

1.2) *5 days cumulative number of buds :*

The cumulative number of buds produced by schyphistomae (Fig. 11) on the following 5 days after pulse was significantly different only between salinity 10 treatment and control ***(p-value: 0.04747)*** (Fig.12). Here, it is important to hi-light the absence of budding for all replicates on treatment 10, what bring questions that will be discussed .

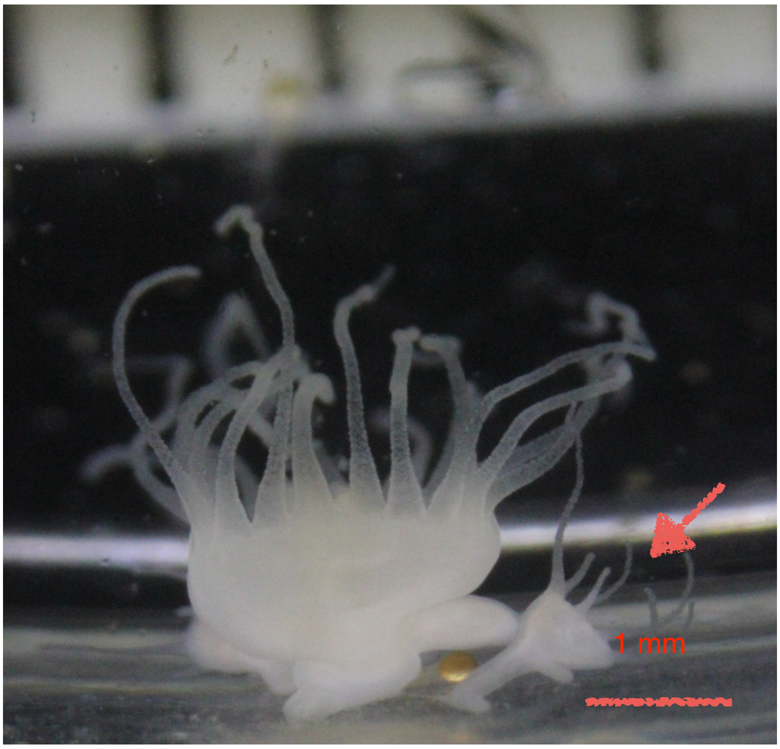

Fig.11 - Picture of Scyphistoma and its new bud (pointed by arrow).

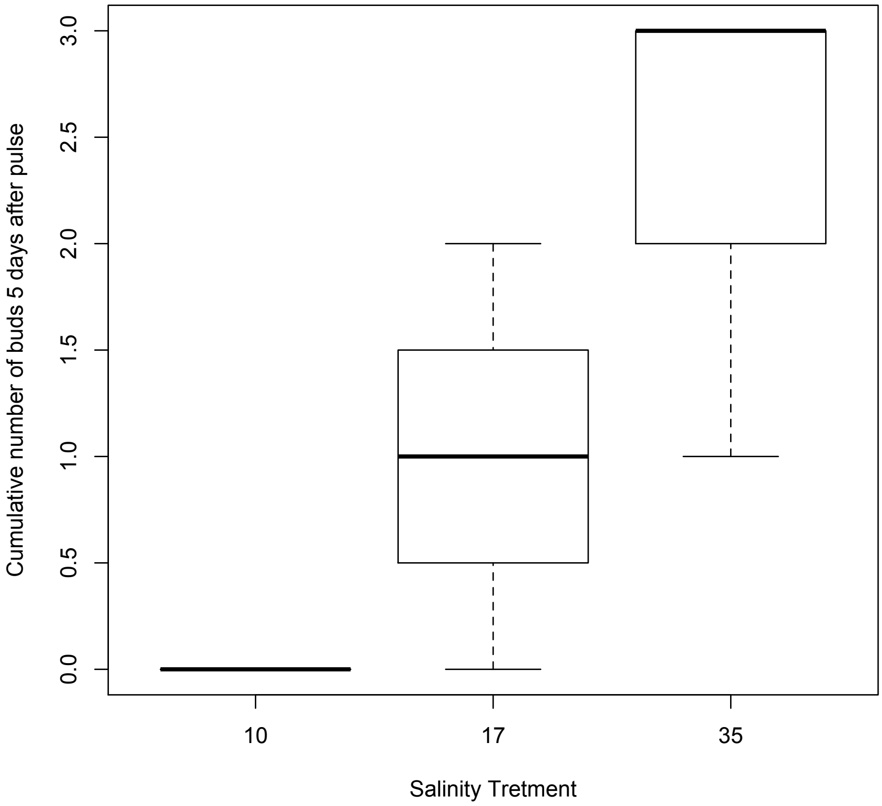

Fig.12 - Graphic of number of buds produced by Scyphistoma replicate for each treatment (salinities 10,17) and control (salinity 35)

1.3) *12 days cumulative number of produced ephyrae and number of strobilating scyphistomae (Fig.15):*

The cumulative number of produced ephyrae was not significantly different among treatments ***(p-value: 0.3285)***. (Fig.13). In addiction, the number of scyphistomae that has strobilated did not differ among treatments, they even have presented the same average ***(p-value: 0.824)***. (Fig.14).

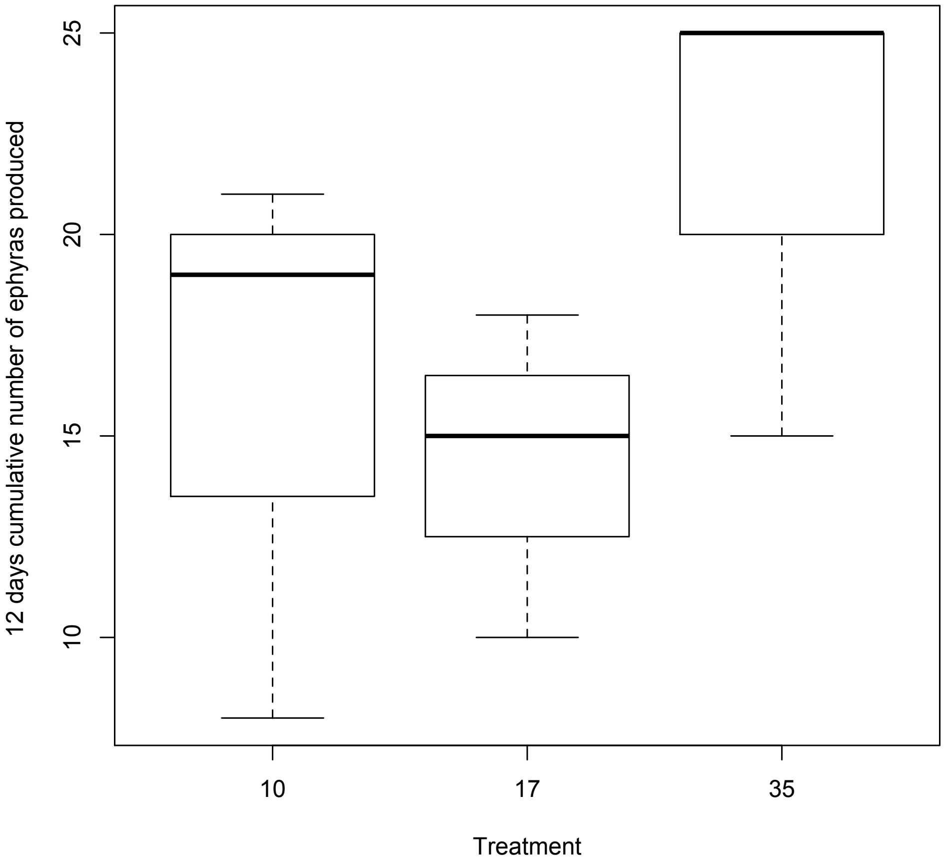

Fig.13 - Graphic of cumulative number of ephyrae produced by Scyphistoma replicate for each treatment (salinities 10,17) and control (salinity 35)

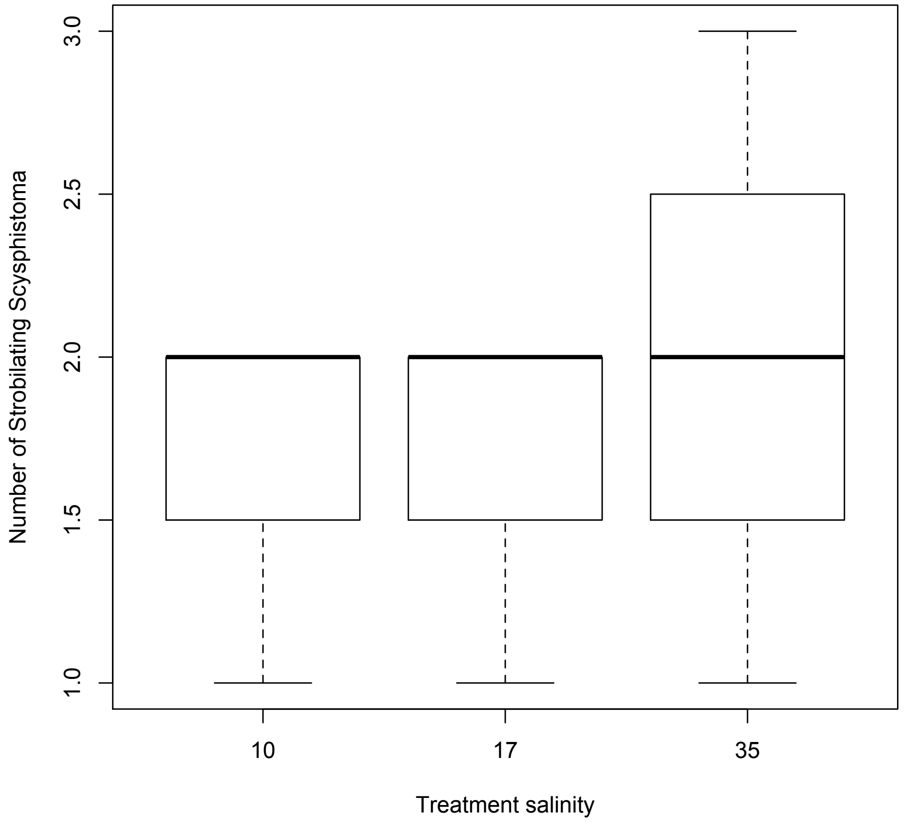

Fig.14 - Graphic of number of strobilating individual per Scyphistoma replicate for each treatment (salinities 10,17) and control (salinity 35)

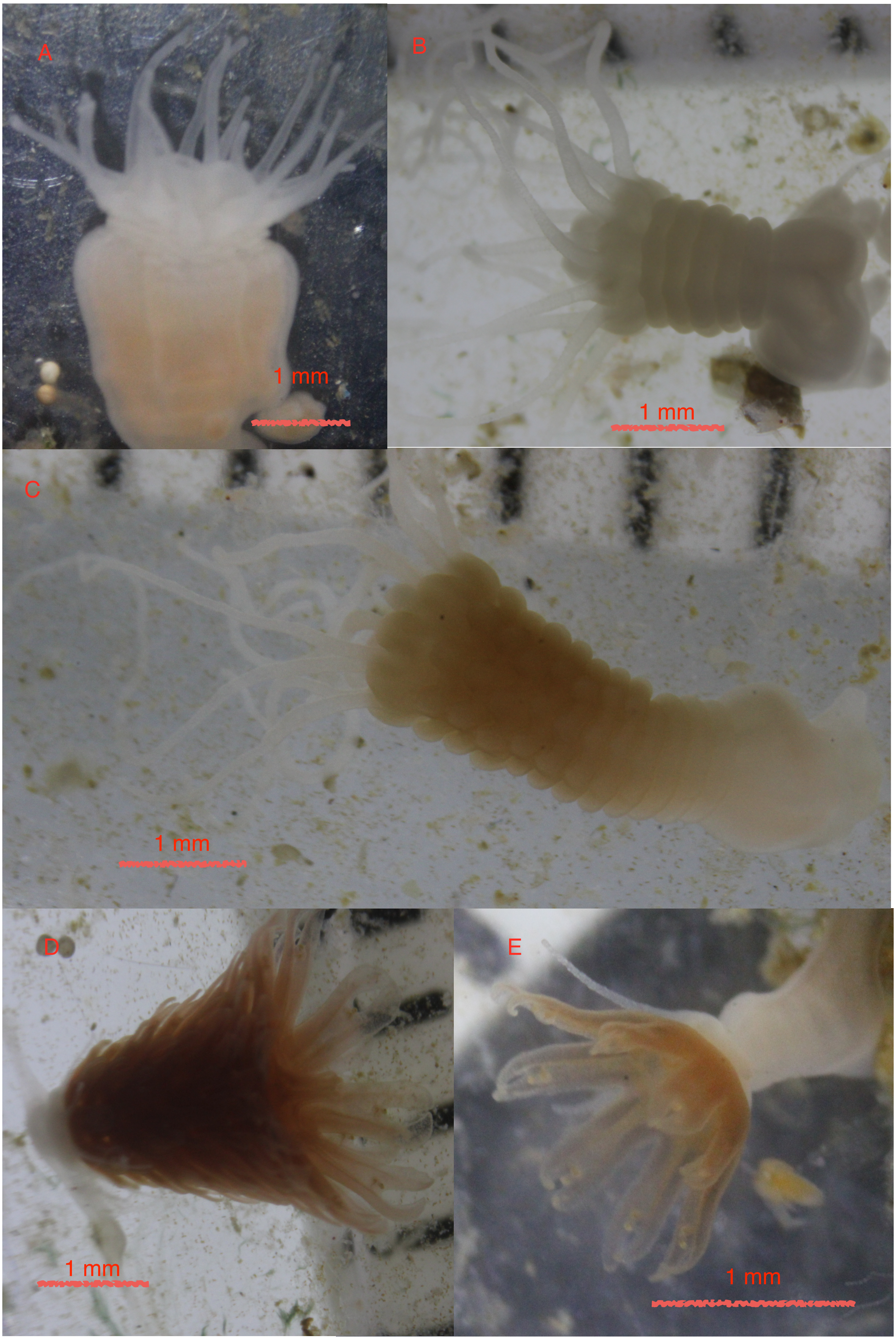

Fig.15 - Scyphistoma into strobilation process from first strobila sign (A) disks formation (B), advanced disk formation (C), disks transformed into ephyrae (D), strobila releasing the last ephyrae (E).

1.4) ***Temporal variation of the ephyrae production:***

It seems to exist a time delay of release of ephyrae in salinity 10 treatment compared to control (Fig.16).

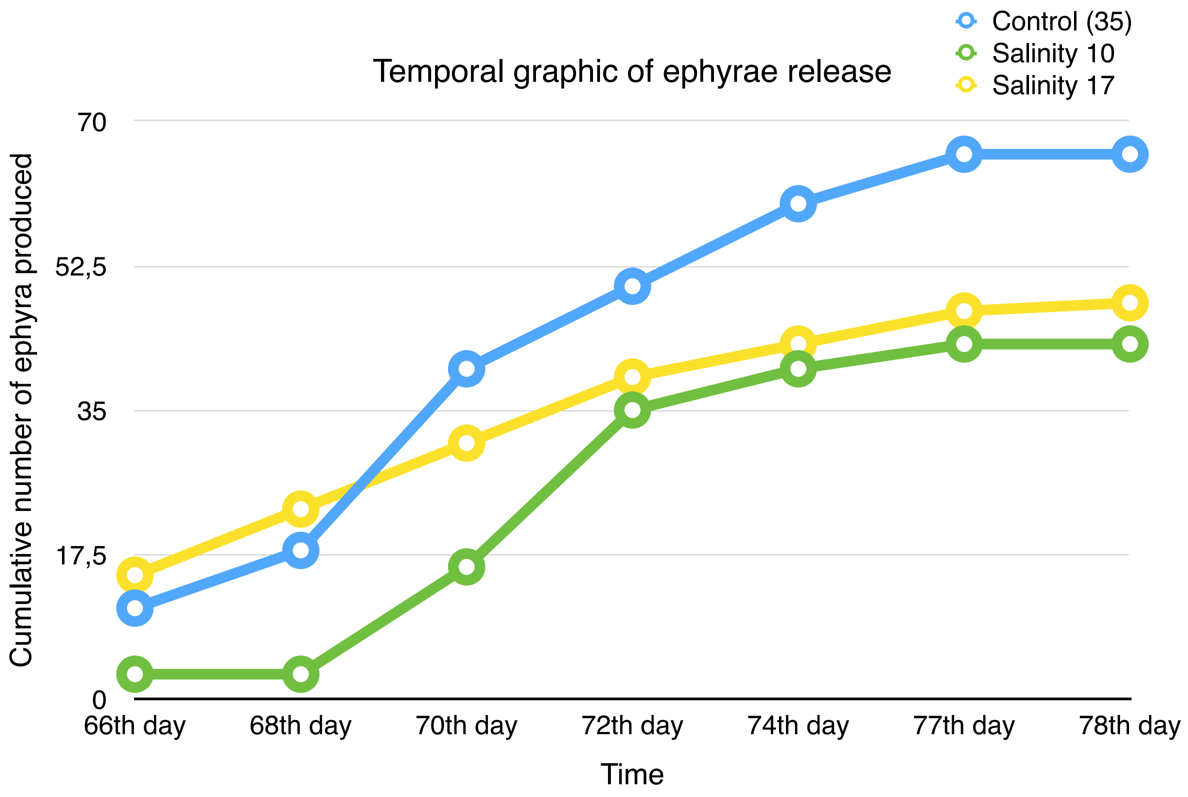

Fig.16: Graphic of cumulated number of ephyrae released per salinity treatment.

2) *Experiment II - "Salinity 10 treatment and individual scyphistoma response"*

2.1) *Ecophysiological response :*

The number of ingested *Artemia salina* nauplii were significantly lower on salinity 10 treatment compared to control ***(p-value: 9.324e-05)*** (Fig. 17).

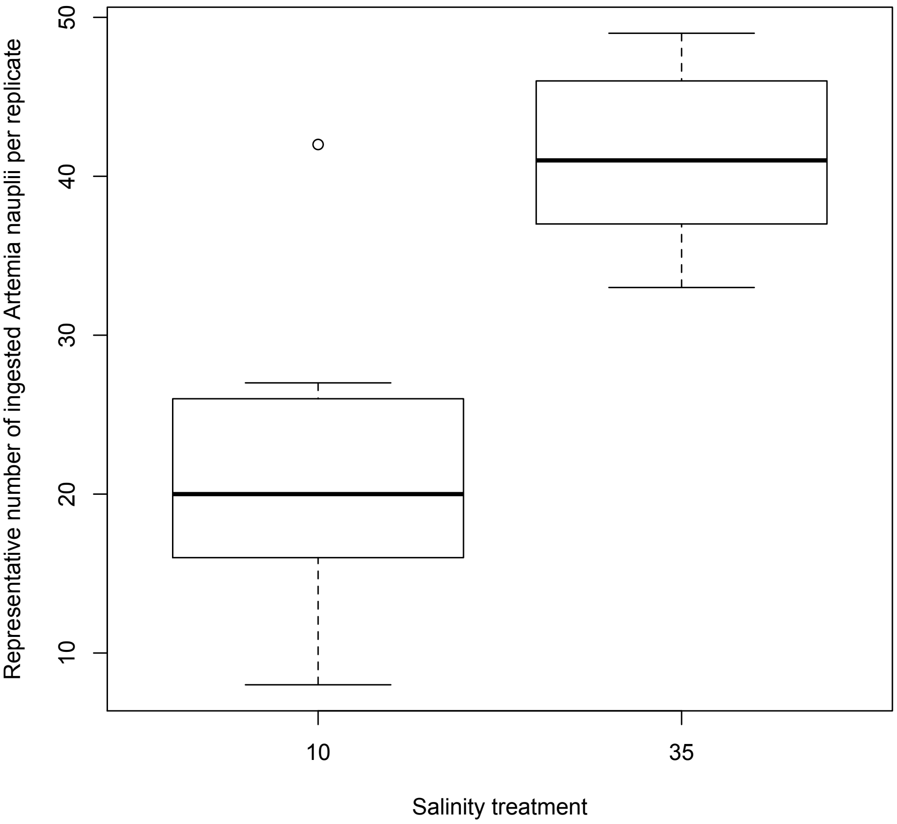

Fig. 17 - Number of ingested *Artemia salina* nauplii by replicate for Control (salinity 35) and treatment (Treatment 10).

2.2) *10 days and 5 days of cumulative number of buds:*

In this experiment the results regarding 10 days cumulative budding had ***p-value: 0.0259,*** showing significant difference. Also, the 5 days cumulative budding presented even higher significant difference between treatment and control ***(p-value: 0.009995)*** (Fig. 18 and Fig. 19). However, R-squared were respectively 0,2283 and 0.307, what may be explained by the fact that numerous scyphistoma presented absence of budding.

This situation opened the discussion of influence of salinity on the number of actively and non actively budding Scyphistomae. Such point of view is the reason why the next

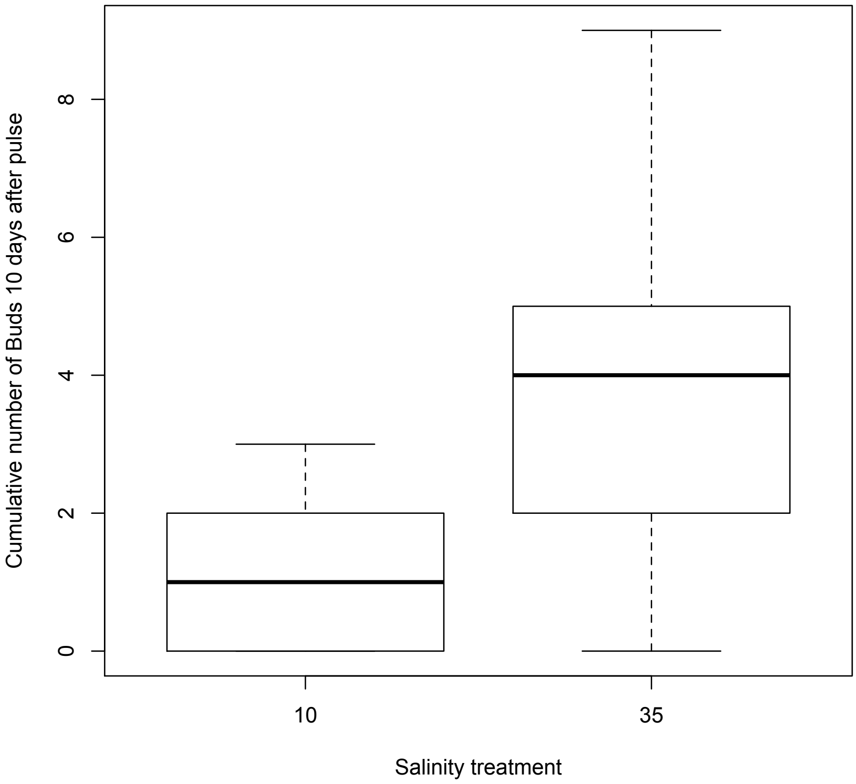

Fig. 18 - 10 days Cumulative number buds produced per replicate for Control (salinity 35) and treatment (Treatment 10).

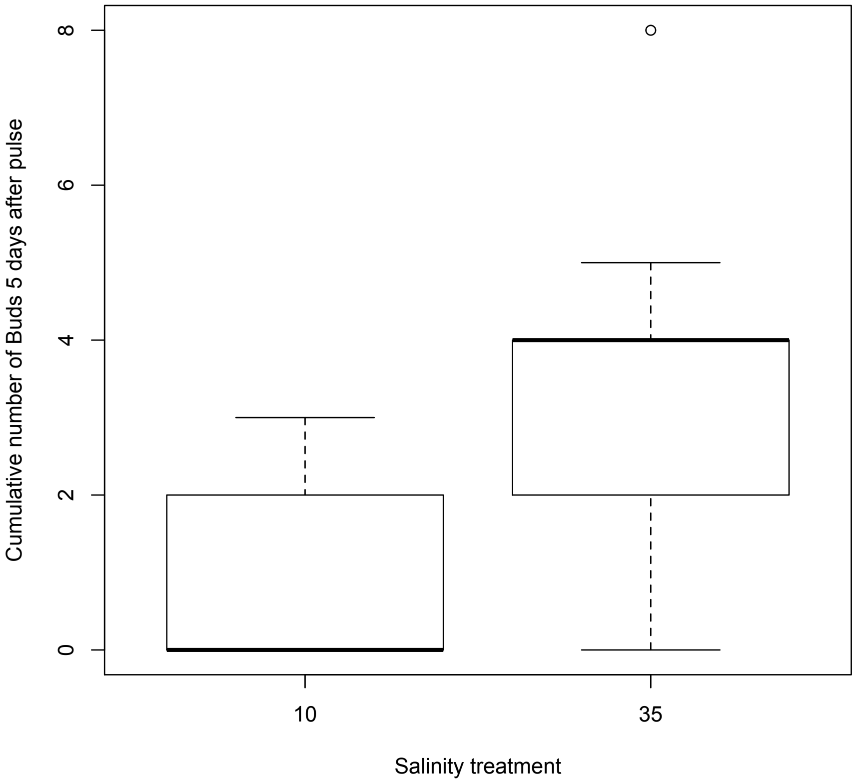

Fig. 19 - 5 days Cumulative number buds produced per replicate for Control (salinity 35) and treatment (Treatment 10).

topic will address “actively budding scyphistoma”.

2.2.1) *Actively budding Scyphistomae:*

The number of actively budding scyphistoma (with at least one bud production) in salinity 10 treatment was about half of the number of the Control. This difference was considered significative (p-value: 0.04829) (Fig.20).

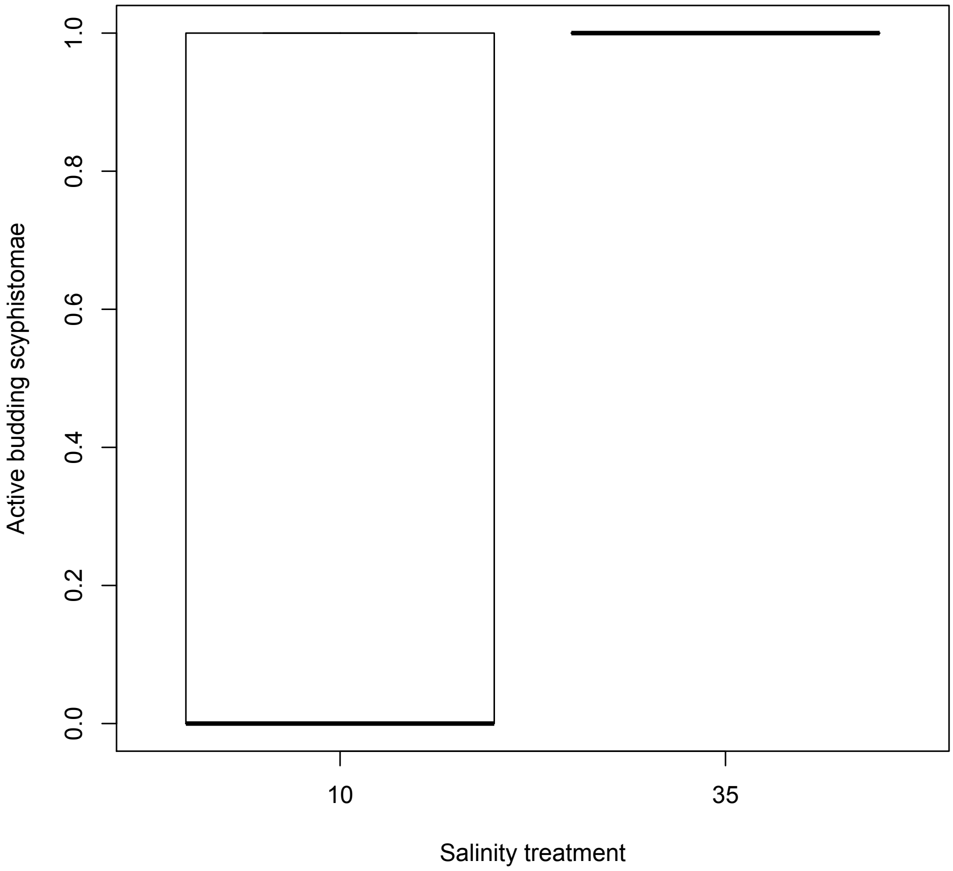

Fig. 20 - Number of active budding Scyphistoma for Control (salinity 35) and treatment (Treatment 10). Number 1 means Actively budding Scyphistoma, number 0 means inactive.

2.3) *12 days cumulative number of produced ephyrae and the number of strobilating scyphistomae:*

The cumulative number of produced ephyra was not significantly different between treatment and control (p = ***0.3552***) (Fig.21). In addiction, the number of scyphistomas that have strobilated did not differ among treatments (p-value: 0.3464*)* (Fig.22).

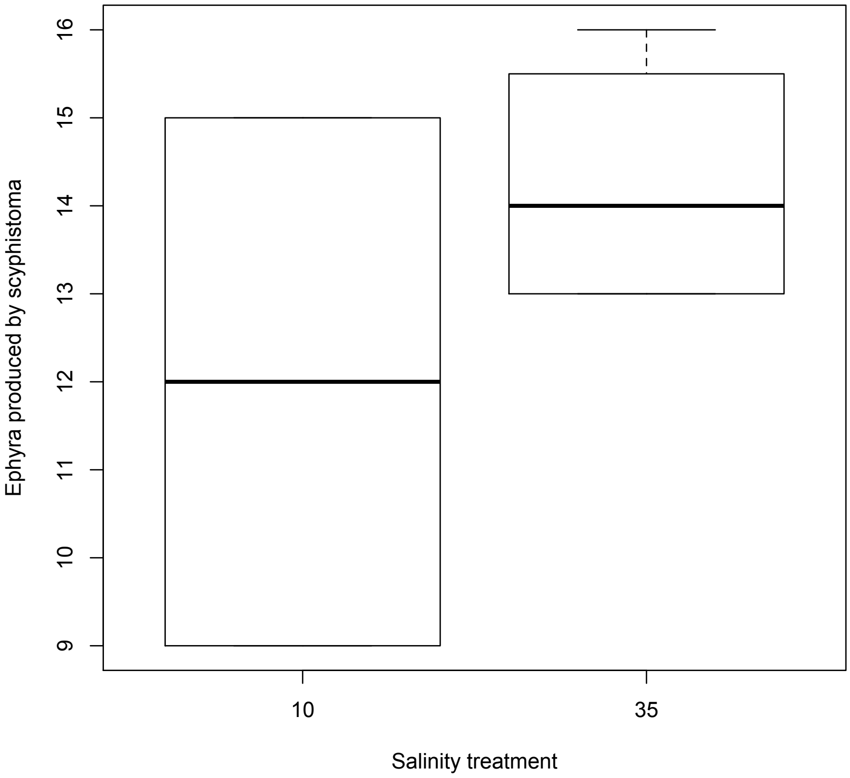

Fig. 21 - Graphic of cumulative number of ephyrae produced per Scyphistoma replicate for salinity 10 treatment and control (salinity 35).

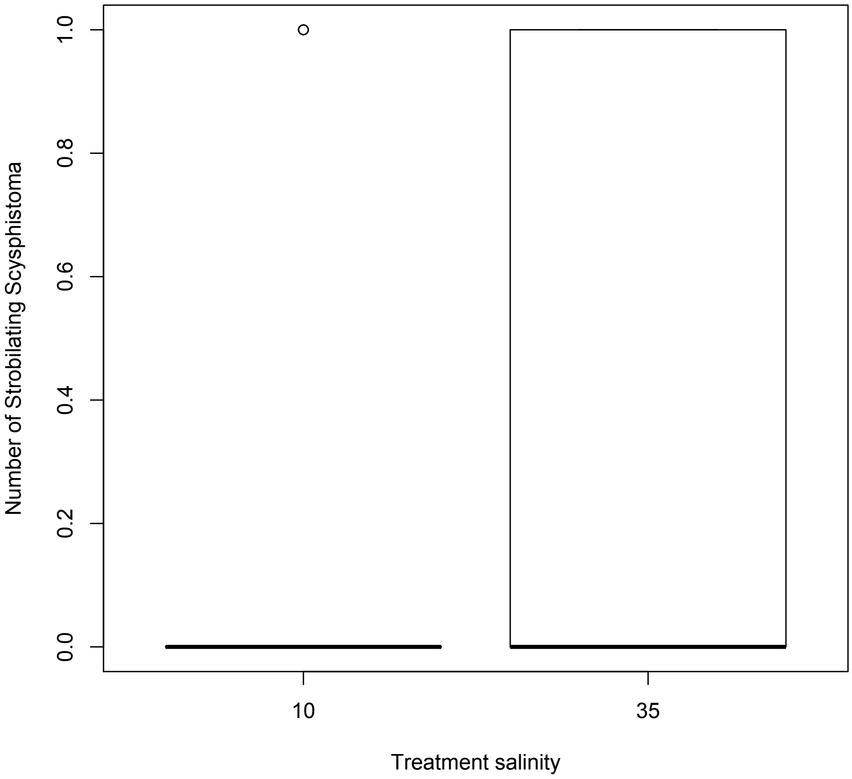

Fig. 22 - Number of strobilating Scyphistoma for Control (salinity 35) and treatment (Treatment 10). Number 1 means strobilating Scyphistoma, number 0 means that individual Scyphistoma did not strobilate.

2.4) *Temporal variation of produced ephyrae:*

Again, it seems to exist a delay of ephyrae release in salinity 10 treatment compared to control (salinity 35) (Fig. 23).

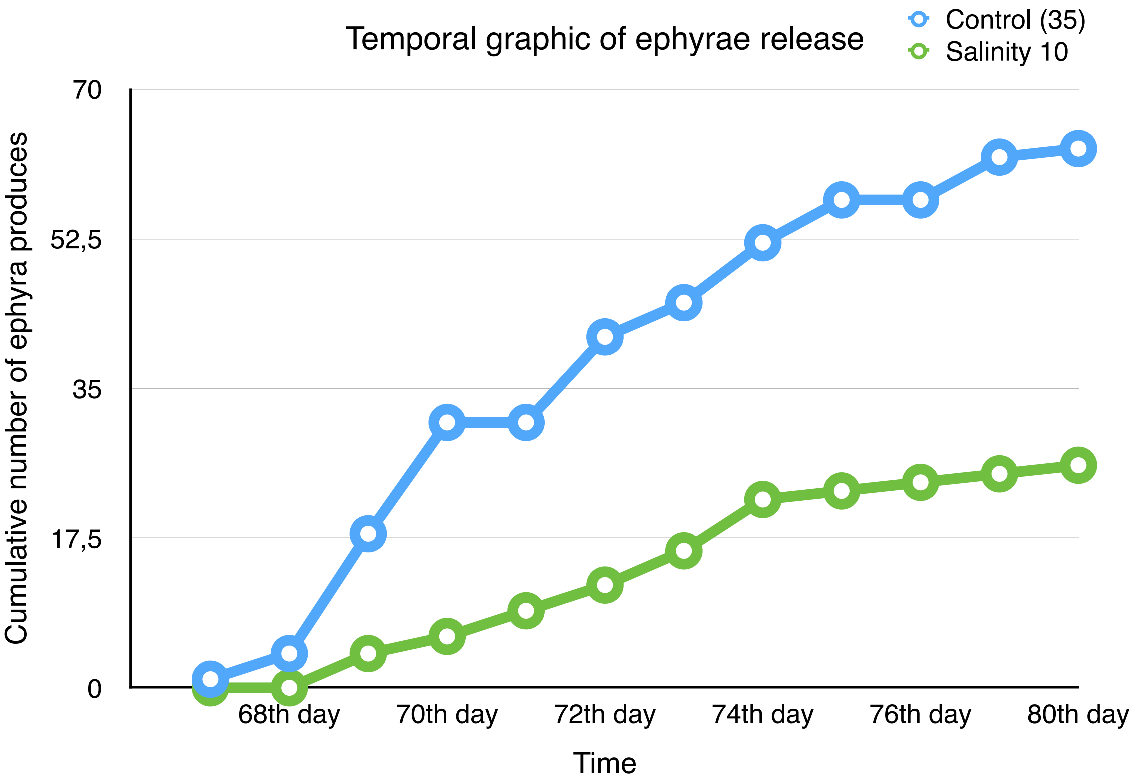

Fig.23: Graphic of cumulated number of ephyrae released per salinity 10 treatment and control (salinity 35).

3) *Experiment III - "Four different salinity treatments and individual ephyra response"*

3.1) *Ephyra survival:*

Ephyra survival was significantly different among some treatments, being 100% in 17 treatment and Control (35) and 0% in 3 and 10 treatments ***(p-value: < 2.2e-16)*** (Fig.24).

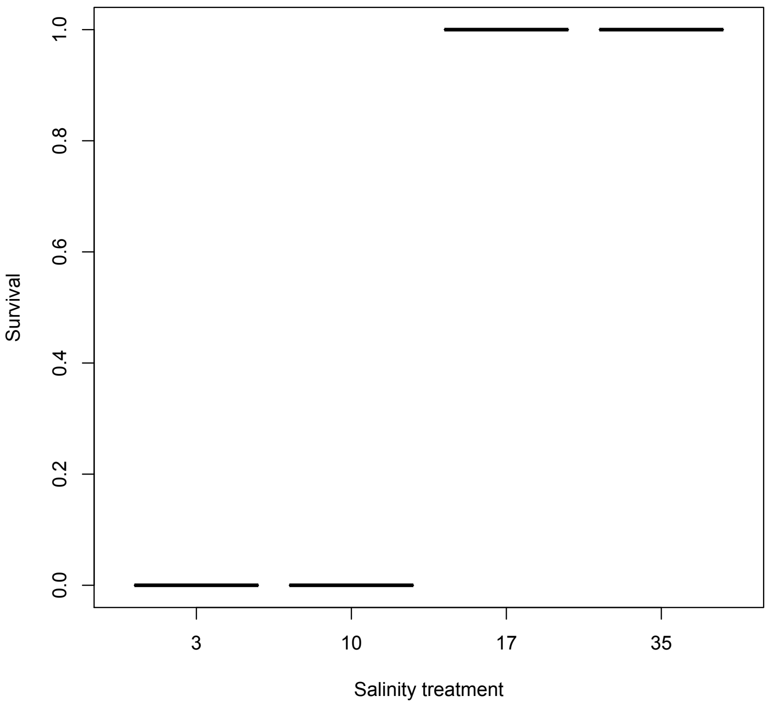

Fig.24 - Graphic of Ephyrae survival proportion for each treatment (salinities 3,10,17) and control (salinity 35)

3.2) ***Ephyra swimming ability:***

Ephyrae swimming ability was significantly lower on salinity 10 treatment compared to control, it was lower (p-value: 0.003538)(Fig.25).

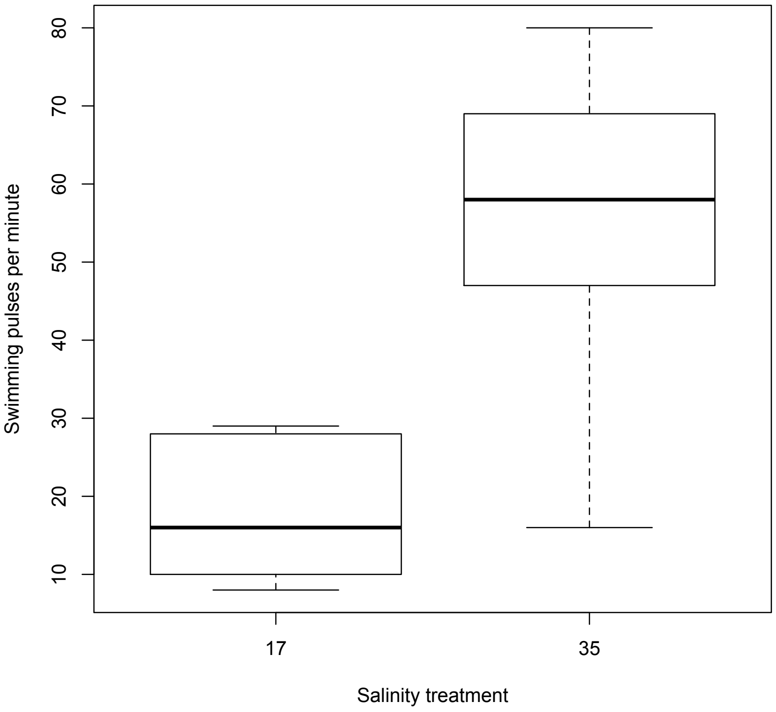

Fig.25 - Graphic of Ephyrae's number of swimming pulses for each treatment (salinities 3,10,17) and control (salinity 35)

Discussion:

1) *Experiment I - "Four different salinity treatments and scyphistoma response"*

The dynamics of jellyfish benthic populations is an outcome of increasing abundance by recruitment of newly settled planula larvae and asexual reproduction and reduction or maintenance of the population by predation, inter- and intra-specific competition for space and food as well as physiological stress (Lucas *et al.,* 2012). Laboratory experiments have assessed the influence of temperature in budding production, in some cases associated with interacting variables, such as food (Han & Uye, 2010), light (Liu *et al.,* 2009; Purcell, 2007) and salinity (Purcell, 2007; Willcox *et al.,* 2007). In this report we focused on short term salinity pulses as management tool which may impact survival , physiological response of and asexual reproduction of benthic stage of *Aurelia aurita*.

In our experiments, *Aurelia aurita* have not survived under salinity 3. Therefore it can be suggested that high intensity short term freshwater discharge may control benthic stage of *A. aurita* in estuaries if it is considered that the some parts of an estuary can reach even 0,5 º% (Chícharo *et al.,* 2006) of salinity during high freshwater discharge. However scyphistomae survival may not be affected by freshwater discharge that would produce estuarine brackish water of salinity higher than 10. This observations indicate that scyphistoma is highly tolerant to salinity short term variation. Therefore, freshwater pulse may be a management strategy for controlling scyphistoma populations when the freshwater pulse is high enough to produce brackish water of salinity 3 in areas of scyphistoma occurrence.

However, freshwater pulse that produces salinity 10 brackish water, may reduce scyphistomae reproduction as long as budding was 0 under salinity 10 and significantly higher on salinities 17 and 35. Then, brackish water ranging from salinities 17 to 35 seems to be the natural range that scyphistoma can deal with. While salinity 10 brackish may reduce scyphistoma budding reproduction. This results agree with Purcell *et al.* (2007) which has found effects of salinity on asexual reproduction of *Aurelia labiata* scyphistomae. What is expected once decrease in salinity concentration in brackish water habitats cause physiological stress on organisms in several marine animals that immigrates into brackish water, e.g. polychaetes, starfish and mussels (Groth & Thede, 1989). Something important to mention is that, such difference should be not a consequence of lower feeding on treated scyphistoma, as long as budding difference is marked since the very first day after freshwater pulse. In addiction, after (Tsikhon-Lukanina *et al.,* 1995) , energy and mass are not a determinant factors for budding, since the new bud takes less than 4,5% of parental body Scyphistoma.

However, the quantity of ephyrae produced, neither the number of strobilating scyphistoma varied with salinity. This result disagrees with (Purcell *et al.,* 2009), probably because such study performed long term and constant salinity experiment. The scyphistomae of the present study have taken 1 month to start strobilation after treatment. So that, 1 month without any salinity shock may be enough for scyphistoma get recovered from freshwater pulse treatment regarding strobilation and ephyrae production.

Those results also allow us to infer that freshwater pulse may not stimulate strobilation as a “dispersion strategy” to adverse situation. This hypothesis can come out if it is taken into account the Clonal theory, that suggest release of dispersive rametes is favored in hard times (Stearns, 1992), and other authors that suggests that the avoidance of saline conditions can be made by dispersion strategies (James *et al.,* 2003). Therefore, in an applicable way, freshwater pulses may be utilized without concerning about strobilation as a “dissipation strategy” response of jellyfish .

Therefore, if it is hypothesized that freshwater pulse would produce brackish water with salinity smaller than 10, the budding reproduction would be reduced, then the scyphistomae colonies would be smaller. If we have smaller colonies and we consider that proportion of strobilation is not population density dependent (Purcell *et al.,* 2009), we would have minimized number of strobilating individuals. As long as it was observed that strobilation and number of produced medusa per scyphistoma are not significantly different among treatments, and it is considered that the number of strobilating individuals are proportional to colony size, it could be inferred that these smaller colonies controlled by salinity 10, may produce smaller quantities of medusa.

By analyzing the cumulative graph of ephyrae production (Fig.12), it is possible to suggest the existence of a delay of ephyrae production. It seems to exist a 2 days delay between control and salinity 10 treatment. It is also interesting to look up the third day of salinity 10, where the ephyrae production had at least six times fewer ephyrae (3 ephyrae) compared to the other treatments (Control: 18 ephyrae ; Salinity 17: 32 ephyrae). Purcell (2007) found a 20 days delay of strobilation on salinity 20 compared to salinity 27. Such high delay may be a consequence of their longer term constant salinity treatment. From my results, I would suggest that this delay is about time necessary for recovering from salinity pulses for later to allocate energy for strobilation. However, another possible hypothesis was inferred by Purcell *et al.* (2009) that such time delay related to salinity may be an adaptation for estuarine species which may need to avoid to be swept offshore by seasonal floods.

2) *Experiment II - "Salinity 10 treatment and individual scyphistoma response"*

It is extremely important to know reproduction also at the individual scyphistoma level to understand the colony dynamics, and that is still poorly studied (Lucas, 2001). By such kind of experiment it is possible to accurately control the variables. As an example, it is possible to have an accurate perception of the budding production and budding activity of scyphistomae (Wilcox, 2007) , in other words, whether Scyphistoma is producing more or less buds, or just not producing any bud because it might be allocating energy to other functions. In this section we focused on short term salinity 10 pulses as management tool by impacts on physiological response of individual replicates of benthic stage of *Aurelia aurita* regarding its asexual reproduction and feeding rate. It also presented natural substrata into the plaques in order to simulate a more natural environment.

The brackish water of salinity 10 treatment presented a significant smaller number of ingested *Artemia salina* nauplii. Such reduction on feeding behavior was also observed in Hydrozoa *Moerisia lionsy* polyps (Ma & Purcell, 2005) and Scyphozoa *Aurelia aurita* scyphistomae (Holst, 2010). This result is probably a consequence of the clear reduction of scyphistoma tentacles just after freshwater pulse (Fig. 26), situation

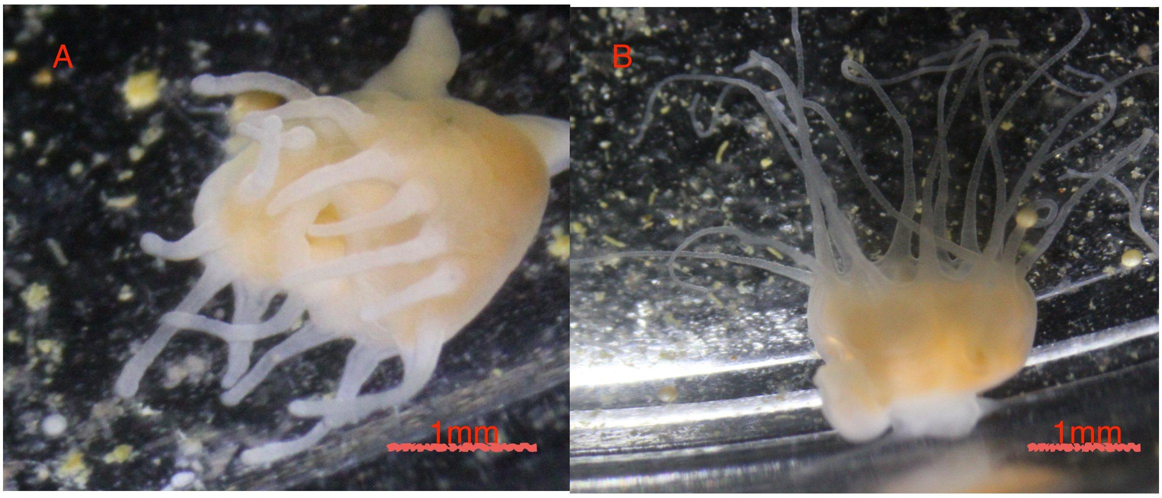

Fig. 26 - Pictures showing tentacle difference between Scyphistoma treated with salinity 10 pulse (A) and Control (B).

that was also observed by Holst (2010). Such reduction of tentacles is described as a sign of stress (Johnson & Wuensch, 1994) and it probably affects foraging. The smaller number of ingested nauplii and the tentacle reduction are negative signs of physiological response. This fact may have consequences on reproduction efficiency of the animal. If it is considered that the scyphistomae from the control are better nourished , and that well fed scyphistomae have greater reproductivity capacity (Keen & Gong, 1989), it is possible to assume that scyphistomae submitted to freshwater pulse have lower reproductivity capacity. However, we did not have significative results on ephyrae production, therefore such difference on feeding rate may be restored after certain period of time when scyphistoma get recovered.

ANOVA test showed significant difference between scyphistomae from control and salinity 10 treatment regarding 5 and 10 days budding reproduction, however the R-square were higher than 0,2. This situation may be a consequence of low replicates number but also may be a consequence of absence of high variation encountered in such individual experiments.

A possible explanation for such R-square is that this experiment has faced some technical problems with temperature variation, having a variation from 22ºC to 10ºC. The very first experiment presented a total of 9 scyphistomae, temperature constant of 10 degrees, and absence of budding reproduction under salinity 10 treatment, and the present experiment had 9 scyphistomae, oscillation on temperature and under salinity 10 treatment high budding reproduction variation. So that, it is possible to infer the existence of an interaction between temperature and salinity when it comes to the budding reproduction. This inference agrees with Purcell *et al.* (2007) that has performed experiments with one 1 month of treatments interacting salinity and temperature. This study has found that temperature and salinity have significant effects and strong interaction on budding of *Aurelia labiata.* This results have shown low temperature and low salinity causing decrease on budding reproduction. In addiction, Purcell *et al.* (2012) has shown that budding reproduction is significantly higher at 21ºC compared to 14ºC. Other empirical evidences have shown that temperature between 16 and 28ºC promote the budding reproduction of *Aurelia* spp. (Willcox *et al.,* 2007; Liu *et al.,* 2009; Han & Uye 2010; Purcell *et al.,* 2012). The reason for that result is suggested by Han & Uye (2010) that states that polyps allocate their energy to production of ephyrae instead buds when temperatures are lower than 14ºC.

The technical problems occurred in the beginning of the experiment caused temperature varying between 22º C and 10ºC. As long as this experiment presented higher "**5 days cumulative budding**" reproduction compared to the first experiment (total absence of budding), and that temperature is considered more impactful on budding reproduction than salinity (Purcell, 2007), it is possible to infer that the occurrence of high temperature periods allowed some scyphistoma to overcome salinity effects and to produce buds.

Another discussion about variability on budding reproduction is about "actively budding scyphistomae". In fact some authors mention that some scyphistoma are actively budding and other are not (Wilcox, 2007). The actively budding scyphistomae are some specifics individuals which are compromised with producing buds and other that are not. They also mention that the salinity may have effect in the number of scyphistoma which are active budding or not. After Wilcox (2007) the number of actively producing buds is inversely proportional to salinity. This situation indicates that high salinity may contribute to scyphistoma population growth in naturally colonies by controlling the number of scyphistoma actively reproducing.

In such way, if it is compare treated scyphistoma with control, it is presented 100% of actively budding scyphistoma in control and only 55% of active budding scyphistoma in salinity 10 treatment. In dynamic of models of energy allocation (reviewed by Perrin & Sibly, 1993) , the assimilated energy is allocated to maintenance of somatic growth, storage or reproduction and that this allocation changes with the organisms necessities. For example , when facing adverse situation some animals allocates energy for reproduction, others may allocate energy for getting recovered. In this way, it is possible to infer that the lower budding reproduction on treated scyphistoma may be due to their allocation of energy from reproduction to recover. Scyphistoma might have mechanisms of allocating energy for specific types of reproduction, or somatic growth or just physiological maintenance according to their necessities. Probably, in this case, scyphistoma allocated energy for morphological or physiological recovering after osmoregulation chock. Therefore, salinity pulse would induce scyphistoma allocation energy to functions rather than budding, and then it could control scyphistoma colony growth.

The present experiment did not present significant difference on the quantity of ephyrae produced , neither the number of strobilating scyphistoma varied with salinity. This result disagrees with other studies with scyphozoans (Purcell et al., 1999; Purcell, 2007) and even Aurelia aurita (Holst, 2010). Holst (2010) has found that the number of strobilated scyphistoma and number of produced ephyrae under salinity 12 was significantly smaller compared to salinities 20 and 35, perhaps such experiments had stepwise salinity reductions and higher term salinity treatments (3 months). Then, those results disagreement are probably due to their long term and stepwise salinity reduction experiment design. This situation brings the hypothesis that time of exposition or time of salinity reduction are important factor for strobilation. For direct management approaches, this result shows that the freshwater pulse may not stimulate strobilation as a “dispersion strategy”. The produced ephyrae results also presented somehow a delay in the ephyrae release temporal graphic.

Increased occurrence of jellyfish blooms have been reported in semi-enclosed bodies of water and that has been linked to changes in salinity as consequence of climate variation and human perturbations. Among the consequences of climate change there is the big scale changes in rainfall regime, glaciers and packs of ice melting which alters salinity regimes, especially in estuaries and coastal areas where jellyfish populations are more prone to be affected by such salinity changes. Therefore, it is important to have a frame of the possible responses of jellyfish populations when they face such situations. In addiction, those correlations give us signs of possible measures that we can take for control such populations, for example to use freshwater for controlling species.

Freshwater pulses are proposed as a possible ecohydrological tool for controlling jellyfish. Freshwater is currently and routinely being used for the port border management of biofouling species such as the invasive coral *Tubastraea* spp. (Moreira *et al.*, 2014). Moreira et al. (2014) studies found significant impact on controlling such corals with low salinities treatment application. It has proposed the use of such treatment as a routine in biosecurity management tool to be applied in pre-border shipping vectors transporting non indigenous marine biofouling species. Also freshwater treatment is suggested to be used against an invasive tunicate *Didemnum vexillum* colonies that with 4 h soaks is 100% killed (McCann *et al.*, 2013). This propositions of freshwater treatments can be effectively applied to other organisms control. However, the ecohydrological approach proposed by the present study would have a large scale impact despite it be considered a soft way of species control. Large scale because it would impact the whole embayment of freshwater inflow, and soft because it is actually controlling many other dominant species and it is a natural pulse, which was blocked in the past due to hydrotechnical constructions. Chícharo *et al.* (2006) has proposed freshwater pulses released by hydrotechnical structures aiming to control the dominance of species in Guadiana estuary. This kind of ecohydrological approach also helps to avoid invasions because invaders are benefitted by stable conditions during low inflow periods (Bunn & Arthington, 2002).

However, some caution have to be taken regarding impacts on food web, once some authors has mentioned starvation as a mechanism of stimulation of strobilation. According to Demographic Theory of life history evolution (reviewed by Olive, 1984), when food is limiting, organisms allocates efforts on reproduction. Then this allocation of energy leads to increase fecundity and/or increased survivorship of the offspring. Therefore, it is necessary to understand how salinity would affect food availability for scyphistoma. In case that scyphistoma do not die but enter into starvation after freshwater pulse, it is possible that pulse indirectly stimulates strobilation. It the other hand, some authors states that higher food availability increases the asexual reproduction of jellyfish (e.g. Purcell *et al.,* 1999, Stibor & Tokle, 2003). Therefore, a food web model taking into account scyphistoma diet should be done in order to predict whether this animals will enter in starvation after freshwater pulse. Another caution that needs to be taken is about temperature variation with salinity pulses. In other words, it is necessary to know wether the pulses of water will change dramatically the temperature of estuary, as long as most authors agree that scyphistomae of many jellyfish species strobilates after a dramatic reduction of temperature (Purcell *et al.*, 1999). Several colonies of *A. aurita* starts to strobilate after critical winter minimum reached (Omori *et al.*, 1995; Kroiher *et al.*, 2000).

3) *Experiment III - "Four different salinity treatments and individual ephyra response"*

When is just released, it is supposed to stay very close to the scyphistoma due to their limitations on swimming abilities (Kamiyama, 2011). Research performed in Mikawa Bay, Japan support the hypothesis that the spatial patterns of ephyrae in sheltered areas are associated with polyps distribution, as shown in (Toyokawa *et al.,* 2011). Therefore, the same brackish water that would impact scyphistoma may impacts ephyra. According to our results, ephyrae are even more sensible for salinity variation than scyphistoma. They die just after the first freshwater pulse under salinity 3 and salinity 10 , while scyphistomae are alive under salinity 10 exposition. Therefore, the salinities 10 and 3 are suitable brackish water salinities for controlling jellyfish blooms as long as it reduces recruitment of ephyrae. It is also interesting that ephyrae here survived to salinity 17 , and after Dillon (1977) ephyrae dies under salinity 15, therefore, salinities 15 and 17 might be the range of salinity which ephyrae get more ecophysiological stressed.

Another ecological observation about salinity range and ephyra survival is the fact that ephyrae die and scyphistomae do not die under salinity 10 pulse. This result shows the more delicate status of sexual medusa and the strength of benthic stage, as proposed for numerous authors (Cargo & Schultz, 1966; Hofmann *et al.,* 1978; Madin & Madin, 1991; Purcell *et al.* 1999; Ma & Purcell 2005). The same sensitive status to acute salinity changes is observed in the scyphozoan *Chrysaora quinquecirrha* ephyrae in Chesapeake Bay (Cargo & King, 1990). This observation is important due to the fact that, it can be infer that this benthic stage can survive to adverse situations such as extreme salinity changes. Then, the jellyfish may have advantage over the organisms which does not present such kind of resistant stage.

The present results showed significant decrease in number of swimming pulses done by ephyrae after salinity 17 treatment, and none pulsation during the 17 treatment. The fact that ephyra stops to move when it is under salinity 17 treatment may be a stress response or may be considered a behavioral response for their survivorship aiming to sink in the water column as long as more salty water should be in deeper depths (Diego & Alison, 2009). This hypothesis can be supported by Albert (2012) that states that adult *Aurelia labiata* in Roscoe Bay, Canada, swims downwards whenever it has contact with low salinity waters.

After freshwater pulse there is still a significant difference on pulsation between ephyra of the control (35) and salinity 17 treatment, what seems to show some physiological recovering of freshwater pulse, therefore to stop swimming may be a stress response. This result agrees with Mayer (1910) that reported a linear and direct initial effect of salinity on pulsation rate of scyphozoan *Cassiopea xamachama*. Mayer (1910) also demonstrated certain ions function responsibility for producing rhythmic bell contraction and that the ions magnesium, potassium and calcium are inhibitory. Therefore, salinities change may cause an unbalance on ions composition of ephyrae and impact pulsation.

Dillon (1977) has performed experiments with ephyra pulsation and different salinity levels with 24 hours treatment, and it has initially found the same results. However, after 24 hours the pattern of pulsation changed and ephyra under lower salinity level presented higher pulsation pattern compared to treated ephyrae. This may be explained by the difference of methods, as long as Dillon (1977) performed experiments with constant salinity levels during 24 hours. Probably in their experiment ephyrae had time to get acclimatized to the constant low salinity level.

In case of swimming ability to be negatively affected by salinity pulses (not only a behavioral adaptation response) it should be considered for recruitment success. Swimming abilities in zooplankton has been associated with essential functions for species success such as feeding, sink avoidance and predation avoidance. The zooplankton swimming ability is very important for vertical positioning that will allow organisms to take advantage of favorable currents for migration or position retain. Migration and position retention may affect ephyrae recruitment success, then the population size of adult medusa. Recruitment also could be affected by decreasing in foraging activity, as long as swimming behavior is responsible for producing currents that aggregate food items (Southward, 1955) . Bamstedt *et al.* (2001) has found significantly higher feeding behavior in ephyra under salinity 35 compared to the ephyra under salinity 17,5. Purcell *et al.* (2007) had found significant salinity effects on feeding and growth rates of *Aurelia aurita* ephyrae when submitted to 17 salinity pulses (Bamstedt *et al.,* 2001). Therefore, the *Aurelia aurita* recruitment is probably affected by the level of nourishment of the organism, which is affected by swimming abilities that in turn is affected by salinity changes.

Therefore, freshwater pulses of salinity 3 and 10 impacts recruitment of ephyrae, and the salinity 17 is prejudicial for recruitment by limiting ephyrae swimming abilities.

Conclusions:

It can be concluded that salinity 3 is a salinity level that may control jellyfish by causing scyphistomae death. This is due to the fact that every scyphistomae and ephyrae did not survive under salinity 3 treatment. By this conclusion, it can be inferred that a freshwater pulse originated from a dam that would produce brackish water of salinity 3 in an estuarine areas may be a jellyfish ecohydrological management tool. Moreover, it is possible to predict that the scyphistoma will not occur on areas of salinity equal or lower than 3 into the estuary.

Regarding asexual reproduction, it was concluded that salinity 10 treatment controls such variable and that it was the best salinity concentration for performing more detailed experiment. Despite individual replicates experiment have detected high, variability the budding reproduction was smaller on scyphistoma submitted to salinity 10 freshwater pulses. It also can be concluded that more the number of active or non active budding scyphistoma are impacted by salinity 10 treatment.

Taking strobilation and ephyrae production into account, the freshwater pulses (salinity treatments) did not present any effect on those variables.

Regarding feeding response we concluded that Scyphistoma have significant decrease on feeding rate when submitted to salinity 10 pulse.

Based on our experiments with ephyrae, it can be concluded that, differently than scyphistoma, ephyra dies under salinity 10 pulse. We also conclude that swimming ability is significantly and negatively affected by salinity 17 pulse.

Chapter IV - Scyphistoma and ephyra feeding ecology

Introduction

Food, temperature, salinity and photoperiod play important roles in synchronizing annual cycles of reproduction and influencing reproductive output in marine invertebrates, being the evidences based on correlations between gonad maturation, spawning and environmental variables (Olive, 1984). On jellyfish case, the considered most important factors causing a bloom's collapse are senescence after spawning, infestation with parasites, low salinity water, high water temperature, predation, food limitation and intertidal stranding (Pitt *et al.*, 2014). Thus, diet combined with ecophysiological responses to environmental changes and nutritional condition studies may provide knowledge about connections among physiological conditions, trophic interactions and population dynamics.

Aurelia aurita diet is considered to include a large range of sizes and type of preys (Moller, 1980; Olesen *et al.,* 1994; Sullivan *et al.,* 1994), and they generally preys using mechanisms of mucous and ciliary current prey capturing, what means that they can be considered generalist feeders with wide range of zooplankton (Sullivan *et al.*, 1994; Båmstedt, 1990; Möller, 1980) observed in their diet. Only one author has mentioned that scyphistomae feeding strategy is selective (Sullivan *et al.*, 1997). Nevertheless, just like most of gelatinous predators, *Aurelia aurita* can quickly deplete their prey population (Båmstedt, 1990). Therefore, they can easily utilize high densities of zooplankton population and be efficient on predation (Lucas, 2001). In this way, it is important to understand the types of prey ingested by jellyfish because gelatinous plankton occurrence causes high impact on their prey population. This information is also important to understand what are the preys that supports jellyfish blooms.

As long as *Aurelia aurita* relies on fluid dynamic feeding, prey selectivity is determined by size of *Aurelia aurita* (Sullivan *et al.*, 1994). Then such factor varies between different life stages of *Aurelia aurita*. For the present study, it is specially important to understand the life stages that are the major responsible for medusa recruitment: Scyphistoma and Ephyrae. Their feeding strategies are still not clearly understood. It is importance to understand feeding strategies in order to predict whether how blooms occurrence or absence of *Aurelia aurita* alters zooplankton community.

In addiction, the freshwater pulse impacts the whole ecosystem community. In this way, it is important to understand food items that scyphistomae and ephyra are eating for future modeling the direct impacts of freshwater pulse on *Aurelia aurita* food availability in order to predict trophic cascades. Thus, knowing what the different life stages of jellyfish are eating and also their feeding strategies provides better knowledge on the impacts of blooms or absence of them, and to predict whether they can enter in starvation or over fed after freshwater pulse. In addiction our experiment differ from many other reports because it did not considered only gut content since the present study performed the experiment with alive organisms and natural zooplankton.

Hypothesis:

(i) Scyphistoma feeding strategy is generalist.

(ii) Ephyra feeding strategy is generalist.

Aims:

i) To identify the preys of *Aurelia aurita* scyphistoma and ephyrae and their relative importance.

ii) To determine whether the feeding strategies of *Aurelia aurita* scyphistomae and ephyrae are specialist or generalists.

Methods

Scyphistoma diet experiment:

Natural plankton was collected at Low Guadiana estuary with a 150 mm net under high tide condition. It was picked up 6 scyphistoma settled individually in plaques with 10 mL from our culture with sizes ranging from 1,5 to 2,2 mm. It was dropped 2 mL of zooplankton solution into Scyphistoma plaques and also into 6 other plaques without Scyphistoma, which was called as "Branco" (Fig. 27). After 1:00 hour it was counted the number os remained plankton into every plaque. In order to have the number of individuals of each different taxon eaten by Scyphistoma , it was firstly summed up the number of individuals of each taxa remained in Scyphistoma plaques, then the same was done for "Branco" plaques. Afterwards, the number of ingested individuals of each different taxon was obtained by subtracting the number of remained plankton from "Branco" plaques and the number of remained plankton from Scyphistoma plaque for each zooplankton taxon.

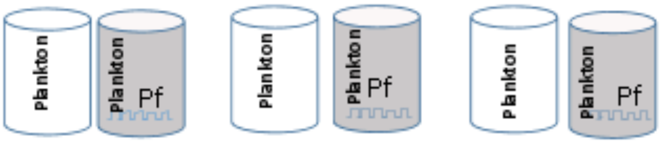

Fig. 27 - Illustration demonstrating Ingestion experiment. White recipients, called as "Brancos", were the control and contained natural zooplankton without Ephyrae, while grey recipient contained Scyphistoma and zooplankton.

After estimating the number of eaten individuals per taxon , it was calculated the Relative Abundance (percentage of a eaten prey taxon in relation to all eaten prey items) and the Frequency of Occurrence (%F) of prey items. Those index were plotted into Costello biplot (Costello, 1990). Such plotting was done in order to analyze the relative importance of a prey type (dominant or rare) against the degree of homogeneity in prey selection by predator population, and thus determines if a species is either specialist or generalist predator (Costello, 1990).

Ephyra diet experiment:

Natural plankton was collected at Low Guadiana estuary with a 150 mm net. It was picked up 12 ephyra of 2-3 mm size which were released from our scyphistoma culture. Each ephyrae was individually placed into 3 mL plaques. It was dropped 1 mL of zooplankton solution into Ephyrae plaques. Before and After 1:00 hour it was counted the number of individuals of each different taxon inside the plaque by stereo microscopy. It was also checked taxa inside ephyrae gastric cavity. After obtaining the number of eaten individuals per taxon by Ephyrae , it was calculated the Relative Abundance (percentage of an ingested prey taxon in relation to all eaten prey items) and the Frequency of Occurrence (%F) of prey items. Those index were plotted into Costello biplot (Costello, 1990). Such plotting was done in order to analyze the relative importance of a prey type (dominant or rare) against the degree of homogeneity in prey selection by predator population, and thus determines if a species is either specialist or generalist predator (Costello, 1990). If the points are spread along and below the diagonal originating at the origin, this suggests that the feeding was homogeneous amongst the predators. In the other hand, if the points are spread along and bellow the feeding

Results:

After providing natural macro zooplankton from high tide of Guadiana estuary, Scyphistomae have ingested the following taxa: cirripede nauplii, copepod crustaceans, ostracoda and veliger larvae (Fig.28).

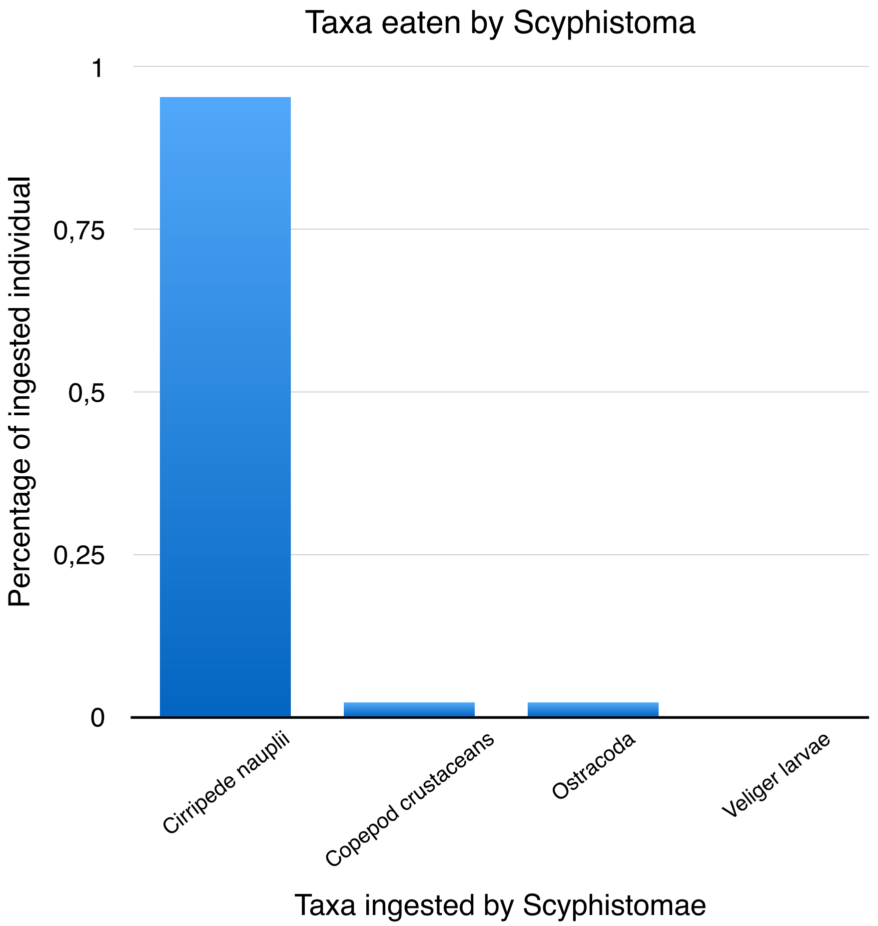

Fig.28 - Graphic of percentage of different ingested taxa by Scyphistoma.

Taking into account Costello plot (Fig. 29), it is possible to consider as scyphistoma an generalist predator slightly selective for Copepoda, since those taxa were localized above the diagonal line crossing the origin. However, this tendency can not be generalized because cirripede nauplii taxon was localized below the diagonal of feeding strategy, and differently then the other taxon, cirripede nauplii was considered highly dominant taking almost 100% of the occurrence .

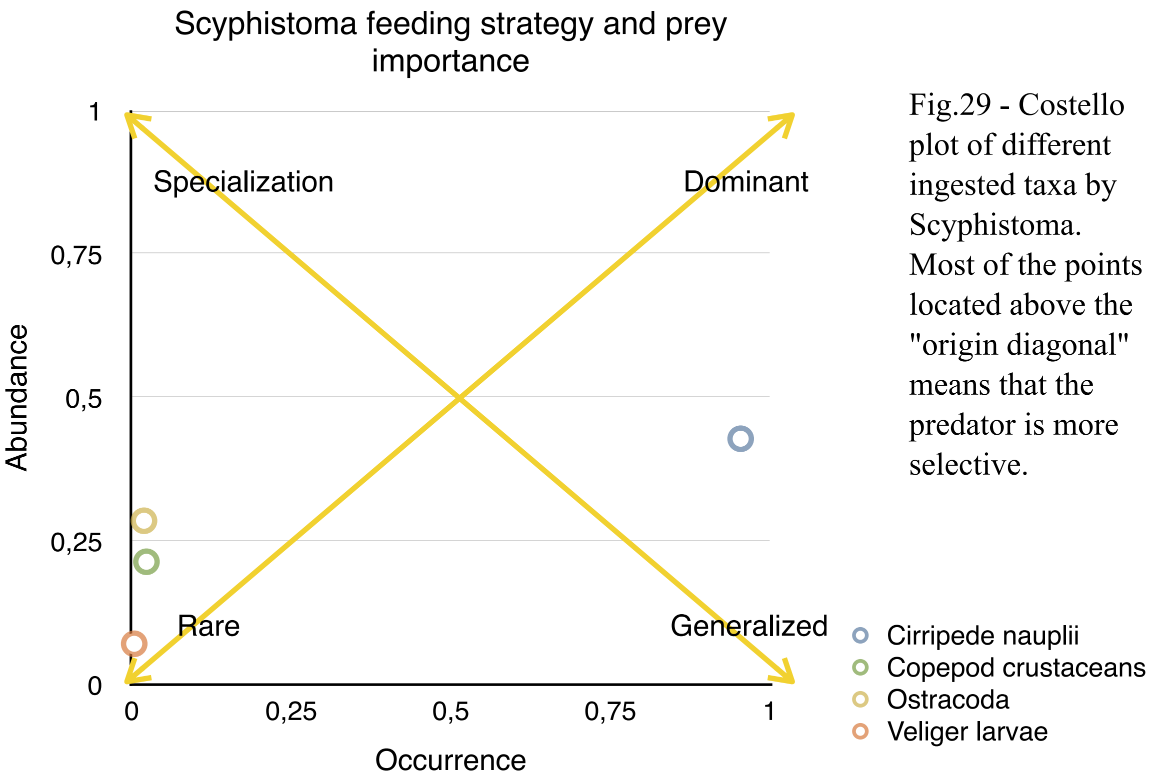

Fig.29 - Costello plot of different ingested taxa by Scyphistoma. Most of the points located above the "origin diagonal" means that the predator is more selective.

Ephyrae after fed with natural zooplankton from low Guadiana estuary, they have actually eaten the following taxa: Nauplii, copepod crustaceans and ciliates (Fig. 30).

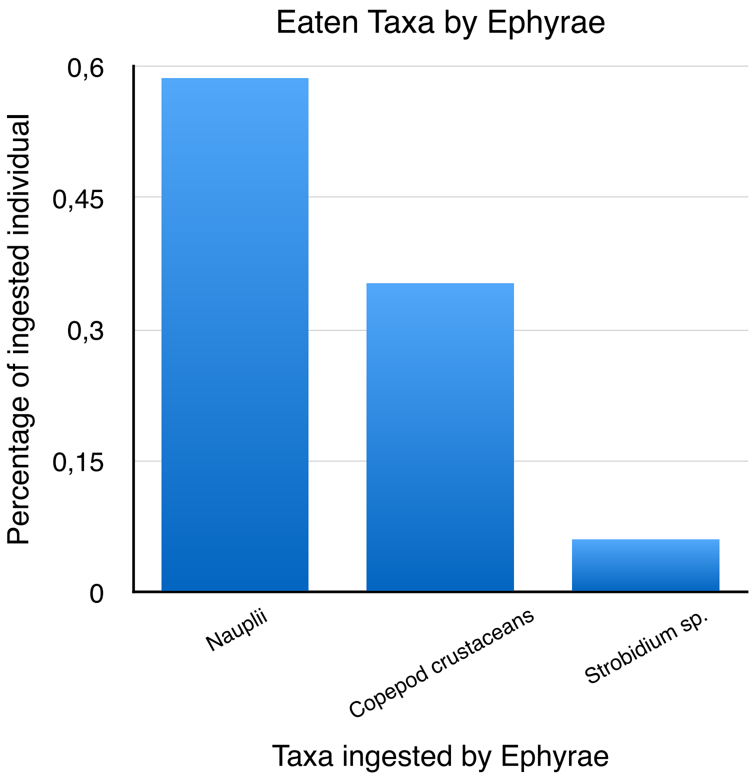

Fig. 30 - Graphic of percentage of ingested taxa by Ephyrae.

Taking into account Costello plot, we can consider Ephyrae as a generalized predator, since most of the taxa were localized under or below the diagonal line crossing the origin (Fig.31).

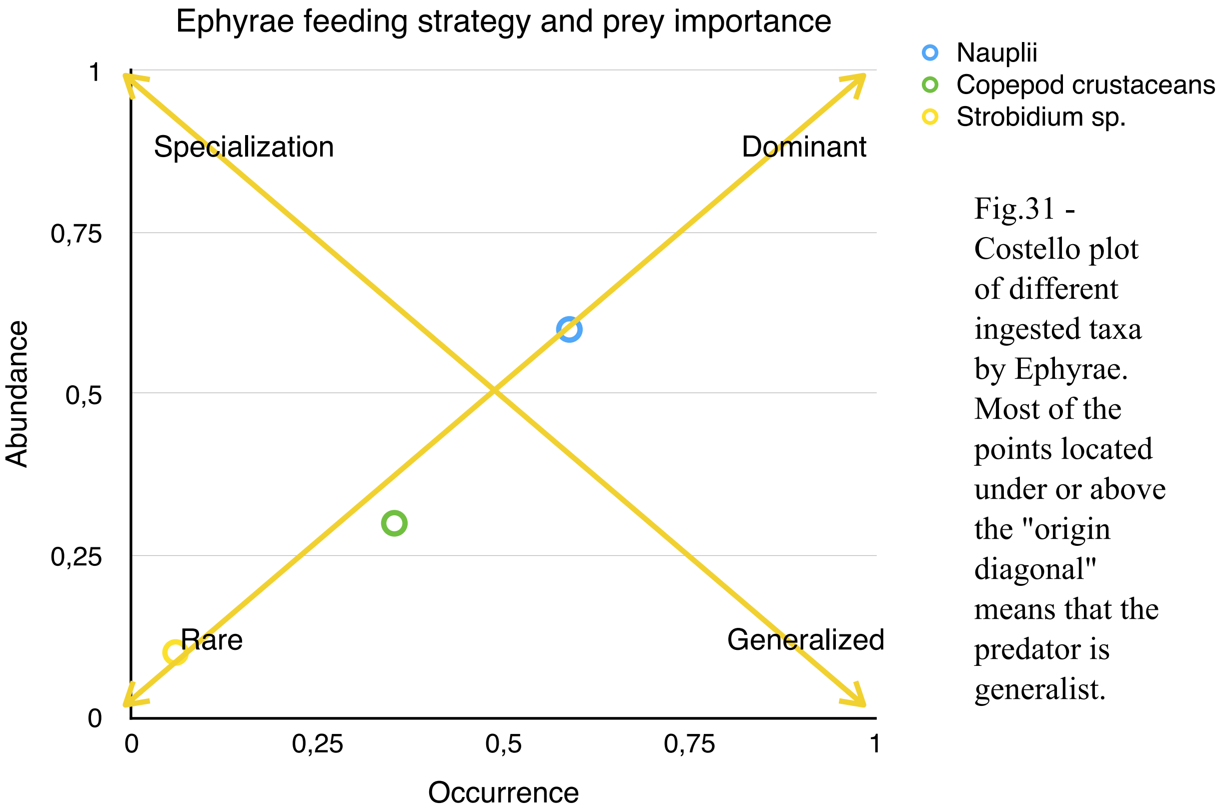

Fig.31 - Costello plot of different ingested taxa by Ephyrae. Most of the points located under or above the "origin diagonal" means that the predator is generalist.

Samples of zooplankton from Ria Formosa low tide dominated by ciliates *Strobidium sp.* was offered for ephyrae, and through direct observations we could affirm that ephyrae can eat numerous of ciliate, at least when they are very abundant (Fig.32).

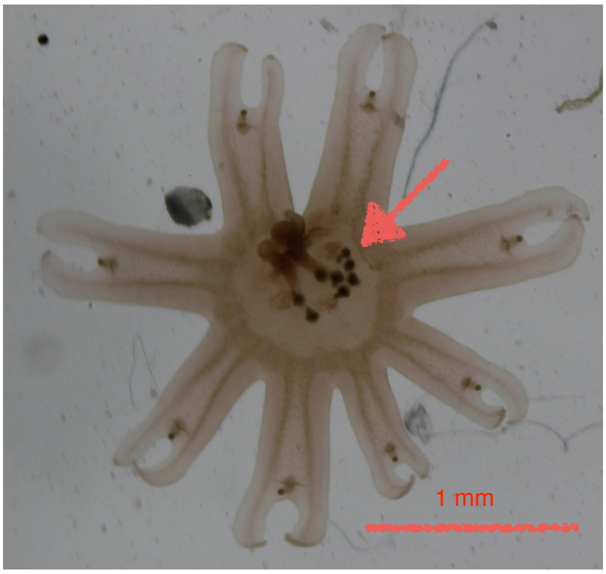

Fig.32 - Clear image of ingestion of *Strobidium sp*. (pointed by an arrow) by Ephyrae.

Discussion:

As it was not performed an experiment comparing scyphistomae and ephyrae diet , it is not possible to assure that scyphistoma is more selective than ephyra. However, by analyzing the Costello's plot results it is possible to observe a slight selective feeding strategy for Scyphistoma, while ephyra was considered generalist. Those results are enforced by Tsikhon- Lukanina *et al.* (1995) that states that scyphistoma is low food selective, but undoubtedly more selective than ephyrae. Therefore, the different life stage of *Aurelia aurita* may have different diets and ecological roles in the food web.

Compared to the medusa, scyphistoma diet is poorly understood. However, it is believed that *Aurelia aurita* scyphistoma feeds mostly on zooplankton, just like medusa (Grönhdal, 1988). According to Tsikhon- Lukanina *et al.* (1995) scyphistoma with more than 8 tentacles feeds only on mobile organisms being it considered as predator. In the present experiment, the preys ingested by scyphistoma (16 tentacles) were all mobile organisms. They did not seem to feed on dead organisms, despite sample contained some dead zooplankton. Maybe this feeding behavior is a consequence of the impossibility of scyphistoma to move towards its prey. In case of an active prey, it swims in water column and eventually enter into the field range which scyphistoma tentacle can reach them. In this way, we may consider scyphistoma as predators. The suggestion of scyphistomae as a predator is also mentioned by Kamiyama (2011).

Another observed fact on scyphistoma diet is that the scyphistoma ingested some taxa that were holoplanktonic and others that were meroplanktonic, such as cirripede larvae and veliger larvae of gastropod and bivalves. Therefore, benthic organisms may be important on scyphistomae diet, at least during their planktonic larval stage. In this way, it is possible to infer that scyphistoma may have impact on recruitment of these benthic organisms.

In the present experiment, Copepoda were ingested by high proportion of scyphistoma when compared with Copepoda low occurrence. This result was not expected, once it is known that adult crustaceans have very well developed escape response. In this way, scyphistomae should not be selective for this groups. However it is possible to speculate that the high swimming activity of adults, compared to nauplii, may enhance chances of Copepoda encounter polyps tentacles. Fast swimmers organisms can be more susceptible to scyphistoma predation due to the high chances of they encounter scyphistoma tentacles (Suchman & Sullivan, 2000). Such preference for Copepoda crustaceans is also observed for juvenile and adults *Aurelia aurita* (Pereira, 2014). Maybe Copepoda escape response is low effective when predation is done through water currents production.

This slight feeding selectivity for Copepoda may imply on impact of scyphistoma on ecosystem grazers population. Since diatoms seem to not be part of scyphistoma diet (Kamiyama, 2011) and scyphistoma preys grazers, the decrease of scyphistomae may imply on increase of diatoms that may be accumulated, decomposed on the bottom and mineralized by bacteria. As a possible result, a small portion of primary production is transferred to top predators in the ecosystem.

Another hypothesis for this slight selectivity may be a critic to the present experiment. It also can be speculated that such selectivity is just an artifact of experiment. It had only six replicates and a extremely higher proportion of cirripede nauplii compared to the other plankton taxa into the zooplankton solution (cirripede nauplii had about 95% of occurrence). All the 6 replicates ingested Nauplii and a big proportion of this replicates ingested other taxa. Supposed that the experiment had higher number of replicates, probably the number of replicates that would ingest nauplii would be much higher, therefore the points may be better spread in the chart.

The *Aurelia aurita* ephyrae have fed on nauplii, Copepoda and *Strobidium* sp. and it was indicated that ephyrae's diet is generalist. That result is understandable for nauplii and *Strobidium* sp. preys, because nauplii are considered to have slow or no escape response (Costello & Colin, 1994) and *Strobidium* sp. is also considered to have low escape response (Jakobsen, 2001). However, as mentioned before, Copepoda is a distinct group because it presents high escape response. Our results do not support Costello & Colin (1994) hypothesis that states that copepod crustaceans are avoided by ephyrae due to their well developed escape response with good escape abilities or playing dead behavior. However, the present results may be explained after Sullivan (1997) and Purcell & Arai (2001), that states that prey vulnerability is not just consequence of prey escape velocity, as predicted by Costello & Colin (1994), but also on amount of swimming activity of prey in the flow field surrounding ephyrae. Therefore, despite copepod crustaceans present high escape response to predation, their high swimming activity may play against copepod and enhance the chances of it to be predated. Such balance may be the reason why copepod were predated by ephyra according their availability.

Samples of zooplankton from Ria Formosa low tide with dominance of *Strobidium* sp. were provided for ephyrae. It was observed on stereomicroscope that ephyrae have highly ingested it. Bamstedt (1990) also did the same observation and it stated that ephyrae ingests certain number of ciliates according to ciliates abundance. This predation intensity linked to high ciliate occurrence in the samples might be explained by the fact that ciliates do not seem to have an effective escape response to ephyrae predation. After Jakobsen (2001), *Strobidium* sp. is a kind of ciliate which do not jump as a escape response , differently than other ciliates and mesozooplankton. It is interesting to highlight that these ciliates are typical of eutrophic environment and also *Aurelia aurita* blooms has been highly connected with eutrophic embayment (Kamyiama, 2011). In this way, once such ciliates are the major grazers of phytoflagelates and non diatoms phytoplankters (Granéli *et al.*, 2002), the control of ephyrae may imply on increase of ciliate which in turn would decrease algae abundance and harmful algae blooms.

Finally, considering ephyra and scyphistoma as generalist it is possible to infer that the control of them may imply on the increase of consumers diversity in the food web. However, it is important to highlight that generalist organisms may be important for controlling invasive species. Their control also may imply on increase of Copepoda group, then the higher food availability for fish and control of primary production.

Conclusion:

Based on our observations it is possible to conclude that scyphistoma's diet includes Copepoda , Ostracoda, and pelagic larvae from some meroplanktonic organisms (veliger of mollusca and cirripedia nauplii). About scyphistoma feeding strategy it was concluded that it is generalist with slight selectivity for Copepoda.

Taking ephyra diet into account, it was concluded that its diet includes Nauplii, Copepoda and Ciliates (*Strobidium* sp.). Here it is concluded that Copepoda is an important prey for ephyra (about 35% of ingested taxa). About ephyra feeding strategy, it is possible to conclude that it is generalist.

General conclusion:

It has been concluded that freshwater pulses can control *Aurelia aurita* blooms by reducing scyphistoma survival when it is exposed to salinities equal or lower than 3, and by reducing ephyrae survival when it is exposed to salinities equal or lower than 10. Those results also allow us to hypothesize the higher sensibility of ephyrae larvae to freshwater pulses compared to scyphistoma. Freshwater pulse of salinity lower than 10 until 3 also may control jellyfish blooms by impacting negatively the ecophysiological performance of scyphistomae and budding reproduction. While, freshwater pulse of salinity lower than 17 until 10, negatively impacts ephyra ecophysiological performance. It also was concluded that ephyra and scyphistoma have generalist diet , although scyphistoma was slightly selective for Copepoda, and that salinity decrease also reduce the feeding abilities of these organism. Then freshwater pulses can be proposed as an ecohydrological management tool to control jellyfish blooms.

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