UNIVERSIDADE DO ALGARVE

DYNAMICS OF PRODUCTION AND MORTALITY OF AURELIA SP. *IN THAU LAGOON, NORTHWERSTERN MEDITERRANEAN*

Raquel Fonseca da Silva Marques

Dissertação para obtenção do grau de:

Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:

Delphine Bonnet

Alexandra Teodósio

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Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída

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ABSTRACT

Jellyfish are conspicuous components of the marine ecosystems and can have severe impacts on ecosystems functioning and human activities. With the apparent increasing of jellyfish blooms, scientific interest on jellyfish ecology is mounting worldwide. However, investigations are still required with regards the processes of top-down and bottom up control of jellyfish populations. Here we focused on the jellyfish *Aurelia* sp., which is responsible for numerous outbreaks around the world. Thau lagoon, Northwestern Mediterranean, harbours a resident population of *Aurelia* sp., as the entire life cycle is suggested to occur in the lagoon. Therefore, this coastal lagoon offers an ideal framework to investigate the complex benthic-pelagic life cycle of *Aurelia* sp.. We used a multiple approach, comprising field and laboratory investigations, to assess processes of bottom-up and top-down control of *Aurelia* sp. population. The distribution, habitat use and population dynamics of *Aurelia* sp. benthic population were assessed *in situ* by free diving and underwater picture analysis, between April and June 2014. We revealed a well established population of polyps over the entire lagoon and underlined the crucial role of man-made structures to their development. A 4-year field survey (2010-2014) of the pelagic stages unveils the dynamics of the population and its driving forces. Finally, to explore top-down control, fish gut contents were analysed using PCR-based techniques, to identify potential natural fish predators of *Aurelia* sp.. This approach was further complemented by laboratory experiments on fish predation and selectivity on different life stages of *Aurelia* sp.. We provide evidences that jellyfish might be a source of food, potentially important for opportunistic fishes. Moreover, we hypothesise that diversity of fish predators is largely underestimated, as even herbivorous fishes might consume gelatinous organisms. Overall, these results further contribute to the mounting acknowledgment that jellyfish are not ‘dead end’ in food webs.

Keywords: Jellyfish, polyps, ephyrae, medusae, population dynamics, fish predation.

RESUMO

O grupo dos organismos gelatinosos é composto por diversas espécies, que têm em comum o facto de serem compostos maioritariamente por água, o que lhes confere o característico aspeto gelatinoso. Entre a grande diversidade destes organismos, encontram-se espécies capazes de formar grandes agregações, conhecidas por blooms, que afetam fortemente o funcionamento dos ecossistemas e prejudicam diversas atividades humanas. Uma destas espécies é a *Aurelia* sp. utilizada neste estudo como modelo biológico. O seu ciclo de vida é muito complexo e caracteriza-se por duas fases: a fase adulta, que se reproduz sexualmente, e a fase betónica (pólipos) capaz de se reproduzir assexuadamente. Esta complexidade do ciclo de vida torna o seu estudo difícil, nomeadamente no que diz respeito à população betónica, a qual tem um papel fundamental como base no desenvolvimento dos blooms. Poucas investigações *in situ* direcionadas à avaliação da população bentónica foram realizados até à data e a sua maioria concentram-se na zona oeste do Oceano Pacifico. O Mar Mediterrâneo é um dos locais mais sensíveis aos impactos antropogénicos e um dos locais onde os blooms de gelatinosos parecem estar a aumentar. No entanto, apenas um estudo envolvendo avaliação *in situ* de pólipos foi até agora aqui desenvolvido. Apesar de as fases pelágicas destes organismos terem merecido de maior atenção por parte do mundo científico, estudos a pequena escala são ainda necessários para compreender os percursores dos blooms de gelatinosos. Adicionalmente, devido ao seu baixo valor nutricional e elevado conteúdo em água, os gelatinosos foram vulgarmente caracterizados como o fim da rede alimentar. Assim, a sua importância como item alimentar foi largamente ignorada. No entanto, investigações recentes têm vindo a revelar que a importância destes organismos na transferência de energia para níveis tróficos superiores pode ser maior do que o conhecido. O objetivo principal deste estudo foi então compreender a dinâmica da população de *Aurelia* sp. na lagoa de Thau, situada no sul de França, noroeste do Mar Mediterraneo, avaliando processos de *top-down* e *bottom-up* que controlam a dinâmica das populações. A lagoa de Thau é uma lagoa costeira onde existe uma população residente de *Aurelia* sp., uma vez que todo o seu ciclo de vida parece ocorrer dentro da lagoa, providenciando assim um lugar perfeito para o estudo de todos os estados do ciclo de vida deste organismo. Toda a lagoa foi explorada através de mergulho livre, entre Março e Junho 2014, de forma a identificar a distribuição e habitat dos pólipos de *Aurelia* sp.. Uma colónia de pólipos foi

adicionalmente monitorizada semanalmente entre Abril e Julho 2014, através da análise de fotografias subaquáticas registadas por mergulhadores, determinando assim a densidade de pólipos ao longo do tempo. A proporção de pólipos em reprodução assexuada (*budding* e estrobilação) foi determinada através da observação de amostras recolhidas no mesmo local de monitorização. Os resultados permitiram verificar que apesar da ausência de substratos duros naturais, a população bentónica de *Aurelia* sp. encontrava-se largamente distribuída por toda a lagoa. Tal só é possível devido à elevada presença de substratos artificiais, construídos pelo homem, que beneficiam a população de pólipos, providenciando superfícies adequadas à sua fixação e desenvolvimento. A monitorização da dinâmica da população bentónica revelou a importante influência da temperatura, especialmente no aumento da reprodução assexuada, no entanto estudos mais extensos são ainda necessários para avaliar a dinâmica anual do desenvolvimento de pólipos na lagoa de Thau. Quatro anos de dados (2010-2014) referentes à abundancia e crescimento das fases pelágicas da *Aurelia* sp., determinados através de amostragens quinzenais de zooplâncton e complementados com dados ecológicos (*i.e.* temperatura, salinidade, concentração de clorofila e abundância de zooplâncton) foram também analisados. Desta forma foi possível avaliar a sua dinâmica de populações e os fatores que a influenciam. Os resultados indicam que a população pelágica de *Aurelia* sp. é muito variável no espaço e no tempo, sendo bastante influenciada pelos parâmetros ambientais, sublinhando a importância da temperatura e disponibilidade de alimento. Por fim, para explorar a mortalidade, estudos complementares *in situ* e em laboratório foram realizados. Conteúdos estomacais de peixes recolhidos na lagoa foram analisados através de técnicas de PCR (*Polymerase Chain Reaction*), de forma a identificar a presença ou ausência de material genético de *Aurelia* sp.. Adicionalmente diversas experiencias laboratoriais foram realizadas de forma a compreender o potencial de predação de *Sparus aurata* sobre diferentes fases do ciclo de vida de *Aurelia* sp.. Três experiencias distintas foram desenvolvidas: diferentes items alimentares foram testados através de dietas mono específicas (*Monospecific diets experiment*); a avaliação da ingestão de acordo com a disponibilidade de alimento foi estudada através do gradiente de concentração de presas (*Gradient of concentration experiment*) e experiencias com dietas mistas permitiram avaliar a seletividade de presas (*Selectivity experimets*). A determinação dos possíveis predadores naturais de *Aurelia* sp. através de técnicas moleculares, revelou que até um peixe herbívoro (*i.e. Sarpa salpa*) consome, pelo menos ocasionalmente, organismos

gelatinosos. Estes resultados mostram que a diversidade de predadores de *Aurelia* sp. na lagoa de Thau, e possivelmente de outros organismos gelatinosos em outros locais do mundo, está provavelmente subestimada. Finalmente, as experiencias laboratoriais de predação de peixes sobre *Aurelia* sp. evidenciaram que todas a fases do seu ciclo de vida foram aceites por *S. aurata* como fonte de alimento. Os pólipos são vulneráveis à predação acidental, deliberada ou indireta, enquanto que a vulnerabilidade das fases pelágicas parece estar relacionada com o seu tamanho. Este estudo sugere que em situações de bloom, onde a concentração de gelatinosos é muito elevada, estes peixes poderão ter a capacidade de aumentar a ingestão, compensando assim o seu baixo valor nutricional. Assim sendo, peixes com uma dieta mais generalista poderão ser beneficiados da alta disponibilidade de alimento, sem desperdiçarem energia na sua procura e captura. Embora presas de alta qualidade nutricional serem preferencialmente consumidas, em situações de bloom, a disponibilidade destas poderá ser drasticamente reduzida, como consequência do impacto de predação dos próprios organismos gelatinosos. Neste caso, os gelatinosos poderão então fornecer uma possível fonte de alimento alternativo. Por outro lado, estes resultados evidenciam o potencial impacto da sobre-exploração dos recursos pesqueiros, que ao removerem os predadores dos organismos gelatinosos, favorecem a sua proliferação e o desenvolvimento de blooms.

De um modo geral, este estudo enaltece os efeitos do impacto antropogénico nas populações de gelatinosos, quer através das alterações climáticas, quer através de sobre-exploração dos recursos marinhos. Estes fatores influenciam fortemente os processos de *bottom-up* e *top-down* que controlam a dinâmica de populações dos organismos gelatinosos e particularmente da *Aurelia* sp. na lagoa de Thau. Os organismos gelatinosos podem assim ser considerados como bio-indicadores da condição dos ecossistemas e carecem de adicional investigação relativamente à sua ecologia. Só assim será possível prever eventuais blooms e compreender os seus efeitos.

Palavras-chave: Organismos gelatinosos, pólipos, éfiras, medusas, dinâmica de populações, predação.

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CHAPTER I: GENERAL INTRODUCTION

JELLYFISH ECOLOGY AND IMPACTS

Jellyfish ecology

Jellyfish are important components of ecosystems, identified as the group of pelagic animals composed mainly by water, which gives them the typical gelatinous appearance. They are what biologists call gelatinous macrozooplankton (Boero, 2013) and belong to the phyla Cnidaria and Ctenophora (Richardson et al., 2009). Jellyfish are known for their sudden and dense aggregations followed by rapid collapse of the population (Boero et al., 2008). This phenomenon, named bloom event interferes, directly or indirectly, with several human activities (Purcell et al., 2007; Richardson et al., 2009; Purcell, 2012) and can have a great impact on ecosystem functioning (Richardson et al., 2009; Boero, 2013). Increasing evidence of synantropic condition of jellyfishes is promoting a rising concern related to jellyfish blooms. Publications related to jellyfish proliferations have been increasing (**Fig. I.1**), presenting evidence that gelatinous organisms benefit from human-mediated changes on the ecosystems (Mills, 1995, 2001; Arai, 2001; Purcell et al., 2007; Pauly et al., 2009; Richardson et al., 2009; Purcell, 2012; Boero, 2013).

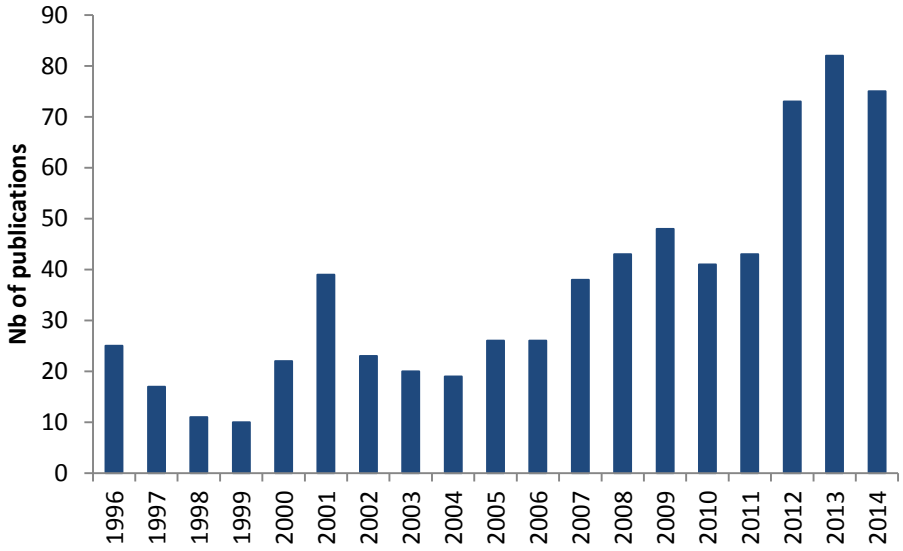

Fig. I.1: Number of publications related to jellyfish blooms, from 1996 to 2014. Search performed in Science Direct with 'Jellyfish blooms' as key words.

Brotz et al. (2012) did the first rigorous demonstration that jellyfish populations appear to be increasing in coastal marine ecosystems at a global scale. However, it is still controversial. Condon et al. (2012) stated that the recent paradigm might be based on perception, since there is a lack of long-term data at a global scale. Condon et al. (2013) further reported that jellyfish populations might undergo long term oscillations, with an approximate 20 years periodicity and underlined the mandatory accurate investigation on jellyfish abundance for the next 40 years and so on to confirm the above predictions (Purcell, 2012; Condon et al., 2013). Still, since the human impact on marine environment is expected to continue, it is suggested that the frequency and magnitude of jellyfish blooms may increase, heading to a further gelatinous future (Purcell et al., 2007; Pauly et al., 2009; Richardson et al., 2009; Purcell, 2012). The highly impacted Mediterranean Sea, where jellyfish blooms appear to be increasing (Kogovšek et al., 2010), is therefore an ideal place to study jellyfish ecology. This Sea is considered as a miniature ocean, suitable as a potential model, providing clues of the impact of climate change in the global oceans and their associated biota (Lejeusne et al., 2010).

Jellyfish life cycle

Jellyfish community is highly diverse, comprising many different species with assorted life cycle patterns. Ctenophores are usually holoplanktonic, remaining in the pelagic realm their entire life. Cnidarians though, are generally meroplanktonic, including benthic and pelagic stages. Cnidarians life cycle usually consists of two main phases: the polyp (benthonic) and medusa (pelagic), which perform asexual and sexual reproduction, respectively. After planulae settlement and metamorphosis into polyps, each polyp undergo asexual reproduction by budding and strobilation (Arai, 1997; Collins, 2002; Pechenik, 2005) which enhance the production of young medusa (*i.e.* ephyrae). Ephyrae grow in the water column reaching the adult stage (*i.e.* medusae), which, after sexual reproduction, produce new planulae, closing the life-cycle (see **Fig. I.2** for an example). The production of cysts (podocysts) may also be included (Arai, 2009), being an outcome of a life-cycle adjustment in order to overcome periods of unfavorable environmental conditions (Boero et al., 2008). Nevertheless, this general life cycle is not applicable to all species of Cnidaria and each stage can be reduced or absent (Arai, 1997). In this report I will focus on Cnidarians, especially Syphozoans (*i.e. Aurelia* spp.), since they are the most common bloom forming gelatinous

organisms and can have severe impacts on human activities and ecosystem functioning (Mills, 2001).

Possible causes of jellyfish outbreaks

Several reviews have identified the possible factors that might influence jellyfish blooms (Purcell et al., 2007; Richardson et al., 2009; Purcell, 2012; Boero, 2013), pointing out the potential effect of climate change, overfishing, eutrophication, introduction of alien species and habitat modification as the most important.

Climate change

Climate has been claimed as one of the most important triggers for jellyfish outbreaks (Purcell, 2005). The ocean warming appears to endorse the magnitude and frequency of jellyfish outbreaks around the world (Molinero et al., 2009; Kogovšek et al., 2010; Licandro et al., 2010; Lynam et al., 2011), by direct effects on their population dynamics or indirect effects on the ecosystem functioning (Purcell, 2005). For instance, increasing temperatures affect benthic stages of jellyfish by boosting the production of ephyrae through asexual reproduction, underlining the crucial role of the benthic stages in blooms shaping (Lucas et al., 2012; Purcell, 2012). The indirect impact might be perceived by a shift in the structure of zooplankton community, as an outcome of increasing temperatures and consequent water column stratification, which benefit gelatinous populations and simultaneously impair their competitors (*i.e.* zooplanktivorous fishes) (Lynam et al., 2011; Reygondeau et al., 2015).

Climate change might be generally represented by global warming. However, not only the temperature *per si* may affect jellyfish populations, but also the changing in chemical and physical characteristics of the oceans, such as ocean acidification (Winans & Purcell, 2010) and currents pattern shifts. The latter one generates processes of aggregation and advection, often misinterpreted as increasing jellyfish blooms (Graham et al., 2001; Suchman & Brodeur, 2005).

Climate change is therefore expected to influence jellyfish populations, heading to a future with better conditions for their proliferation. However, among the *ca.* 3700 species of pelagic cnidarians (Daly et al., 2007) the responses may not be equal within and among jellyfish species and further research at local/regional and global scales is required (Purcell, 2005).

Overfishing

Marine resources have been under some kind of fishing pressure everywhere in the world (Pauly et al., 1998, 2002), leading to many situations of fish stock collapses (Mullon et al., 2005). Overfishing may positively affect jellyfish populations in two ways, by removing their predators, and their competitors (Purcell et al., 2007; Richardson et al., 2009; Purcell, 2012; Boero, 2013). Pauly et al. (2009) predict that the removal of jellyfish predators will lead to future dominated by jellyfish. However, much attention has been focused on the removal of their competitors. With their removal, an ecological niche is offered to jellyfish which allows them to increase in abundance, benefiting from high food availability (*i.e.* zooplankton). Some authors reported overlaps of jellyfish and zooplanktivorous fish diets, stressing the potential high level of competition (*e.g.* Purcell & Sturdevant, 2001; Brodeur et al., 2002) and it is also common in literature to report jellyfish outbreaks after local fish stocks collapse (*e.g.* Shiganova, 1998; Daskalov, 2002; Lynam et al., 2006; Daskalov et al., 2007). Since jellyfish may also be predators of their own competitors by eating their eggs and larvae (Purcell, 1985; Purcell et al., 1987; Hansson et al., 2005; Gordoa et al., 2013), the impact of jellyfish on fish stocks might be drastic, dropping the resilience of the already fragile fish stocks (Boero, 2013).

Eutrophication

The most simple and direct consequence of nutrients enrichment (*i.e.* eutrophication) is the enhancement of primary production, which fuels jellyfish populations through rising food availability (Arai, 2001; Purcell et al., 2007; Purcell, 2012). However, the characteristic small sized community composition, may offer an inter-specific competitive advantage for jellyfish, since, unlike other organisms, they are capable of feeding on wide range of prey, from POM to macrozooplankton (Båmstedt et al., 2001; Hansson, 2006; Kamiyama, 2011; McNamara et al., 2013; Morais et al., 2015). Eutrophication is also commonly associated with depleted oxygen zones. Most organisms do not survive in this environment, but many species of jellyfish are tolerant to low oxygen concentration environment (Purcell & Arai, 2001; Purcell et al., 2001) and even capable of asexual reproduction (Condon et al., 2001). With the possible expansion of eutrophic and hypoxic zones, as a consequence of human impacts (Diaz & Rosenberg, 2008), it is likely that habitats suitable for jellyfish dominance will increase.

Habitat modification

In order to cope with the increasing human population, infrastructures in the coastal landscapes have been developed (Bulleri & Chapman, 2010). With global warming, sea level is expected to rise and climate extremes events are expected to occur more frequently (IPCC, 2007), implying the reinforcement and protection of coastal areas. The expansion of breakwaters, jetties and seawalls in coastal areas is pointed as one promising source of suitable substrates for jellyfish polyp settlement, promoting their proliferation and contributing to jellyfish outbreaks (Purcell et al., 2007; Duarte et al., 2012; Purcell, 2012; Boero, 2013; Gibbons & Richardson, 2013). Not only coastal constructions, but also the reduction of freshwater inflow variability caused by dams, increase the stability of flow and salinity, which favor estuarine jellyfish by increasing survival of polyp phase (Chícharo et al., 2009; Barbosa & Chícharo, 2011).

Introduction of alien species

Shipping, both by ballast waters and hull fouling, together with aquaculture, seems to be the most common vectors of introduction of nonindigenous species in new habitats around the world (Gollasch, 2008). Graham & Bayha (2008) considered the physiological, ecological and life-history traits of jellyfish (*i.e.* rapid growth, asexual propagation, intensive predators, crypsis and morphological plasticity), and concluded that they are perfectly suited as invasive organisms. There are several reports of jellyfish introduction in many locations around the world (see Purcell et al., 2007), with some species occurring currently at a global scale, such as *Aurelia* spp. (Dawson, 2003).

Addressing the real cause of jellyfish outbreaks is intricate, though. Interactions between gelatinous organisms and climate, overfishing, eutrophication, habitat modification and introduction of alien species are extremely problematical and predictions are difficult to make. Human impact on marine ecosystems is highly concentrated in coastal areas where those different parameters interfere with each other and may influence jellyfish ecology in a synergetic way (Arai, 2001; Purcell et al., 2007; Purcell, 2012).

Impacts of Jellyfish outbreaks on human activities and ecosystems

Theoretical predictions reveal a future with more frequent and larger blooms of jellyfish. The high abundance of these gelatinous organisms, even periodically may

interfere directly or indirectly with human activities, presenting adverse or beneficial consequences.

Among the negative side, fishing is probably the most impacted activity. Competition with jellyfish may have serious consequences on zooplankton, diminishing their availability for fish stocks and consequently reducing fish landings (*e.g.* Shiganova, 1998; Purcell & Arai, 2001; Purcell & Sturdevant, 2001; Brodeur et al., 2002; Hansson et al., 2005; Hamer et al., 2011). Predation on ichtyoplankton larvae and eggs (Purcell & Arai, 2001) has also been claimed as one of the most serious impacts on fish stocks recruitment (Purcell et al., 2007). In addition, the aggregation of jellyfish in large numbers can affect directly fishermen by clogging fishing nets, and spoiling the quality of the catch (reviewed by Purcell et al., 2007). Like fishing gears, cooling systems of industrial factories, which use the coastal waters to cool down their engines, are also clogged by large aggregations of jellyfish (Dong et al., 2010), causing elevated economic losses all over the world (reviewed by Purcell et al., 2007). Tourism is one of the most important activities in numerous countries around the world and episodes of jellyfish stings might cause reduction of their attractiveness (Boero, 2013). Furthermore, jellyfish outbreaks may also decimate entire cultures of fishes in extensive aquaculture systems, damaging the skin and gills of the caged fishes through their stinging cells (*e.g.* Båmstedt et al., 1998). Because most of these activities are located in coastal waters, their susceptibility is high to negative impacts of jellyfish outbreaks.

However, jellyfish blooms can also have positive impacts, especially as a food source for a variety of organisms (Purcell & Arai, 2001; Cardona et al., 2012; Milisenda et al., 2014), as a refugee for several juvenile fishes (Lynam & Brierley, 2007; Masuda, 2009), as a food source for human consumption (Hsieh et al., 2001) which supports an alternative fishing and aquaculture activity (Omori & Nakano, 2001; You et al., 2007), as a source of important molecules for biotechnology and medicine (*e.g.* Jones et al., 1999; Ding et al., 2011) and finally as touristic attraction (*e.g.* Palau; Dawson et al., 2001).

BIOLOGICAL MODEL: *AURELIA* SP.

A large number of jellyfish blooms in coastal areas and semi-enclosed seas are performed by scyphozoan species of the genus *Aurelia* (Mills 2001). For a long time, *Aurelia aurita*, the most studied jellyfish species, was considered as cosmopolitan, capable of local adaptation due to its phenotypic plasticity (Lucas, 2001). However,

recent studies have addressed the biogeography of the genus *Aurelia* and reported that is actually a species-complex embracing numerous locally adapted species (Dawson and Jacobs, 2001; Dawson and Martin, 2001; Schroth et al., 2002; Dawson, 2003; Dawson et al., 2005; Ki et al., 2008). The South coast of France, where Thau lagoon is situated, is suggested to be inhabited by *Aurelia* sp.1 (Schroth et al., 2002; Dawson, 2003; Dawson et al., 2005). However, further locally molecular studies are required, since isolated populations inhabiting coastal lagoons were already shown as different cryptic species (*e.g.* Mljet lagoon in Croatia is inhabited by *Aurelia* sp.5) (Dawson and Jacobs, 2001). As a result, here I will consider *Aurelia* sp. until the existence of molecular evidence of the specific taxonomic identification of the *Aurelia* population present in Thau lagoon.

Aurelia spp. inhabits nearshore waters, especially closed basins, such as coastal embayments, fjords and estuaries, occupying a great variety of habitats worldwide (Lucas, 2001). They inhabit coastal waters of western and central Mediterranean areas, as well as the Black Sea (Mutlu, 2001). They are common in the Adriatic Sea and in coastal lagoons such as Mljet Island, Lake of Verano, Berre, Bages-Sigean and Thau lagoon (Bonnet et al., 2012; Boero, 2013; Bonnet, D., personnal observation), showing an increasing trend of recurrent blooms in recent decades (Kogovšek et al., 2010). The Mediterranean Sea is one of the most sensitive areas to the combined effects of anthropogenic disturbances, *i.e.* habitat modification and climate change (Lejeusne et al. 2010), which warns on potential favourable conditions for jellyfish blooms and provides the ideal environment to study them.

Aurelia spp. life cycle

The life cycle of *Aurelia* spp. is composed by both benthic asexually-reproducing polyp and pelagic sexually-reproducing medusa. The different stages can be defined as egg, planula, polyp (scyphistoma and strobila), ephyra and medusa (**Fig. 1.2**). The sexual medusa is dioecious and releases sperm in the water column. The sperm fertilizes the ovocytes within the females and the egg is then released in the water column. The egg becomes planula larvae, which settles on benthic substrate after 7 to 10 days in the water column. Planulae metamorphoses into a polyp (scyphostoma) and through asexual reproduction several ephyrae are produced and liberated in the water column, a process called strobilation. Ephyra develops into an adult medusa, closing the life-cycle (**Fig. I.2**; Arai, 1997).

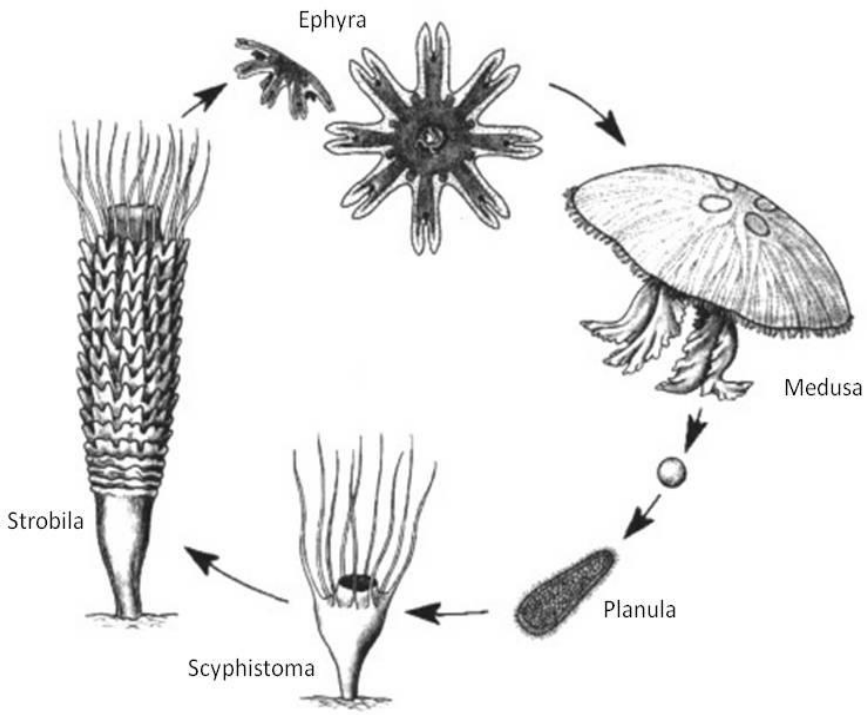

Fig. I.1: Life cycle of *Aurelia* spp. (Scyphozoan). (Adapted from Ruppert et al. 2005).

Aurelia sp. in Thau Lagoon

Bonnet et al. (2012) studied the pelagic phases of *Aurelia* sp. in Thau Lagoon, reporting the seasonal pelagic dynamics of this species (**Fig. I.3**). In Thau Lagoon, *Aurelia* sp. life-span is 7-8 months. The first ephyrae released in the water was reported in November, representing the initiation of strobilation process, possibly as a result of decreasing temperatures, as suggested by other authors (*e.g.* Watanabe & Ishii, 2001; Miyake et al., 2002). The presence of ephyrae was recorded until April with maximum abundance in February and April months. Immature individuals (without developed gonads) were present in spring, between March and April, when they reach maturity. Sexual medusae were observed from April to June, showing a drastically decline in abundance after this period. Both ephyra and adult stages were completely absent from July to October. According to Bonnet et al. (2012), rising temperatures and food availability explain the higher growth rates of *Aurelia* sp. in Thau lagoon. Ephyrae are tolerant to temperatures from 5 to 26°C but their maximum abundance occurs at 16°C, after which the abundance declines, giving rise to adult medusae. Medusae are present in warmer waters but their abundance peaks at 19°C and is followed by a sharp decline, likely due to natural mortality which occurs soon after eggs release (Bonnet et al.,

2012). Thau lagoon seems to provide a good environment for *Aurelia* sp. proliferation, since it is a sheltered semi-enclosed system with high food availability and suitable environmental conditions.

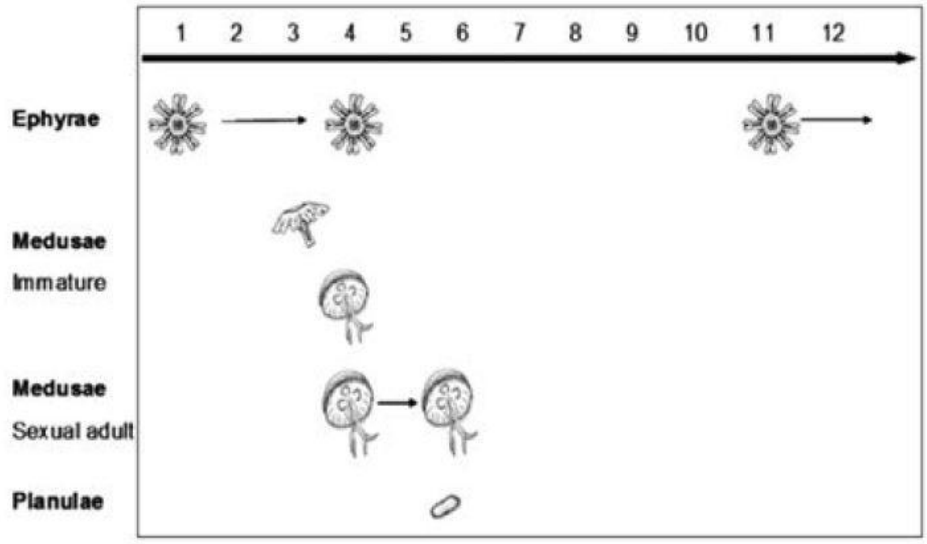

Fig. I.2: Seasonal presence of pelagic life-cycle in water column in Thau Lagoon. (After Bonnet et al. 2012).

STUDY AREA

Thau lagoon is a semi-enclosed coastal lagoon (43°23'59.10'' N 3°36'37.15'' E) with 75 km^2 and connected to the Mediterranean Sea by three narrow channels (Canal de Sète, Canal des Quilles and the Grau de Pisses-Saumes). It is a shallow environment with a mean depth of 4m and a maximum depth of 24m (at one specific location) (**Fig. I.4**). The lagoon has a weak tidal range (<1m), which promotes a high residence time of water masses (1-4 months) and is highly influenced by seasonal strong wind events (Fiandrino et al., 2012). Thau lagoon is under heavy human pressure, as shellfish farming is one of the most important economic activities, with a production of 15 000 tons of oysters and mussels per year, representing 10% of the French national production (Bonnet et al., 2012; Mongruel et al., 2013). Despite of being connected to the sea, there is no advection of *Aurelia* sp. from and to the Mediterranean Sea, which implies that both pelagic and benthic phases of *Aurelia* sp. occur inside the lagoon (Bonnet et al., 2012).

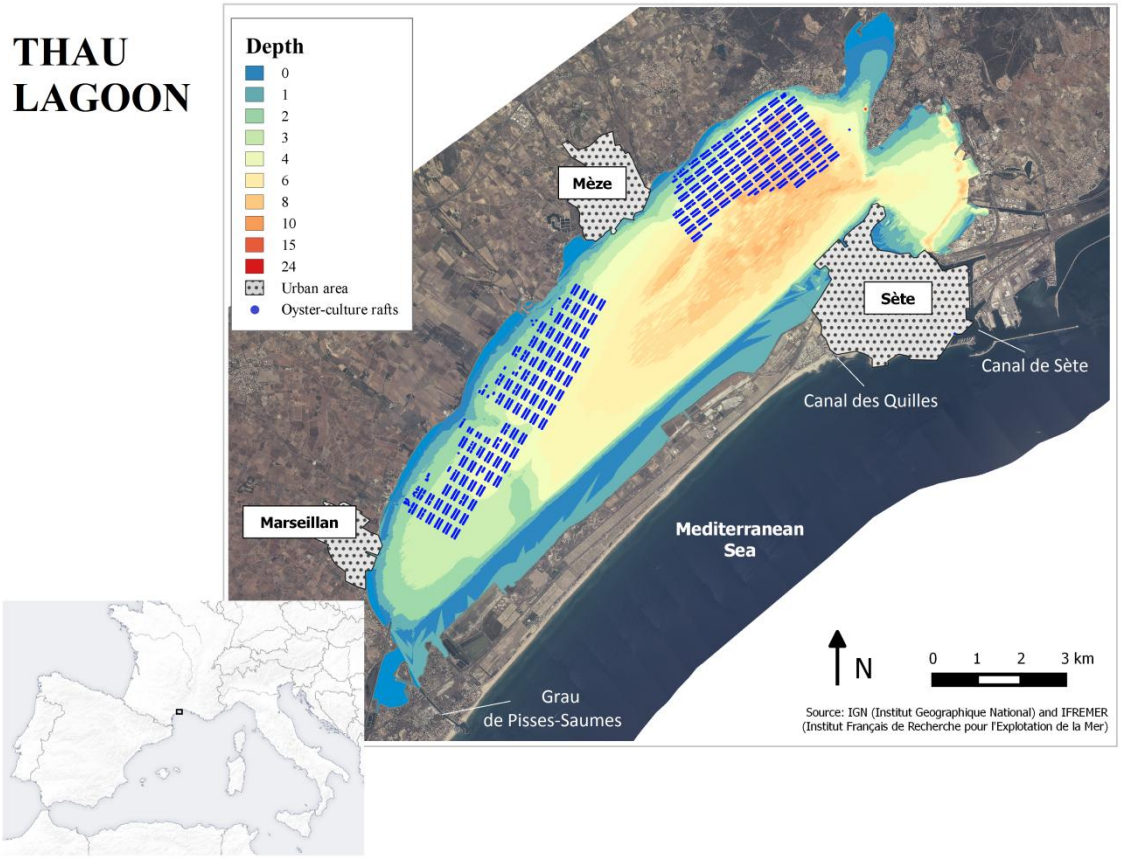

Fig. I.3: Map of the study area.

OBJECTIVES

Aurelia spp. are probably the most studied species of jellyfish in the world. However, critical periods of their complex benthic-pelagic life cycle have been little investigated. Research efforts have rarely targeted the benthonic stage and the *in situ* dynamics of the *Aurelia* spp. polyps population have been overlooked. Furthermore, local to regional level studies on pelagic dynamics are still required to understand the ecology of jellyfish blooms, the triggers of their outbreaks and their impact on different ecosystems. Finally, little is still known regarding the trophic relationships between fish predators and jellyfish and its impact on energy pathways.

Thau lagoon, a semi-enclosed system that harbours a resident population of *Aurelia* sp., is in essence a natural laboratory that offers an ideal framework to investigate the dynamics of *Aurelia* sp. population to assess processes of top-down and bottom-up control. Therefore, this report will be divided in three main subjects with their own specific objectives (**Fig. I.5**):

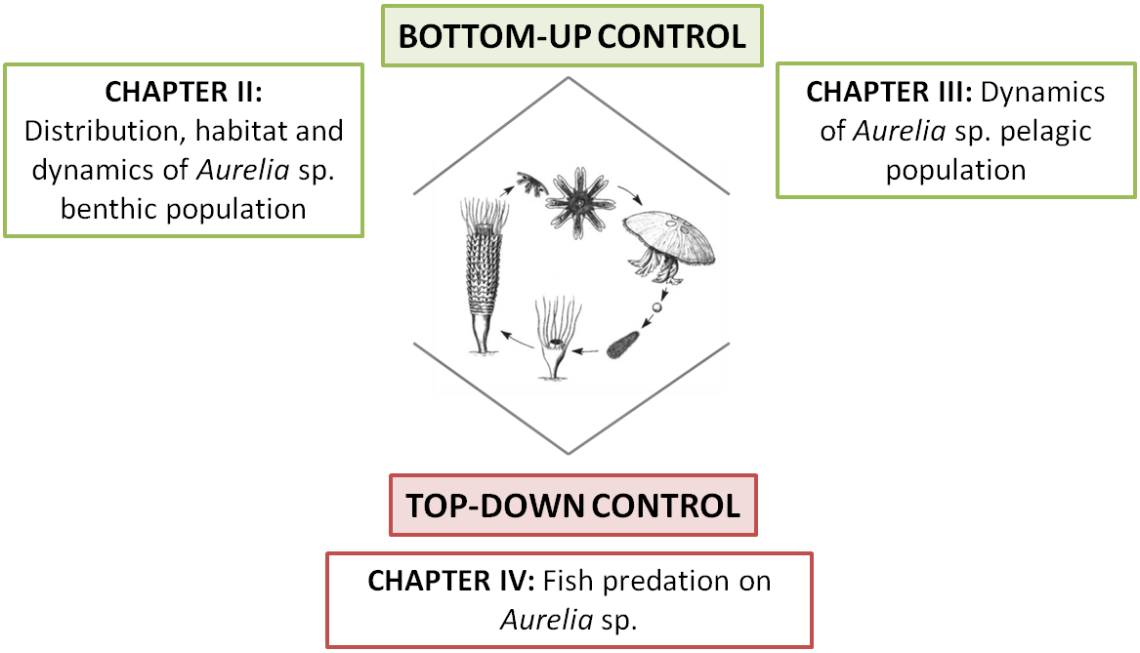

Fig. I.4: Schematic representation of the thesis structure.

Chapter II: Distribution, habitat and dynamics of *Aurelia* sp. benthic population

The benthic asexual reproducing stage (polyp) of *Aurelia* spp. is acknowledged fundamental in bloom onset (Lucas, 2001), although field investigations remain scarce. In Thau lagoon, pelagic monitoring suggests that the benthic stage of *Aurelia* sp. should be present in the lagoon (Bonnet et al., 2012), however direct investigations on polyps population are completely absent. Thus, this chapter is assessing three main questions:

- Are the polyps of *Aurelia* sp. present in Thau Lagoon?
- What is the distribution of polyps in Thau Lagoon?
- On what type of substrates do the polyps settle?
- What is the dynamics of *Aurelia* sp. benthic population in Thau lagoon?

Chapter III: Dynamics of *Aurelia* sp. pelagic population

While the question of polyps occurrence have been tackled, the next main objective of this report is to understand the population dynamics of the pelagic stages of *Aurelia* sp. in the lagoon. Regional and inter-annual variability in *Aurelia* sp. pelagic populations dynamics around the world have been recognized and reported in the literature (Lucas, 2001). This variability promotes the needs to develop local *in situ* investigations and to compare population dynamics in contrasted ecosystems. Therefore, three main objectives will be addressed in this chapter:

- What is the dynamics of *Aurelia* sp. pelagic population in Thau lagoon?
- What are the environmental forces acting on such dynamics?
- To compare population dynamics among different habitats around the world.

Chapter IV: Fish predation on *Aurelia* sp..

Jellyfish were largely described as 'dead ends' of the food webs, as a result of their high water content and low nutritional value (Doyle et al., 2007). However, some reports have demonstrated that jellyfish might be an important source of energy, especially during bloom events (Arai et al., 2003; Arai, 2005; Cardona et al., 2012). Chapter IV addresses fish predation on different life stages of *Aurelia* sp. in Thau lagoon, using complementary field and laboratory investigations (using *Sparus aurata* as biological model), in order to answer five main questions:

- What is the diversity of *Aurelia* sp. fish predators in Thau Lagoon?
- Can *Aurelia* sp. be a non-negligible source of food for *Sparus aurata* population in Thau lagoon?
- What are the development stages of *Aurelia* sp. preferred by *S. aurata*?
- Are *S. aurata* able to favour *Aurelia* sp. diet?
- What is the theoretical grazing pressure of *S. aurata* community on *Aurelia* sp. populations in Thau lagoon?

CHAPTER II: DISTRIBUTION, HABITAT AND DYNAMICS OF *AURELIA* SP. BENTHIC POPULATION.

INTRODUCTION

Despite the recent scientific interest in jellyfish ecology, some critical periods of their complex life cycle have been overlooked. Most studies focused on medusa stage and although *Aurelia* spp. is the most studied species of jellyfish, field investigations of benthic population dynamics remain scarce and mainly restrained to the western Pacific Ocean (Watanabe & Ishii, 2001; Miyake et al., 2002; Willcox et al., 2008; Ishii & Katsukoshi, 2010; Toyokawa et al., 2011; Duarte et al., 2012). In the Mediterranean, there is only one *in situ* study on *Aurelia* sp. polyps (Malej et al., 2012).

The abundance of adult medusae is tightly dependent on ephyrae production, which in turn is totally reliant on the polyps population. Thus, improving the knowledge on polyps ecology is a research priority (Condon et al., 2014). According to Lucas et al (2012), the distribution and abundance of the benthic population is determined by recruitment of planulae to the sea bed, asexual reproduction of the scyphistoma and inter- and intra-specific trophic relationships.

Previous studies suggest that Thau lagoon harbours a resident population of *Aurelia* sp. (Bonnet et al. 2012). Although the presence of *Aurelia* sp. benthic population was never demonstrated, the presence of ephyrae in sheltered areas might be an indication that polyps are present in this ecosystem (Toyokawa et al., 2011).

Ocean sprawl is pointed as a major contribution to increase jellyfish outbreaks by providing higher availability of settlement substrates (Duarte et al., 2012; Janßen et al., 2013; Makabe et al., 2014; Qingdao, 2014). Likewise, Lo et al. (2008) investigated the possible triggers of *Aurelia aurita* blooms in Taiwan, and reported that the presence of extensive oyster-culture rafts was probably the crucial feature that enhanced jellyfish populations. Considering the high anthropogenic impact and particularly the wide presence of artificial constructions in Thau lagoon (*e.g.* oyster-culture rafts), an analogous situation might be occurring in this lagoon. Here we report a field survey, providing a qualitative assessment of the distribution and habitat use of the benthic population of *Aurelia* sp. in Thau lagoon, a semi-quantitative evaluation of colony size and an assessment of benthic population dynamics.

METHODS

Polyps distribution and habitats

Hard substrates potentially suitable for *Aurelia* sp. polyps settlement in Thau lagoon were identified based on man-made structures present in the lagoon (source: IGN-Institut Geographique National) or by local observations, and classified by substrate type: concrete, metal, polyethylene/plastics, breakwater rocks, wood and shell bottom (**Fig. II.1**). They were then mapped, using Quantum GIS program (2.2.0- Valmiera version).

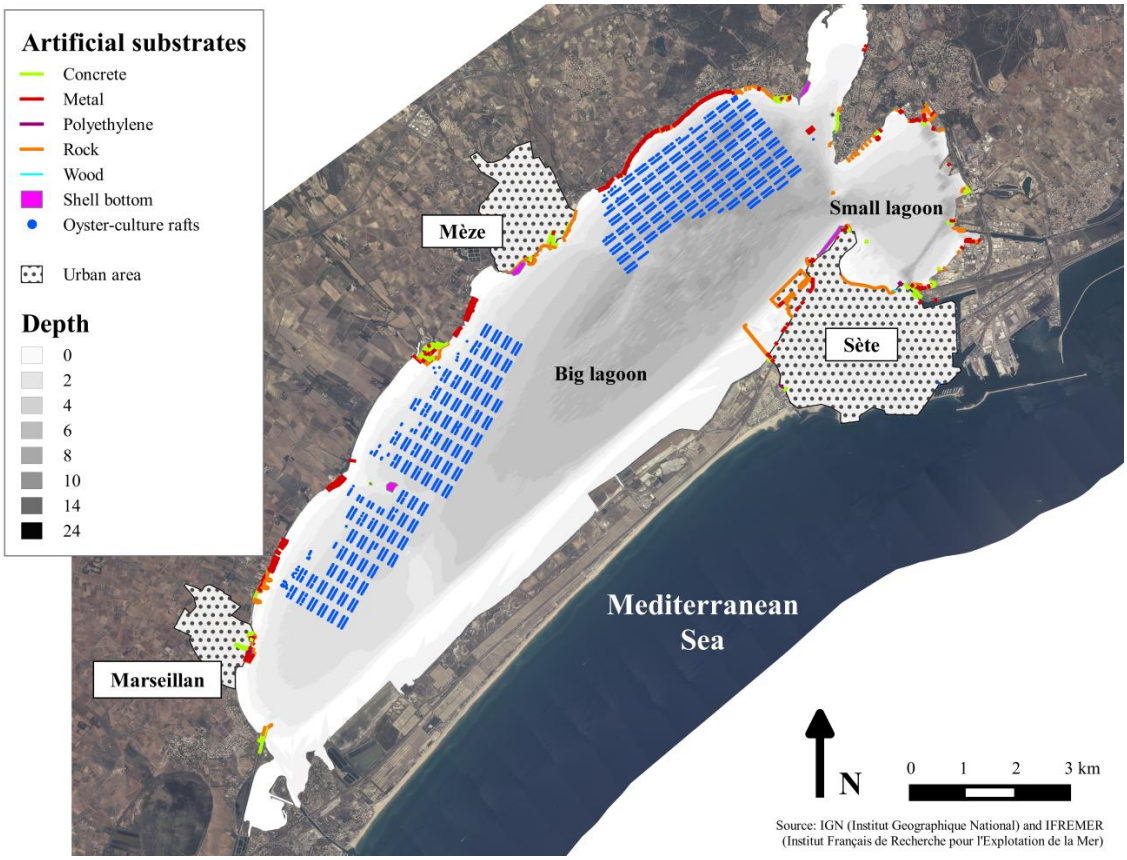

Fig. II.1: Study site and hard substrates identified in Thau lagoon.

To assess polyp distribution, we used a non destructive approach consisting of thirty nine survey dives performed between 14th March and 19th June. Investigations were conducted by free diving in discrete places, selected according to the distribution of previously mapped substrates, in order to cover the entire lagoon and different substrate types (**Fig. II.2**).

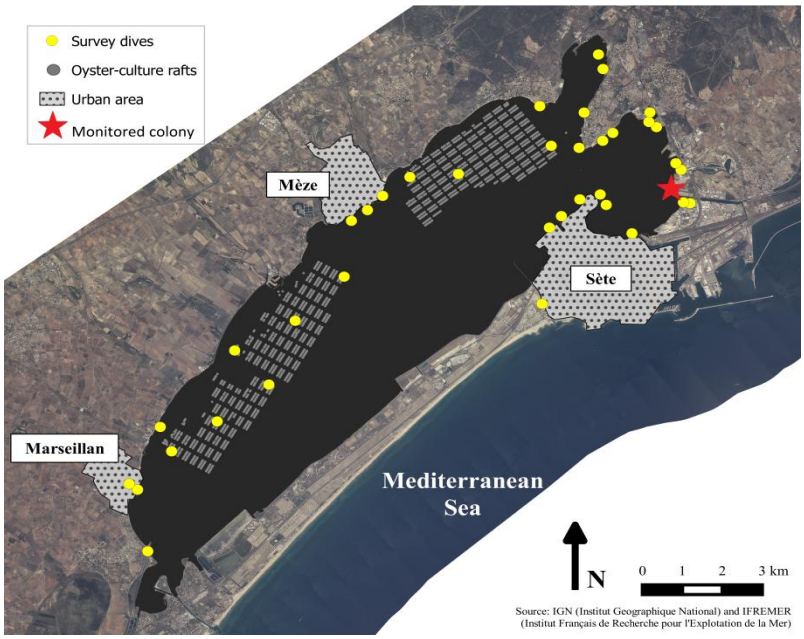

Fig. II.2: Distribution of the survey dives in Thau lagoon and colony monitoring sampling site.

The lagoon was divided in three main zones and chronologically investigated over the surveyed period: the coastal area of small lagoon, oyster-culture tables and coastal area of the big lagoon (**Fig. II.1**). Each dive lasted between 15-60 min, depending on the quantity of potential substrates promising for investigation at each location. Suitable surfaces for polyps fixation (*i.e.* shaded areas of natural or artificial surfaces) were surveyed and all the identified colonies of *Aurelia* sp. were registered. Georeferenced location, depth (recorded by dive computer Suunto, Gekko), substrate type and coverage area of each sub-population were recorded and mapped. A sub-population was characterized by one or several colonies of polyps covering the same continuous substrate type. The substrate type was identified and the nature of the surface directly colonized was registered, including the diversity of biofouling. When possible, *i.e.* with small sub-populations ($<0.5 m^{-2}$), the colonized biofouling organisms were quantified. These sub-populations were composed by one or very few isolated colonies, allowing the identification and additional quantification of the colonized organisms. The size of each sub-population was visually estimated according to a semi-quantitative index system (Index of polyps coverage, IPC), based on four categories **(Table II.1**). This index system was not as accurate as in Purcell et al (2009) or Willcox et al (2008) but it allows describing the distribution and the differences in the habitat use of *Aurelia* sp. benthic population.

At each dive, temperature and salinity were measured in sub-surface at about 0.5m depth (recorded with the probe EC 300 VWR international/ WTW model 350i).

Table II.1: Semi-quantitative index system used to quantify the area covered by each sub-population. A sub-population was characterized by one or several colonies of polyps covering the same continuous substrate type.

Polyps coverage (m^2)	Index
0.01-0.1	1
0.1-0.5	2
0.5-1	3
>1	4

Dynamics of benthic population

A colony of polyps was monitored weekly, from April to July 2014, in one dive site (43°25'31.11"N; 003°42'0.89"E; red star, **Fig. II.2**) where the largest density of polyps and numerous colonies were found providing the best sampling location for surveying *Aurelia* sp. benthic stages. In this site, *Aurelia* sp. polyps covered the underside surface of half submerged boat with maximum depth of 4m. Environmental parameters such as temperature and salinity were registered for every survey dive. Temperature and salinity were recorded in sub-surface (<0.5m) with multiparametric probes (EC 300 VWR International/WTW model 350i). Chlorophyll *a* concentration and zooplankton abundance were determined from samples of station 1 in Thau lagoon (see **Fig. III.1** in Chapter III), which were collected between April and July, throughout the pelagic monitoring study (see section Methods of Chapter III for further details).

Population and Colony densities

To assess the development of the colony, polyps abundance was determined with a quadrat method and underwater image analysis. Underwater images were taken with a Cannon PowerShot G16 camera with Ikelite Canon G16 Compact Housing non-TTL case and a Riff TL-WW light. To photograph the same quadrat (15x15cm) and keep a constant distance from polyps a PVC structure was adapted to the camera case (**Fig. II.3A**). In order to photograph exactly the same colony area the quadrat was marked directly on the substrate by scratching the surface of the wreck. Photographs were pre-treated with Adobe Photoshop CS2 Version 9.0 to improve contrast. The edge of the quadrat was not considered in the quantification to avoid misinterpretation due to shaded areas. Polyps were counted by eye observation. The polyps density was assessed at two levels as in Willcox et al. (2008): population density and colony density. Population density was calculated by dividing the number of polyps within the

photographed quadrat by the area of the quadrat (225cm^2); colony density was calculated by dividing the number of polyps forming the densest area of the colony (hereafter named sub-colony) by the area covered by the sub-colony (**Fig. II.3B**). The surface of the sub-colony was fixed at the beginning of the survey, determined as the area correspondent to a density of polyps >18 ind.cm^{-2} (*i.e.* 27cm^2). It therefore ensures an accurate long-term evaluation of the development of the sub-colony.

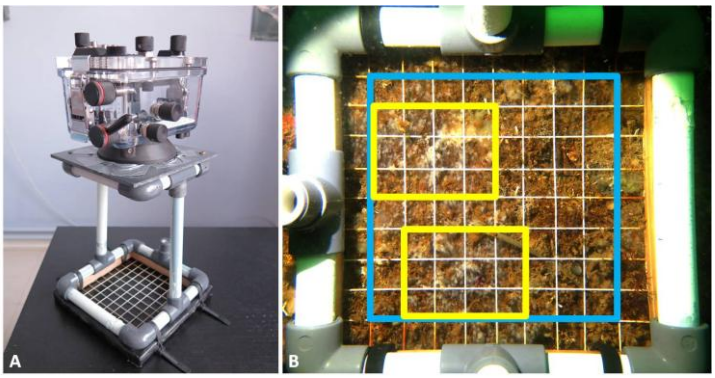

Fig. II.3: Monitoring of benthic population: A) PVC structure and quadrat adapted to the camera case; B) example of an underwater picture with the areas used to calculate population density (Blue Square) and colony density (Yellow Square).

Ephyrae production

The quantitative assessment of ephyrae production was done by means of weekly monitoring dives to collect three samples of polyps (mainly settled on oyster or mussel shells). Samples were placed in 600L tank, at *in situ* temperature and salinity in an open water circulating tank, with polyps facing downwards. Polyps were observed and counted under a dissecting microscope (Olympus SZ40; Olympus KL 1500 LCD) with maximum one day after collection. Proportion of budding (budding of new polyps from the base of the parent and stolon budding) and strobilating polyps was determined, as well as the number of strobila disks and ephyrae of each strobila, when ocurring. When present, strobilating polyps were monitored and photographed every 2-3 days, until no more strobila or ephyrae were observed in the sample (maximum strobila monitoring time was 8 days).

Statistical analysis

Data was first standardized (zero mean and unit variance) in order to acquire dimensionless variables. Then, Principal Component Analysis was performed to examine correlations among the measured physical (temperature and salinity) and ecological (chlorophyll and zooplankton) environment relative to polyps, and to project

polyp descriptors (population density, colony density and mean proportion of budding) into the multivariate environmental space. In addition, the test of hypotheses explaining the polyp changes was done using a General Linear Model (GLM) in a factorial mode, including the above environmental factors as predictors. This allowed assessing the individual effects and their interactions. Linear models were applied in order to assess correlations among polyps descriptors. Statistical analysis was performed using the software R 3.1.1 (The R Project for Statistical Computing 2014) and Statistica v.10 (StatSoft.Inc) and taking $\alpha<0.05$ as the limit for statistical significance.

RESULTS

Polyps distribution and habitats

Thau lagoon is mainly characterized by muddy bottom with few natural rock formations. Among the different structures identified, the most common was metal structures, representing 92.66%, while wood was rarely found in the lagoon (0.06%) (**Table II.2 and Fig. II.1**).

The strong percentage of metal structures in the lagoon is due to the high number of vertical metal pillars used as a support for boats moorage, pontoons, small piers and oyster-production structures. The oyster-culture rafts contribute largely to this high value, since they represent 88% of all metal structures identified in the lagoon. Four sites were additionally identified as shell bottom due to the accumulation of oyster and mussel shells on the bottom. All the identified substrate types were surveyed with higher effort on the most common structures (**Table II.2**). Polyps were found on the underside surface of different hard substrate types distributed across the entire lagoon (**Fig. II.4**).

Table II.2: Percentage of artificial substrates identified and surveyed in Thau lagoon, during the study period. NI: not identified.

Artificial Substrates	Identified (%)	Surveyed (%)
Metal structures	92.66	58.82
Breakwater rocks	2.63	11.76
Concrete	1.51	8.82
Plastics	0.35	11.76
Wood	0.06	0.98
Tires	0.12	3.92
Moored or submerged boats	0.12	3.92
NI	2.54	-

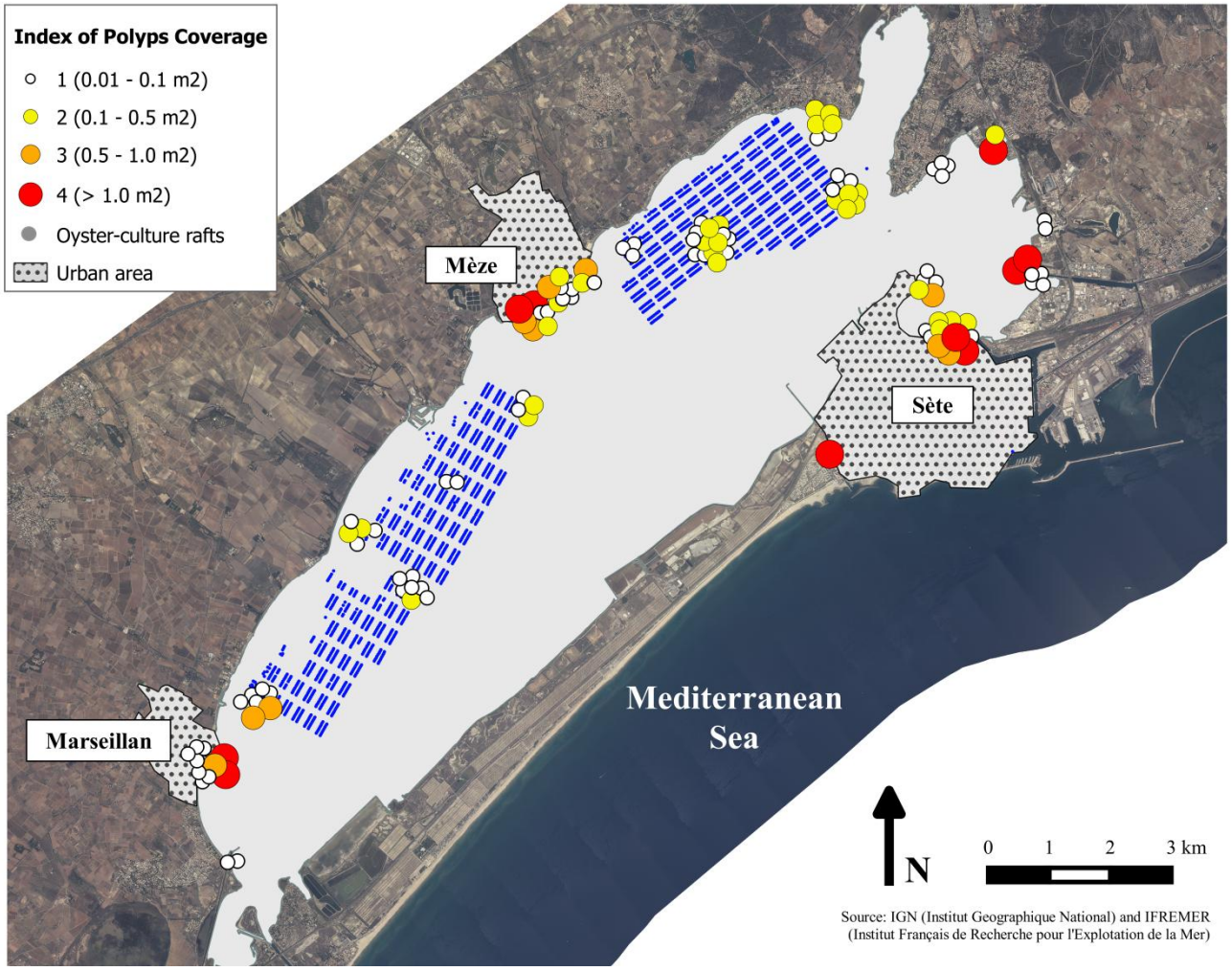

Fig. II.4: Distribution of polyps sub-populations according to index of polyps coverage in Thau lagoon. Points were slightly moved to avoid overlapping.

The largest polyp densities were present in areas experiencing heavy anthropogenic influence, such as harbours and pontoons, mainly located nearby the most populated areas (**Fig. II.4**). It is worth noticing that polyps were not found in natural rocks or shell bottom areas, likely because of the lack of suitable surfaces for downwards settlement. Polyps occurred in patches between 0.2 and 6.1m depth, with most of the sub-populations present in the first 2 m (72.4% at 0.2-2.0 m, 24.5% at 2.1-4.0 m and 3.1% at 4.1-6.1m). Polyps were directly settled on hard substrates or on other fouling organisms growing on those structures (*i.e.* red and brown algae, Ascidia, Porifera, Bryozoa, Bivalvia, Cirripedia, Polychaeta calcareous tubes and Amphipoda muddy tubes). The population of polyps in Thau lagoon was mainly composed by many small sized colonies (55.9% with 0.01-0.1m^{-2}) with fewer sub-populations covering large areas (9.8% with > 1m^{-2}). Among the different artificial hard substrates, metal structures represent the most colonized substrate type, supporting 59% of all sub-populations, followed by plastics, breakwater rocks, concrete, moored or submerged boats, tires and finally wood (**Fig. II.5**).

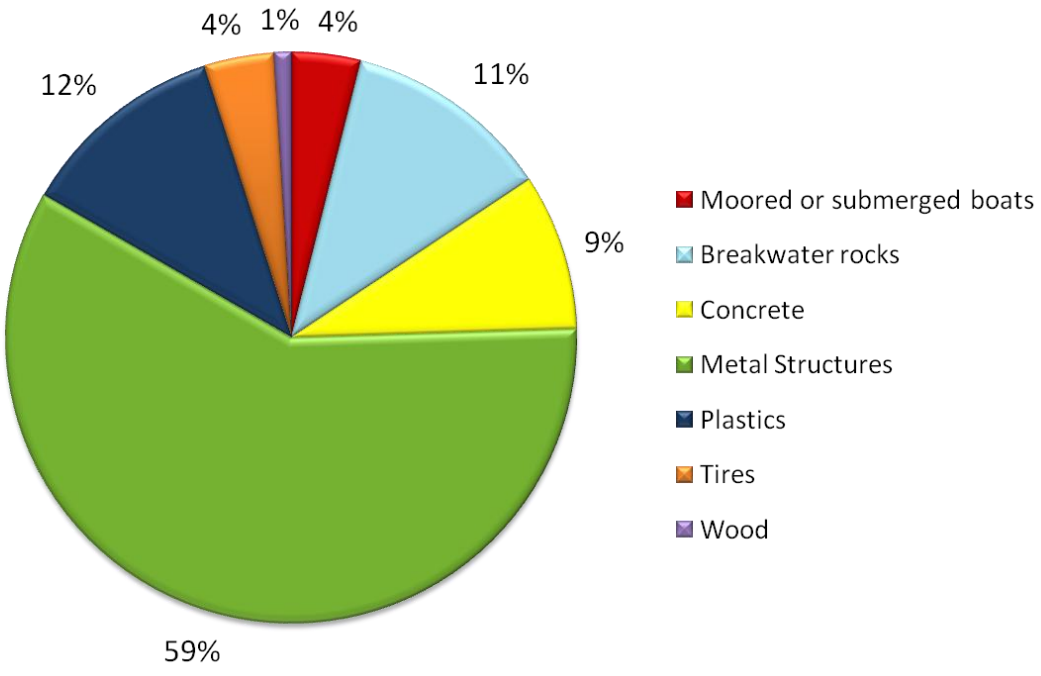

Fig. II.5: Contribution of different artificial hard substrates to total number of sub-populations identified in Thau lagoon.

However, the vertical metal surfaces were not directly colonized by polyps, but rather by other organisms which provided an adequate substrate type (86.6% of the sub-

populations settled on biofouling organisms, among which 90.4% were oysters). The percentage of sub-populations settled on biofouling organisms, considering only colonies with less than 0.1 m^2, was smaller for the remaining substrate types (8.3% of breackwater rocks and 16.7% of plastics). Larger sub-populations were not included since the proportion of biofouling and artificial surface directly colonized by polyps was not possible to quantify by visual inspection, with the exception of metal pillars where populations with size <0.5 m^2 were also considered, since each colony was mainly settled on individual biofouling organisms

Sub-populations covering larger areas were not found on the most common substrate types, but rather on structures with large surface areas faced downwards. Plastic structures (*e.g.* floating piers, plastic sheet cover on the underside of pontoons and plastic debris) were colonized by 30% and 40% of the sub-populations with coverage area superior to 0.5 and 1m^2, respectively (**Fig. II.6**).

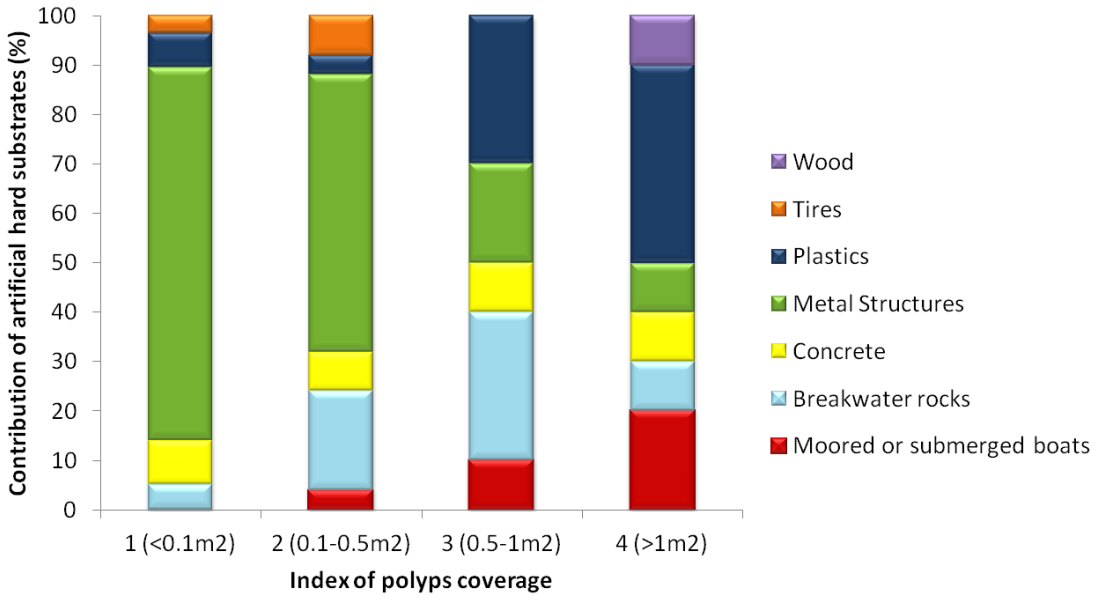

Fig. II.6: Contribution of the different artificial hard substrates to total number of sub-populations by index of polyps coverage.

The most important sub-population of *Aurelia* sp. polyps was found on the underside surface of several submerged boats sunk close to an old industrial concrete pontoon (red star; **Fig. II.2**). By contrast, despite their vast presence in Thau lagoon, metal structures support mainly colonies covering small areas (75.4% of colonies with coverage less than 0.1 m^2, were found in metal structures - **Fig. II.6**). The evaluation of the residence time of the structures supporting big sub-populations (IPC ≥ 3) revealed that 58% were

present in Thau lagoon for more than 20 years, 21% about 20 years and 21% were considerably less than 20 years old (Cantou M, personnal communication).

Temperature and salinity revealed a typical increasing trend over the study period. Temperature ranged from 12.3 to 24.0°C, while salinity ranged from 37.0 to 40.1.

Dynamics of benthic population

The seasonal field survey of *Aurelia* sp. polyps conducted between April and July 2014, showed that polyps population density, colony density and proportion of budding increased over time reaching a peak in June, decreasing afterwards (**Fig. II.7**). Polyps population density doubled from 6 ind.cm^{-2} in April 29 (16.6ºC) to 13 ind.cm^{-2} in June 25 along with highest temperature recorded (23.6ºC). Density decreased to 8 ind.cm^{-2} after three weeks (20.8ºC). Both colony density and proportion of budding maximum values occurred in June 12 (23.4ºC). Colony density revealed a maximum value of 32 polyps per cm^2, while budding polyps reached a maximum percentage of 81.6% ± 4.7 of all polyps in the samples. The lowest density of polyps per cm^2 in a colony (12 ind.cm^{-2}) was recorded in the last two weeks of survey, falling along with the proportion of budding polyps, as well as population density (**Fig. II.7**).

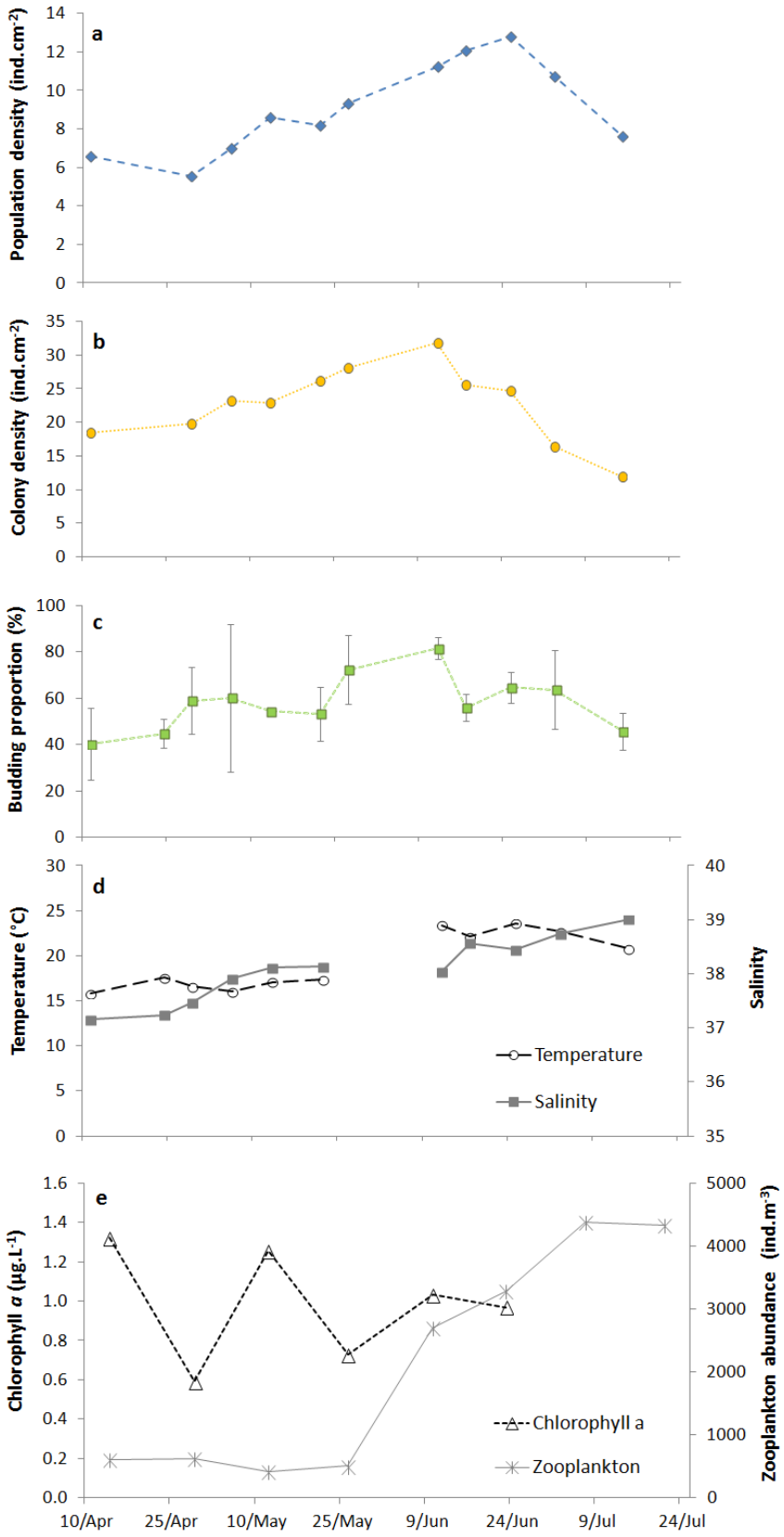

Fig. II.7: (a) Polyps population density (number of polyps per quadrat area) and (b) colony density (number of polyps per colony area) of the same weekly monitored colony, calculated from underwater photographs; (c) mean proportion of budding polyps determined from three samples weekly collected close to the monitored colony. Error bars represent standard deviation; (d) Pattern of in situ temperature and salinity and (e) chlorophyll *a* concentration and zooplankton abundance from bi-weekly collections in Thau lagoon.

The assessment of the potential influence of the environmental variables on population density, colony density and mean proportion of budding was performed by principal component analysis (**Fig. II.8**).

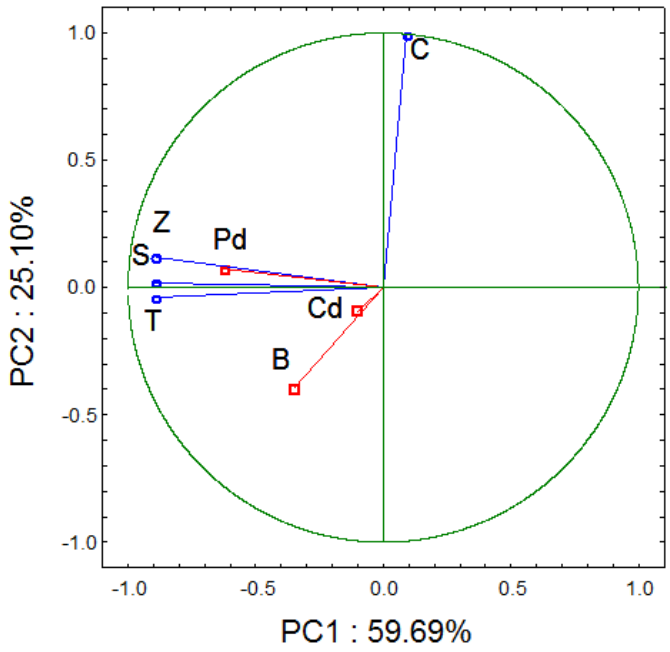

Fig. II.8: Principal Component Analysis results after standardization of data (z-scores transformation). Blue lines represent independent variables: temperature (T), salinity (S), chlorophyll *a* concentration (C) and zooplankton abundance (Z) and red lines represent response variables: population density (Pd), colony density (Cd) and mean proportion of budding (B).

We retained the two first principal components, PC1 and PC2, which accounted for a large proportion of the total environmental variability *ca.* 85%, with PC1 and PC2 representing 59.69% and 25.10% of the variability, respectively. PC1 depicted the pattern of variability of temperature, salinity and zooplankton abundance, while PC2 expressed essentially the variability in chlorophyll *a* concentration. The observed variability of population and colony density appeared related with temperature (positive correlation) and zooplankton abundance (negative correlation), while temperature drove the variability of proportion of budding (positive correlation), as suggested by the GLM assessment (**Table II.3**). It is worth noticing that among biological variables, the only significant correlation was detected between colony density and proportion of budding (**Table II.4**).

Table II.3: Correlation coefficient parameters of General Linear Models used to assess correlation between biological and environmental variables. In bold: Significant correlations (*p-value* <0.05).

Response	Variable	Estimate	Std. Error	t-value	Pr(>|t|)
Population density	(Intercept)	0.00	0.12	0.00	1.00
	Temperature	1.19	0.22	5.52	**0.00**
	Salinity	0.30	0.20	1.53	0.17
	Chlorophyll a	0.27	0.12	2.14	0.07
	Zooplankton	-0.60	0.25	-2.39	**0.05**
Colony density	(Intercept)	0.00	0.16	0.00	1.00
	Temperature	1.46	0.29	4.96	**0.00**
	Salinity	0.20	0.27	0.73	0.49
	Chlorophyll a	0.12	0.17	0.73	0.49
	Zooplankton	-1.65	0.35	-4.78	**0.00**
Proportion of Budding	(Intercept)	0.00	0.21	0.00	1.00
	Temperature	1.21	0.39	3.13	**0.02**
	Salinity	0.13	0.36	0.36	0.73
	Chlorophyll a	-0.24	0.22	-1.07	0.32
	Zooplankton	-0.90	0.45	-1.98	0.09

Table II.4: Correlation coefficient parameters of linear models used to assess correlations between biological variables. In bold: significant correlations (*p-value* <0.05).

Response	Variable	Estimate	Std. Error	t-value	Pr(>|t|)
Population density	Colony density	0.14	0.13	1.15	0.28
	Proportion of Budding	0.09	0.06	1.57	0.15
Colony density	Proportion of Budding	0.34	0.12	2.91	**0.02**

Strobilating polyps were only observed during the first two weeks of the survey period. A total of 3 strobilating polyps were observed in two samples and their development was followed on the next days. Two strobilae presented 1-2 fully developed ephyrae attached, which were released within one day. The third strobila developed strobila disks three days after the first analysis of the sample, which evolved to 2 strobila disks and 5 ephyrae after 3 days. All ephyrae were released 6 days after the beginning of strobilation. As strobilating polyps were very rare during survey period, ephyrae production was not possible to calculate.

DISCUSSION

Distribution and habitat use

Polyps distribution

The survey conducted in Thau lagoon revealed that the benthic population of *Aurelia* sp. are well established in this ecosystem. We found polyps over the entire surveyed

area, indicating that the full life-cycle of this jellyfish occurs in Thau lagoon, supporting what was previously suggested by Bonnet et al (2012). In addition, such results are in line with the lack of evidence regarding potential transport of *Aurelia* sp. from the near coast to the lagoon (Bonnet et al. 2012), implying that this population is geographically and possibly genetically isolated from the remaining populations of Mediterranean Sea. Furthermore, the broad distribution of ephyrae in Thau lagoon (Bonnet et al., 2012) together with our results, provide additional support to the hypothesis that the spatial patterns of ephyrae in sheltered areas are associated with polyps distribution, as shown in Mikawa Bay, Japan (Toyokawa et al. 2011).

All colonies showed a patchy distribution and were settled on the shaded underside horizontal or oblique surfaces of the surveyed substrates, as previously observed in laboratory experiments (Brewer, 1978; Holst & Jarms, 2007) and *in situ* investigations in Japan (Miyake et al., 2002; Makabe et al., 2014). Reduction of sedimentation and space competition with algae (Watanabe & Ishii, 2001; Miyake et al., 2002), as well as more efficient defecation process supported by the force of gravity (Holst & Jarms, 2007), were suggested as possible explanations for this behaviour.

Polyps were found between 0.2 to 6.1 m depth, but more than 70% of the population was in the first 2 m of the water column. To our knowledge, only one study has directly addressed the vertical distribution of polyps population, which, in contrast to our results, reported the absence of polyps in the first depth layers (<4m) (Ishii & Katsukoshi, 2010). Nevertheless, in the latter study, horizontal undersurface of man-made structures were not considered, which are usually preferred as settling substrates even when suitable upper or vertical sides are available (Miyake et al., 2002). In Thau lagoon, the observed vertical distribution of polyps is likely associated with the depth-frequency allocation of the artificial structures, since only a small fraction of the hard substrates (*i.e.* oyster-culture rafts) are present in deeper waters (>4 m, see **Fig. II.1**). In accordance with our results, an ecological survey in the Port of Koper, in the Adriatic Sea, revealed the presence of polyps between 1.5 m and 12 m depth, mainly settled on the underside of oyster shells (Malej et al 2012).

The qualitative assessment of polyps distribution and the semi-quantitative approach used to estimate sub-population size, allows suggesting a broad and relative evenness horizontal distribution of the benthic population over the hard substrates in Thau lagoon. However, polyps demonstrated a particular occurrence in areas exposed to high anthropogenic influence, *i.e*. highly populated areas, where human constructions offered

suitable surfaces for polyps settlement (**see Fig. II.1 and II.4**). The distribution of the benthic population is firstly determined by recruitment of planulae to the sea bed (Lucas 2001). The patterns of large scale distribution of polyp populations have been little reported. Though, it has been related with the distribution of the parental population that influence spatial patterns of planula larvae, and thus the establishment of polyp colonies (Toyokawa et al. 2011). In agreement with previous observations, here the presence of *Aurelia* sp. medusae over the entire lagoon (see chapter III) may also explain the spread distribution of polyps. Such pattern is also likely favoured by the hydrographic characteristics of Thau lagoon (**Fig. II.9**). The relative long residence time of the water masses (*i.e.* 1-4 months), associated with seasonal strong wind events in the lagoon (Fiandrino et al. 2012) may retain and randomly disperse the planula larvae within the lagoon , which can properly settle on the available hard substrates.

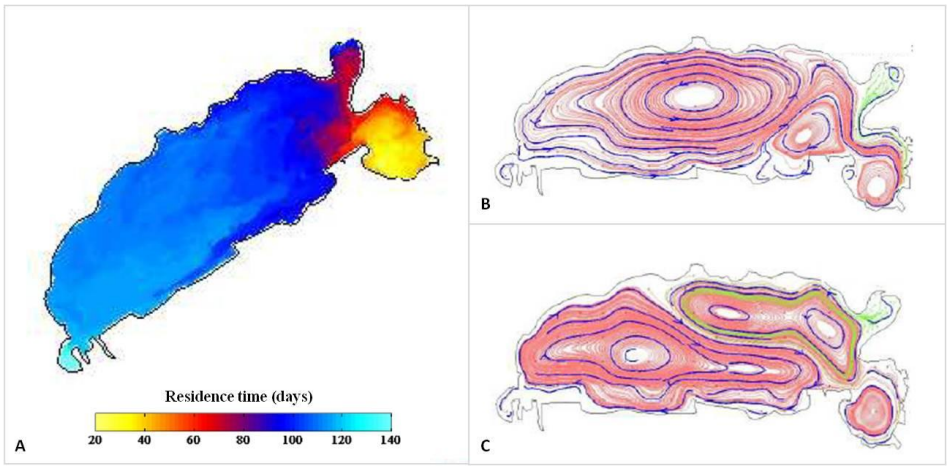

Fig. II.9: Hydrographic features of Thau lagoon. A) Residence time of water masses; Current lines induced by A) Northwestern winds and B) Southwestern winds. (After Fiandrino et al., 2012).

Habitat use

The long term survivorship of jellyfish populations relies on the development of benthic stages. A main feature enabling polyps to increase their distribution and abundance is the ability of settling on a great variety of substrate types, including artificial ones (reviewed by Lucas et al. 2012). Laboratory experiments have shown that artificial substrates are suitable for planulae settlement and often preferred by several jellyfish species (Pitt, 2000; Holst & Jarms, 2007; Hoover & Purcell, 2009). *In situ*, many reports have shown the presence of jellyfish polyps beneath man-made structures (*e.g.* Miyake et al. 2002; Willcox et al. 2008; Purcell et al. 2009; Ishii and Katsukoshi 2010; Duarte et

al. 2012). Here, we show that habitat modification through human constructions was crucial in the expansion of polyps population. Thau lagoon is characterized by sandy or muddy bottoms with very few natural hard substrates, particularly with suitable surfaces faced downwards. Among the natural substrate types surveyed during this study (shell bottom and some natural rocky formations) none were colonized by *Aurelia* sp. polyps. Thus, we hypothesize that in the absence of artificial structures, the available natural hard surfaces could have restraint the long term survivorship of *Aurelia* sp. population in Thau lagoon. This is in agreement with the expected influence that increasing coastal constructions have on promoting jellyfish outbreaks through enlarging settling structures (Duarte et al., 2012; Makabe et al., 2014; Qingdao, 2014).

Among the artificial structures surveyed in this study, metal was by far the most colonized, supporting 59% of all sub-populations (**Fig. II.5**). Vertical pillars represented the big majority of metal structures (*e.g.* support of small pontoons and oyster-production rafts, which alone occupy *ca.* 20% of the lagoon surface; Mongruel et al. 2013; **Fig. II.10**) and was the most common artificial substrate identified in the lagoon, even when excluding oyster-production rafts. Therefore, the elevated colonization of metal structures appears to be associated to their high accessibility.

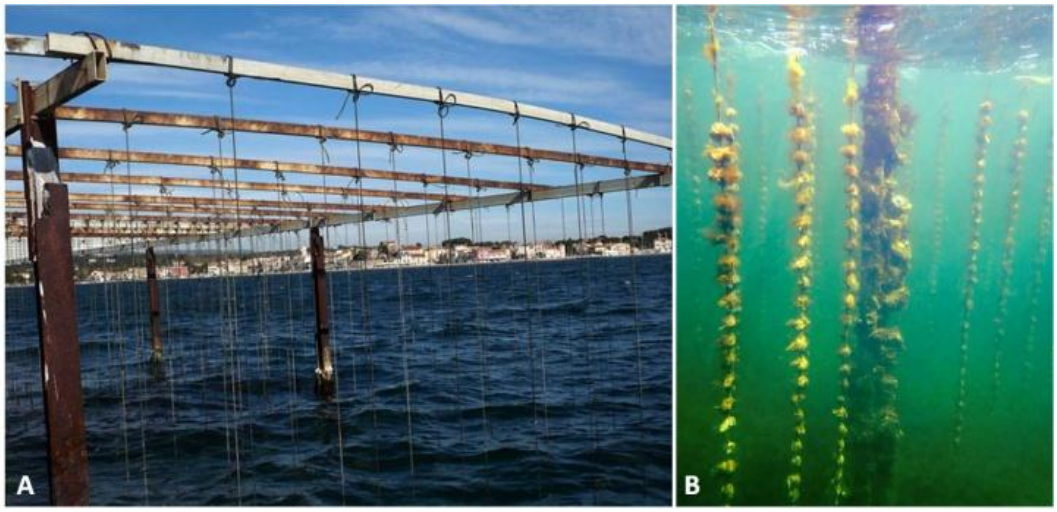

Fig. II.10: Oyster production tables showing metal pillars A) above water and B) underwater, as well as the reared oysters attached to productions lines. (photo credits: A) http://www.arrogantfrog.fr/blog/france/les-secrets-de-lelevage-des-huitres-de-letang-de-thau/; B) R. Marques, ECOSYM).

However, the number of sub-populations present in the different artificial substrates did not follow their availability in the lagoon. For instance, although plastics were one of the rarest substrate types identified, it was the second most colonized, with 12% of all

sub-populations (**Fig. II.5**). Such results might suggest some levels of preference for plastic materials such as polyethylene, as previously observed in laboratory conditions (Holst & Jarms, 2007). The potential impact of pollution is also highlighted, since several colonies were observed on plastic debris (**Fig. II.11B**), tires and particularly on submerged boats. The use of human waste as settling substrate was also reported by Miyake et al. (2002), who described that even the cellophane cover of a cigarette package was suitable enough for polyps fixation.

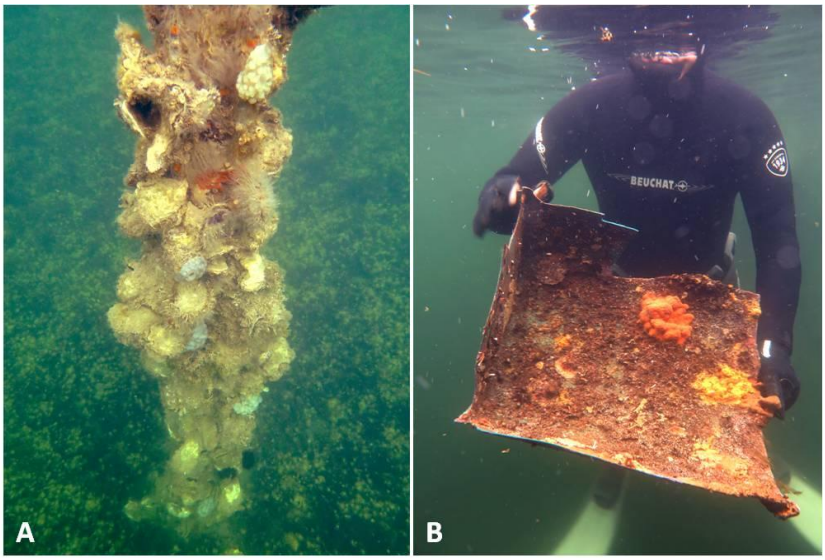

Fig. II.11: A) Structural metal pillar of oyster production tables colonized by wild oysters; B) Plastic debris colonized by *Aurelia* sp. polyps.

The artificial hard substrates were directly and indirectly colonized by *Aurelia* sp. polyps. Hard structures provide settling substrates for other biofouling organisms, which are then directly colonized by polyps. Colonization of natural biofouling was also reported by other authors (Miyake et al. 2002; Willcox et al. 2008; Toyokawa et al. 2011), where bivalves, porifera, polychatea and amphipoda tubes were among the most common colonized organisms. In Thau lagoon, although a quantitative proportion of direct/indirect colonization of polyps was not assessed for all substrate types, similar diversity was observed, with bivalves playing a special role. Indeed, on the common metal structures, 86.6% of the settled colonies were fixed on biofouling organisms. The vertical metal surfaces do not provide the suitable surfaces for polyps, but offer proper substrate for biofouling fixation. Among the colonized organisms attached to metal surfaces, 90.4% were oysters. The massive production of oysters in Thau lagoon supplies large amount of larvae to the surrounding pelagic environment that benefit

from the available hard substrates (**Fig. II.11A**). In the adult stage, oysters provide the necessary shaded surfaces for polyps fixation. *Aurelia* sp. colonies were not found on the reared oysters, likely due to their frequent removal from the water, in order to mimic the tides influence and to reduce the survivorship of biofouling organisms on oyster shells. In agreement with our results, the removal of extensive aquaculture rafts was pointed as responsible for the disappearance of *Aurelia* sp.1 populations in Taiwan, as an outcome of changes in light, water retention and availability of settling substrates (Lo et al. 2008).

Sub-population size

Although metal structures supported most of the surveyed colonies, their size was usually small (75.4% <0.1m^2 and only 10% >1m^2; **Fig. II.6**), likely due to the limited available underside surface area, which was mainly provided by the biofouling, such as oyster shells. By contrast, we found the biggest colonies associated with shaded continuous large surfaces, such as plastics (*i.e.* polyethylene pontoons) and the surfaces of submerged boats. Although direct quantitative evaluation of intra and inter-specific competition is lacking, reduced space competition may have positively influenced *Aurelia* sp. benthic population allowing their expansion and enhancing their survivorship (Toyokawa et al. 2011; Watanabe and Ishii 2001; Willcox et al. 2008). In Thau lagoon, 58% of the structures supporting big sub-populations (IPC $\geq$ 3) were submerged for more than 20 years (Cantou M, personnal communication), which might suggest a long-term establishment of the benthic population. After methamorphosis to the polyp phase the ability of asexual reproduction by budding and the settling of new buds in dense colony formation, might provide an inter-specific competitive advantage allowing their long-term establishment and development, while decreasing the available space for fixation of the remaining benthic organisms.

Our survey of *Aurelia* sp. benthic population was completed within a relatively long period (*ca.* 3 months) during the spring season. The general annual trend of the development of *Aurelia* spp. in temperate areas is generally characterized by decreasing abundance of polyps during autumn and winter and an increase during spring and summer (Willcox et al., 2008; Ishii & Katsukoshi, 2010). In Thau lagoon, the density of polyps increased during the study period (see below). We therefore hypothesize that the size of the populations firstly identified (*i.e.* in the small lagoon) were possibly underestimated, when compared with colonies located in the preceding investigated

areas. Bearing in mind that the small lagoon is the most human impacted area within Thau lagoon, these results underline the potential great impact of artificial structures in the development of jellyfish benthic populations.

Dynamics of benthic population

Polyps density

The survey of a colony of *Aurelia* sp. polyps during four months provided new insights on the benthic population dynamics of this species. The three biological descriptors (population density, colony density and proportion of budding) increased from the beginning of spring, peaking in June, when the population nearly doubled, and decreasing afterwards. To date, only few *in situ* investigations were performed to assess benthic populations of jellyfish. In Japan, *Aurelia aurita* polyps densities varied between 0.005 polyps.cm^{-2} (Ishii and Katsukoshi 2010) to 88 polyps.cm^{-2} (Miyake et al. 2002), showing a large range of variability. In the Mediterranean, only one study evaluated polyps density in northern Adriatic (Malej et al. 2012). These authors reported a maximum average of 27 polyps.cm^{-2} in July, when temperature was higher than 22°C, and minimum densities in April and early May concurrent with temperature between 13.5-17.5°C (6 polyps.cm^{-2}). Our investigation showed similar values (range of population density: 6-13 ind.cm^{-2}; range of colony density: 12-32 ind.cm^{-2}), including maximum and minimum values of polyps abundance obtained in the same season (spring).

The general annual trend of the development of *Aurelia* spp. in temperate areas, is a decrease of polyps abundance during autumn and winter and an increase during spring and summer (Willcox et al. 2008; Ishii and Katsukoshi 2010). Our results indicate a similar trend and agree with previous reports on the annual cycle of *Aurelia* sp. in the northwestern Mediterranean (Bonnet et al. 2012).

Asexual reproduction,

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). Here we found that during the first two months of the study period, the colony showed increasing trends, suggesting that planula larvae settlement and/or asexual reproduction were occurring.

Although planula stage monitoring was not included in the scope of this study, Bonnet et al. (2012) reported that in Thau lagoon planula larvae are released later in the spring, *i.e.* in June. Therefore, we suggest that asexual reproduction was likely the main origin of increasing polyps density, which is further supported by the significant correlation between colony density and proportion of budding (**Table II.4**).

Influence of environmental variables

The assessment of *Aurelia* sp. benthic population dynamics in Thau lagoon between April and July denoted that temperature was the main environmental driver of the population expansion (*i.e.* population and colony density), as well as for asexual reproduction (*i.e.* proportion of budding). Laboratory experiments have assessed the influence of temperature in budding production, in some cases associated with interacting variables, such as food (Han and Uye 2010), light (Liu et al. 2009; Purcell 2007) and salinity (Purcell 2007; Willcox et al. 2007). Empirical evidence has also shown that higher temperatures, usually between 16 to 28°C foster the production of *Aurelia* spp. buds (Willcox et al. 2007; Liu et al. 2009; Han and Uye 2010; Purcell et al. 2012). In our study budding and polyps density increased within a similar temperature range (15.8°C to 23.6°C), which further agrees with field reports in Tasmania coasts (Willcox et al. 2008).

A high rate of asexual reproduction was pointed as an outcome of a combined influence of both high temperature and food availability (Han and Uye 2010). Our results, however, did not show significant correlation between production of buds and zooplankton abundance, but it had a significant negative influence on the density of polyps (*i.e.* population and colony density; **Table II.3**). This may be explained by a possible cascade effect. Polyps appear to have some level of preference for microzooplankton, as suggested by the few existing studies reporting polyps diet (Kamiyama 2011, 2013), which could have been depleted by the increasing zooplankton abundance. However, further investigations are required to clarify the role of meso- and microzooplankton in polyps diet.

The drop of *ca.* 3°C in the last four weeks of the study period may explain the reduction of polyps producing buds, nevertheless inter- and intra-specific competition for space are suggested as underlying causes of such decrease (Willcox et al. 2008). Inter-specific space competition resulting from the establishment of other benthic organisms might also explain the reduction of polyps density observed in the last weeks, as suggested

elsewhere by several authors (Watanabe and Ishii 2001; Willcox et al. 2008; Lucas et al. 2012). Here, an increasing density of polychaeta tubes likely reduced the available space for polyps development (**Fig. II.12**).

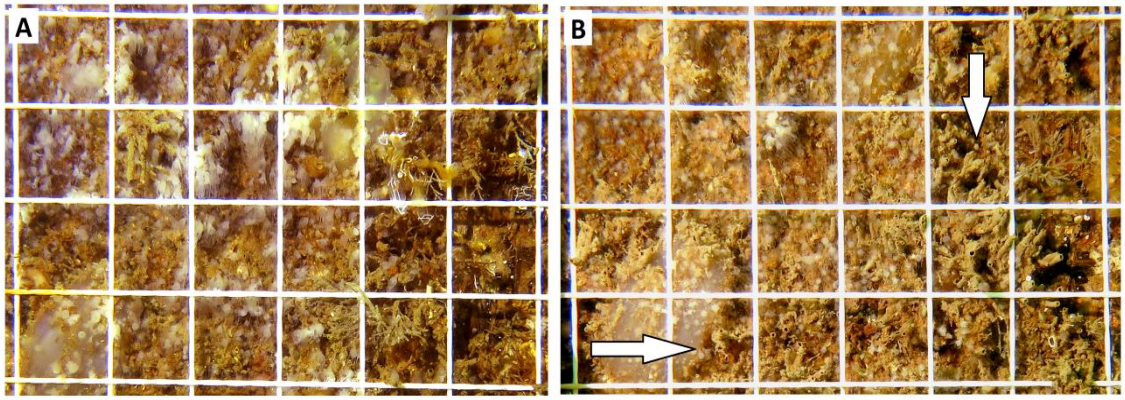

Fig. II.12: Detail of one section of the monitored colony showing the difference of polyps abundance between the sampling date with A) higher abundance (25-06-2014) and B) lower abundance (15-07-2014). In the latter picture is possible to observe many polychaeta tubes (arrows).

Predation, however, is also pointed as one of the most important sources of mortality for benthic organisms and several predators have been identified to consume scyphozoan polyps. In fact, predation was suggested as a possible counter measure to reduce the benthic jellyfish populations and ultimately blooms development (Hernroth and Grondahl 1985; Takao et al. 2014). We identified some potential predators in the monitoring dive site (two nudibranchs and one gastropod), but their impact on benthic population is unknown. Yet, predators diversity and their impact on *Aurelia* sp. benthic populations is most likely underestimated, including the potential influence of fish predation (see chapter IV).

Ephyrae production

As suggested by Han and Uye (2010), polyps allocate their energy to production of ephyrae (*i.e.* strobilation) when temperatures are lower (<14°C). During our survey, strobilation was very rare. Only three strobilating polyps were observed in the samples, which did not allow assessing ephyrae production. Bonnet et al. (2012) suggested that strobilation of *Aurelia* sp. in Thau lagoon occurs in the late autumn early winter, when temperatures are lower. In our study, strobilating polyps were only observed in the first weeks of the study (early spring), which allow suggesting that during the survey the maturity of the population was possibly in the late transition phase, when polyps allocate the energy from strobilating to budding asexual reproduction.

CONCLUDING REMARKS

Our results provide evidence on the crucial role of man-made constructions in expanding new habitats for polyps development, which together with an extraordinary ability of polyps to colonize a variety of substrata favour the ubiquity of *Aurelia* sp. in Thau lagoon. Furthermore, our results provide support to the expected ocean sprawl influence on jellyfish proliferations. In the Mediterranean Sea this may have a much larger significance than previously considered in order to understand the growing jellyfish outbreaks in the last decades along with the anthropogenic modification of Mediterranean coasts. However, although this study provides important indications of the influence of environmental variables on benthic population dynamics, it failed to provide accurate conclusions, since the survey period was too short. A full year monitoring is thus required to evaluate dynamics of *Aurelia* sp. benthic population.

Perceiving the critical role of polyps in jellyfish outbreaks emergence, understanding the population dynamics and repercussions of ecological interactions on jellyfish benthic populations, such as inter- and intra-specific space and food competition, as well as prey and predator trophic interactions are of greatest importance. Failure to understand the ecology of benthic life stages constraint the understanding on jellyfish blooms and particularly their eventual forecast.

CHAPTER III: DYNAMICS OF *AURELIA* SP. PELAGIC POPULATION

INRODUCTION

The lack of biogeographic studies led to misinterpretation of ecological data describing *Aurelia* spp. (Dawson and Martin, 2001). *Aurelia* spp. populations are recognized as very diverse with contrasting dynamics and life histories, such as abundance, growth, life-span and timing of the different life stages (Lucas, 2001). The current recognition of the existence of several cryptic species of *Aurelia aurita* raised the need for further investigations of their population dynamics. Whether it is an outcome of ecological adaptation of a single species or a result of locally adapted cryptic species (Dawson and Martin, 2001), comparisons of *Aurelia* spp. population dynamics are required to understand jellyfish blooms development. Therefore, smaller spatial scale investigations over large areas should be performed and matched. This jellyfish is the most studied in the world. However, population dynamics studies were mainly restrained to the Northeastern Atlantic and Northwester Pacific. In the Mediterranean, despite the manifested importance of jellyfish populations, such studies are scarce: Thau lagoon (Bonnet et al., 2012); Adriatic Sea and adjacent coastal lagoons (Kogovsek et al., 2012; Malej et al., 2012) and Bizerte lagoon (Chakroun and Aloui-Bejaoui, 1995).

Coastal lagoons offer ideal frameworks to study *Aurelia* spp. population dynamics. These ecosystems are highly valuable but also vulnerable to human activities, such as urbanization, agriculture and land-use, as well as industrial development and shipping (Newton et al., 2014). Thau lagoon is privileged ecosystem to study pelagic population dynamics of *Aurelia* spp., since advection to and from Mediterranean Sea does not occur (Bonnet et al., 2012), allowing to study the influence of environmental conditions without external influences.

Growth is one of the most important characteristics of a species dynamics and of great significance in ecosystem based studies. However, for gelatinous zooplankton a consensus regarding the right methodology for growth estimations have not been achieved, leading to a mix of results unsuitable for proper comparisons within and among species.

Here a survey of *Aurelia* sp. in Thau lagoon is reported. The main aim was to identify the patterns and environmental drivers of *Aurelia* sp. pelagic population dynamics and

compare with previous studies. Additionally, growth was specially assessed and a new methodology of growth estimate is proposed.

METHODS

In situ collections

A plankton sampling program was carried out in Thau lagoon (**Fig. III.1**). Bimonthly samplings were conducted in three stations (St1: 43°25'329N, 3°40'862E; St2: 43°24'644N, 3°36'016E; St3: 43°19'082N, 3°33'532E) from January to December in 2010 and 2011. Afterwards, sampling proceeded in St1 from January 2013 until July 2014.

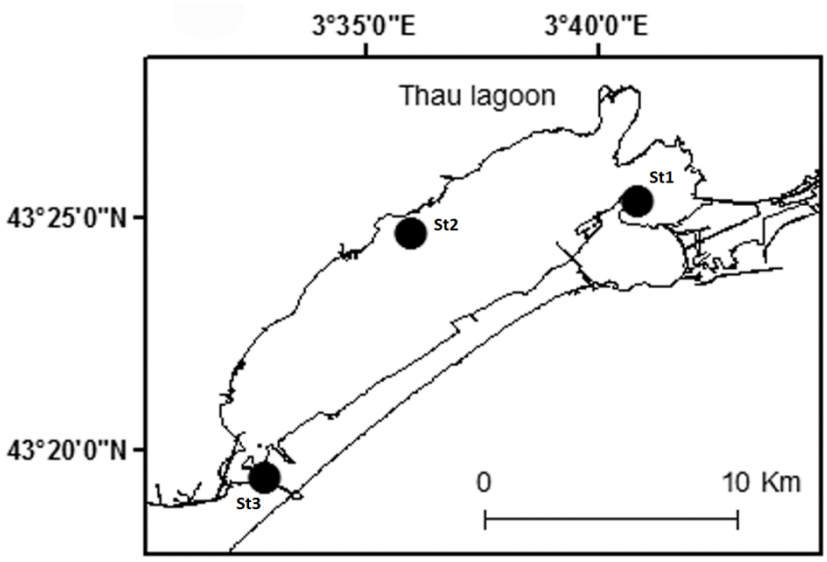

Fig. III.1: Thau lagoon with the indication of the three sampling station (black dots, St1, St2, St3).

Environmental parameters such as temperature, salinity, chlorophyll *a* concentration and zooplankton abundance were monitored.

Temperature and salinity were recorded in sub-surface (<0.5m) with multiparametric probe (EC 300 VWR International/WTW model 350i).

At each sampling station, water samples (150ml) were collected in triplicate in order to quantify chlorophyll *a* concentration. Water was filtered on Whatman GF/F filters and stored at -30ºC. Filters were placed in 12ml tubes with 5ml of 90% acetone. Samples were sonicated and left for 24h at 4ºC for complete extraction. After centrifugation (30min, 3500 rt.min^{-1}, 4°C), 3ml of supernatant were collected and chlorophyll *a* concentration was measured by spectrofluorimetry (LS 50B Perkin Elmer).

Zooplankton was collected near surface by horizontal tows, using a modified WP2 plankton net (1.2 m long, 50 cm opening area and 80 µm mesh size) while a classical WP2 plankton net (2.5 m long, 54 cm opening area and 200 µm mesh size) was used since 2013. All samples were preserved in 4% buffered formaldehyde for further analysis in laboratory. Total zooplankton abundance was determined under dissecting microscope, but only the subsample >200 µm (2010 and 2011) was considered in this analysis, in order to use a homogeneous variable over time.

Gelatinous organisms were collected using a modified Nansen net (100cm aperture, 700µm mesh size) with a plexiglass collector, in order to keep the organisms in good shape. In laboratory, all *Aurelia* sp. individuals were identified. Bell diameter (Bd; cm) and abundance (ind.100m^{-3}) of ephyrae (Bd <1cm) and medusae (Bd >1cm) were determined under dissecting microscope with an eye graticule or a ruler to measure large specimens. Bell diameter was corrected for formalin shrinkage according to **Table III.1** (Möller, 1980).

Table III.1: Coefficient of formalin shrinking adjustment (CA) for each class of *Aurelia* sp. bell diameter (After Möller, 1980)

Bell diameter (cm)	CA
0-0.37	1.182
0.38-0.90	1.168
0.91-2.0	1.264
2.1-5.0	1.274
5.1-11.6	1.399

Analysis of Aurelia sp. growth

Three different stages of *Aurelia* sp. life cycle were identified: ephyrae (Ø <1cm), small medusae (SM: from 1cm until maximum bell diameter) and large medusae (*i.e.* LM: decrease in size after the maximum peak of bell diameter). *Aurelia* sp. growth was calculated using the slope of a regression line, fitted to bell diameter data from all individuals collected, by stage. Growth onset was determined as the date at which the first medusae were recorded in each year.

Data regarding *Aurelia* spp. growth was collected from literature and reviewed (**Table III.7**). Many different growth rate assessments were published leading to incomparable results. Therefore, available data of mean bell diameter were assembled from different studies and growth rates were uniformly re-calculated as previously described. Only SM

phase was considered (*i.e.* bell diameter between 1cm and the maximum reported), and when a continuous period of time was available.

Statistical analysis

Since datasets were not normally distributed, Kruskal-Wallis tests were performed to identify temporal and spatial differences of environmental variables. When testing inter-annual variability of zooplankton abundance in Thau lagoon, only the subset sample comprising organisms > 200µm was considered. Differences of ephyrae and medusae abundances between years were assessed by pairwise comparison using Wilcoxon rank sum test with continuity correction. The same test was also applied to uncover differences between total abundance of ephyrae and medusae for each year.

The test of hypotheses explaining the observed pattern of abundance and bell diameter was done using Linear Models (LMo) or Generalized Linear Model (GLMo) in a factorial mode, including the environmental factors as predictors. This allowed assessing the individual effects and their interactions.

Additional correlations between several variables were also performed using GLMo or LMo:

- Abundance of *Aurelia* sp. (total abundance and by stage, *i.e.* ephyrae and medusa) was correlated with the different environmental variables, considering only the periods of occurrence in order to eliminate the excess of zeros from the data set;
- For each year, growth onset was correlated with different temperature indices, such as mean, cumulative sum and difference between the first and last date (ΔT), over several periods of time potentially influencing growth onset: i) two sample dates before growth onset, ii) period between January and February and iii) period between January and March;
- Mean and individual bell diameter were correlated with total abundance of medusa stage;
- Bell diameter of *Aurelia* sp. was correlated with the different environmental variables.
- Mean growth rate of *Aurelia* spp. inhabiting different habitats (calculated as previously described) was correlated with median and difference of the temperature range presented in **Table III.7**.

All statistical analysis were performed using the software R 3.1.1 (The R Project for Statistical Computing 2014) and Statistica v.10 (StatSoft.Inc) and taking $\alpha<0.05$ as the limit for statistical significance.

RESULTS

Environmental conditions

The annual pattern of temperature follows the normal trend in temperate regions, with lower values in winter months and higher values in summer months (**Fig. III.2**). Temperature ranged from 6.2 to 26.5°C in station 1, 4.6 to 26.7°C in station 2 and 6.4 to 24.7°C in station 3. The maximum and minimum values of temperature in station 2 were observed in 2010 and represent the highest and lowest values among all stations and years.

The general trend of salinity was characterized by higher salinities during winter months followed by decreasing values during spring months, increasing again when temperature rise. This pattern is highly dependent on the low residence time of water masses in the lagoon and the annual trend of precipitation (De Casabianca et al., 1997; Fiandrino et al., 2012). Like temperature, minimum and maximum values of salinity were also recorded in 2010 for station 2 (32.1 and 40.5, respectively).

The general pattern of temperature and salinity were less clear in station 3, which shows higher variability over time. However, either temperature or salinity demonstrated significant differences between stations **(Table III.2B**). Temporal variability was however observed for salinity (**Table III.2A**).

The concentration of chlorophyll *a* and the abundance of zooplankton showed a pattern of high variability over time (**Fig. III.2**). Chlorophyll *a* concentration ranged from 0.2 to 7.7 $\mu g.L^{-1}$, when considering all data set. However in station 3 the concentration was lower, reaching a maximum value of just 3.0 $\mu g.L^{-1}$ in 2011. Significant differences of chlorophyll *a* concentration were found between stations but not between years (**Table III.2**). In contrast, zooplankton abundance, which was assessed only at Station 1, demonstrated to be significant different between years (**Table III.2A and C**). In 2010 zooplankton abundance did not overcome 1102 $ind.m^{-3}$, while in the next years the values were much higher (17 941, 30 470 and 16 990 $ind.m^{-3}$ in 2011, 2013 and 2014, respectively). It is worth noting that, when considering zooplankton abundance <200 µm in 2010 and 2011, zooplankton concentration reached a value of 42 004 and 55 826 $ind.m^{-3}$, respectively.

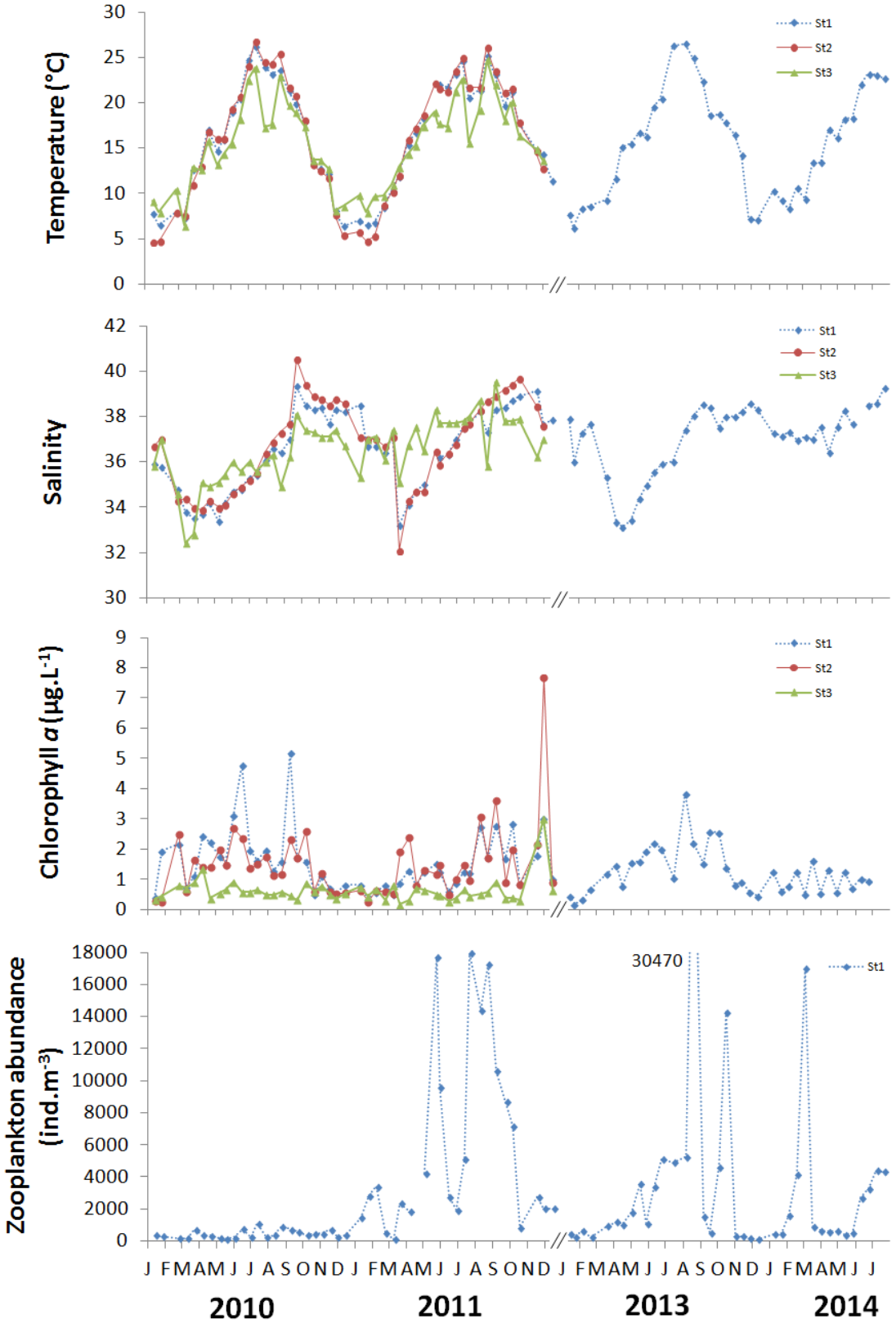

Fig. 3.2: Temporal and spatial (by St) variability of environmental variables in Thau lagoon.

Aurelia sp. abundance

Abundances of ephyra and medusa stages of *Aurelia* sp. are presented in **Fig. III.3**. Ephyrae first appear in November and remain in the water column until early-spring (March-April), when they give rise to adult medusae. Medusae are present until May-June and further disappear until the next generation of ephyrae emerges again. Our results suggest that *Aurelia* sp. life-span ranges from 7 to 8 months (November-May/June). The whole life-cycle of *Aurelia* sp. is considered to occur inside the lagoon, since no advection happens from and to the Mediterranean Sea (Bonnet et al., 2012), and the presence of the benthic population within the lagoon was confirmed (see chapter II).

The abundance of both *Aurelia* sp. pelagic life-stages did not differ significantly between the different stations (**Table III.2B**) and therefore, spatial variability was not further explored.

Maximum annual peaks of ephyrae abundance ranged from 3 ind.100m^{-3} in 2013 to 1472 ind.100m^{-3} in 2014. However, an increasing temporal trend can be observed when excluding 2013 (49, 163 and 1472 ind.100m^{-3} in 2010, 2011 and 2014, respectively), where the maximum abundance was 30 times higher in 2014 than in 2010. Despite such differences, Kruskall-Wallis test did not reveal any significant difference of ephyrae abundance over time (**Table III.2A and C**).

Maximum annual peaks of medusae abundance did not follow the same inter-annual variability as ephyrae abundance. Values were relatively high in 2010, 2011 and 2013 (63, 331 and 75 ind.100m^{-3}, respectively) but medusae were less abundant in 2014 (3 ind.100m^{-3}). Kruskall-Wallis test revealed significant inter-annual variability only when the whole data set (*i.e.* including all stations) was considered (**Table III.2A**).

When comparing the total abundance of ephyrae and medusae for each year, significant differences were not registered, with the exception of 2014, when abundance of ephyrae was significantly higher than of medusae (**Table III.3**).

In order to ascertain what environmental variables influence the total abundance as well as each life stage of *Aurelia* sp., correlations were tested. Results indicate that only the medusae stage was significantly influenced by temperature and zooplankton abundance (**Table III.4**).

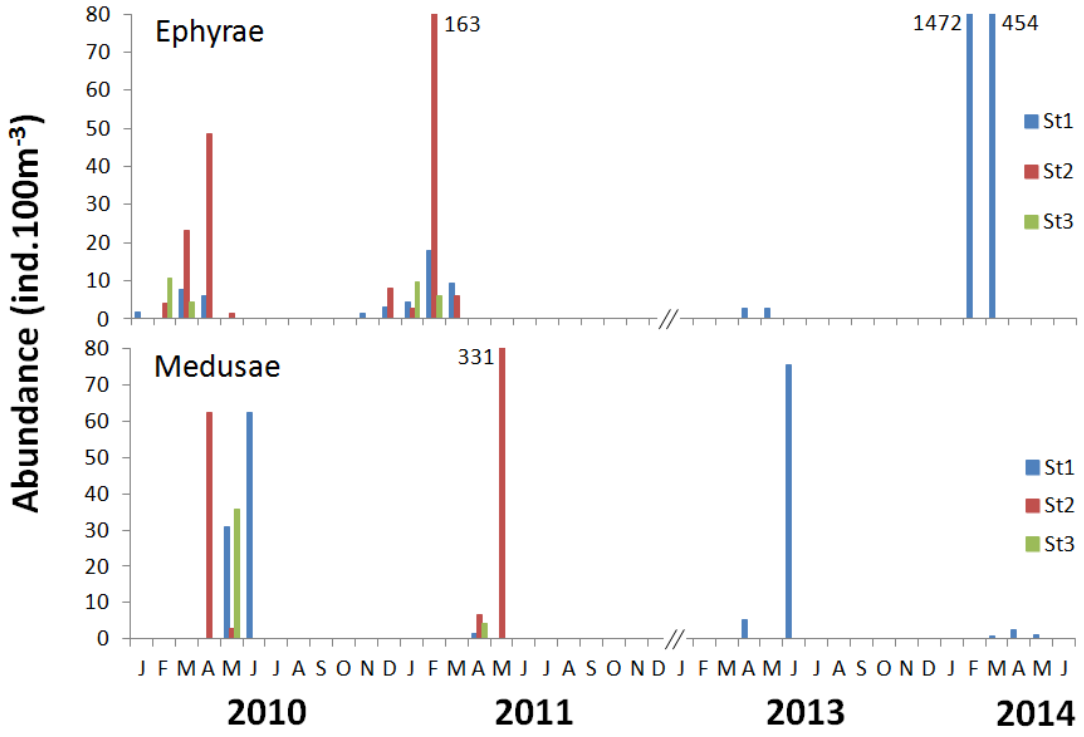

Fig. III.3: Temporal and spatial (by St) variability of Ephyrae (top) and Medusae (bottom) abundance.

Table III.2: Results of Kruskall-Wallis test performed according to different criteria, in order to evaluate significant differences of the environmental and biological variables over space and time. T) Temperature; S) Salinity; Chl a) Chlorophyll *a* concentration; Z) Zooplankton abundance; Eph) Ephyrae abundance; Med) Medusae abundance; St) Station. In bold: significant correlations (*p-value*<0.05).

Criteria	Variable	DF	Test	*p-value*
A) Within the lagoon did it change by year?				
Lagoon	T	3	1.93	0.59
	S	3	16.91	**7.36E-04**
	Chl a	3	2.30	0.51
	Z (>200um)	3	31.26	**7.51E-07**
	Eph	3	5.69	0.13
	Med	3	9.78	**0.02**
B) Within each year did it change by station?				
2010	T	2	0.20	0.91
	S	2	0.65	0.72
	Chl a	2	23.36	**8.44E-06**
	Eph	2	2.97	0.23
	Med	2	0.37	0.83
2011	T	2	0.84	0.66
	S	2	0.08	0.96
	Chl a	2	19.33	**6.36E-05**
	Eph	2	0.20	0.91
	Med	2	0.61	0.74
C) Within each station did it change by year?				

St1	T	3	0.51	0.92
	S	3	6.87	0.08
	Chl a	3	6.26	0.10
	Z (>200um)	3	31.26	**7.51E-07**
	Eph	3	4.96	0.17
	Med	3	6.93	0.07
St2	T	1	0.57	0.45
	S	1	1.30	0.25
	Chl a	1	0.09	0.76
	Eph	1	0.69	0.41
	Med	1	0.00	1.00
St3	T	1	1.16	0.28
	S	1	12.28	**4.58E-04**
	Chl a	1	0.80	0.37
	Eph	1	0.14	0.71
	Med	1	0.39	0.53

Table III.3: Results of Wilcoxon rank sum test to identify significant differences between total (*i.e.* including all stations) ephyrae abundace and total medusae abundance, for each year. In bold: significant correlations (*p-value* < 0.05)

Year	W	*p-value*
2010	27	0.08
2011	21	0.95
2013	0	0.20
2014	25	**0.01**

Table III.4: Correlation coefficient parameters of General Linear Models used to assess correlation between *Aurelia* sp. abundance (by stage and total) and environmental variables. In bold: Significant correlations (*p-value* < 0.05).

Stage	Variable	Estimate	Std. Error	t value	Pr(>|t|)
Ephyrae	(Intercept)	0.00	0.16	0.00	1.00
	Temperature	-0.10	0.19	-0.53	0.60
	Salinity	-0.22	0.18	-1.26	0.22
	Chlorophyll a	-0.01	0.19	-0.05	0.96
	Zooplankton	-0.21	0.17	-1.21	0.24
Medusae	(Intercept)	0.00	0.20	0.00	1.00
	Temperature	1.06	0.41	2.55	**0.03**
	Salinity	-0.08	0.28	-0.30	0.77
	Chlorophyll a	-0.78	0.47	-1.67	0.13
	Zooplankton	1.07	0.39	2.76	**0.02**
Total	(Intercept)	0.01	0.16	0.09	0.93
	Temperature	-0.03	0.20	-0.17	0.87
	Salinity	0.09	0.18	0.50	0.62
	Chlorophyll a	0.06	0.20	0.29	0.78
	Zooplankton	0.29	0.16	1.81	0.08

Aurelia sp. growth

In Thau and Berre lagoons, *Aurelia* sp. population displayed the typical pattern of slow initial growth followed by exponential increase in bell diameter and shrinkage in the end on its life-cycle (**Fig. III.4**). Descriptive values of growth are presented in **Table III.5.** Ephyrae growth showed very low values ranging from 0.004 to 0.08 $mm.d^{-1}$. Bell diameter suddenly increases during SM stage. Maximum growth rate (2.53 $mm.d^{-1}$) was recorded in 2013, which led to a maximum registered bell diameter of 22.38 cm. In contrast, 2014 revealed the lowest values of SM growth rate (0.57 $mm.d^{-1}$) and maximum bell diameter (5.70 cm). Bigger individuals were though observed in Thau lagoon in 2014 but were not collected during our surveys and therefore not included in this analysis. The growth rate of LM, expresses how fast the individuals shrunk at the end of their life-cycle and might reflect the amount of energy they imply in the reproduction process (Lucas, 1996; Hansson, 1997; Aoki et al., 2012). The highest value was registered in 2013 (1.02 $mm.d^{-1}$).

Generalized linear models (**Table III.6**) revealed significant positive correlations between bell diameter and temperature in Thau lagoon ($F=4.79$, p-value < 0.001), suggesting that this environmental variable is responsible for the observed patter of medusa growth.

Growth onset represents the time at which the exponential growth was triggered (**Table III.5 and Fig. III.4**). The results revealed that this transition to adult stage occurs in April. Several temperature indices over different time periods, potentially influencing the growth onset, were tested in order to assess what triggers the exponential growth of medusa. However, significant correlation between growth onset and the different temperature indices were not significant (*p-value* < 0.05; **Table 1** and **2** in Annex).

In order to determine if density-dependent mechanisms regulate the size achieved by *Aurelia* sp. populations, correlations between size and abundance were performed. Mean and individual bell diameter were correlated with total abundance, but significant correlations were not obtained (Linear regression, $r^2=0.13$, $F=2.31$, *p-value*= 0.15 and $r^2= 0.01$, $F= 2.87$, *p-value*= 0.09, respectively).

After growth calculations based on literature data sets (**Table III.7**), correlations between growth and temperature among different habitats, were not significant (Correlation of growth rate with median and difference of temperature of the range correspondent to the growth period; Linear regression, $r^2= 0.02$, $F= 0.18$, *p-value*= 0.68 and $r^2= 0.01$, $F= 0.08$, *p-value*= 0.79, respectively).

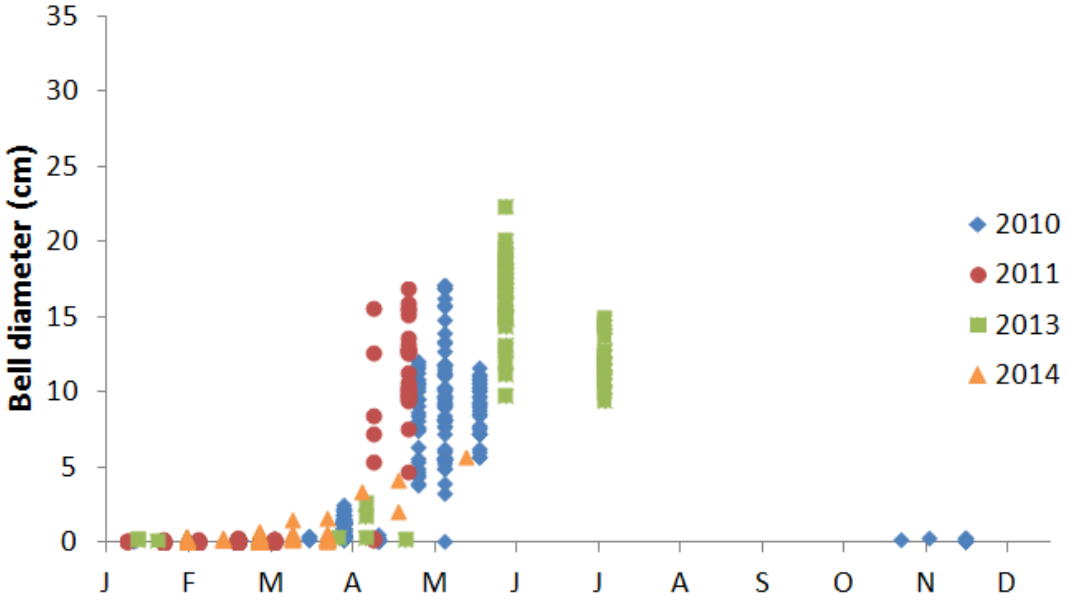

Fig. III.4: Bell diameter of all *Aurelia* sp. individuals in Thau lagoon during the study period.

Table III.5: Growth rate (by growth stage; SM: small medusae, LM: large medusae), maximum registered bell diameter (Max. Bd) and growth onset of *Aurelia* sp. for each year in Thau lagoon. The number of individuals used to calculate growth rate is represented by (n).

Year	Growth rate ($mm.d^{-1}$)			Max Bd (cm)	Growth onset (Julian days)
	Ephyrae (n)	SM (n)	LM (n)		
2010	0.08 (115)	2.02 (131)	- 0.56 (92)	17.07	98
2011	0.004 (158)	1.33 (14)	-	16.93	110
2013	0.01 (6)	2.53 (31)	- 1.02 (46)	22.38	107
2014	0.02 (350)	0.57 (6)	-	5.70*	77

* Larger medusae were observed in 2014 (7-8cm) but were not included in the analysis.

Table III.6: Estimates of Generalized Linear Models used to assess correlation between bell diameter and environmental variables. In bold: Significant correlations (*p-value* <0.05).

Variable	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.04	0.11	-0.35	0.73
Temperature	0.65	0.13	4.79	**<0.001**
Salinity	0.08	0.14	0.58	0.57
Chlorophyll *a*	0.17	0.15	1.15	0.26
Zooplankton	-0.07	0.12	-0.60	0.56

Table III.7: Literature review of *Aurelia* spp. maximum abundances, size and growth rate. Maximum abundances (Max. ab.) of ephyrae and medusae, as well as maximum bell diameter (Bd) represent the highest reported values. Growth rate was calculated based on the slope of a regression line fitted to the assembled reported values of mean bell diameter over time (only SM phase was considered, *i.e.* > 1cm and < maximum men bell diameter). The number of available data (n) used to calculate growth rate per year is also presented. Temperature range (T°C) is correspondent to the SM phase used to calculate growth rate. References: 1) Riisgard et al., 2012; 2) Olesen et al., 1994; 3) Lucas and Williams, 1994; 4) Lucas, 1996; 5) Moller, 1980; 6) Schneider, 1989; 7) Ishii and Bamstedt, 1998; 8) Van Der Veer Oorthuysen, 1985; 9) Lo and Chen, 2008; 10) Toyokawa et al., 2000; 11) Uye and Shimauchi, 2005; 12) Miyake et al., 1997; 13) Aoki et al., 2012; 14) Bonnet et al., 2012; Ts) This study; Ud) Unpublished data. N.A.) Not available.

Region	Location	Max. ab. ephyrae ($ind.m^{-3}$)	Max. ab. medusae ($ind.m^{-3}$)	Maximum Bd (cm)	Growth rate ($mm.d^{-1}$)	n	Months (years)	T°C	Ref.
Northeastern Atlantic	Limfjorden, Denmark	2.2 ± 1.4	14.8 ± 0.8	12.9 ± 3.4	0.63 - 1.14	3-4	Apr-Jul (2009)	20.5-22	1
	Kertinge Nor and Kerteminde Fjord, Denmark	304 ± 129	248 ± 292	5.4	0.40-0.56	4-7	Jun-Sep (1991) May-Jun (1992)	N.A.	2
	Southampton Water, UK	8.71	2.80	14.1	0.42-2.37	3-6	May-Jun (1990,91,93) Mar-May (1992)	10-16	3
	Horsea Lake, UK	-	24.90	10.5	0.19	7	Mar-Sep (1994)	5.8-18.6	4
	Kiel Bight, Germany	0.07	0.16	44	1.9-2.4	3-4	May-Aug (1978) Jun-Aug (1979)	N.A.	5
	Kiel Bight, Germany	-	0.23	ca. 30	2.20-3.05	6-7	May-Aug (1982,84) Apr-Aug (1983)	11.4-16.7	6
	Vågsbøpollen, Norway	-	22.00	11.8	1.24	6	May-Jun (1996)	11-15	7
	Wadden Sea, Netherlands	-	0.49	29	1.75-3.38	6-13	May-Jul (1981) Apr-Aug (1982)	N.A.	8
Northwestern Pacific	Tapong Bay, Taiwan	328.00	14.50	29.7	0.80	5	May-Oct (2001)	27.5-33	9
	Tokyo Bay, Japan	2.40	1.60	28.4	1.0-1.4	3-8	Apr-Aug (1991) Mar-May (1992)	12-25	10
	Inland Sea, Japan	-	-	27.3 ± 4	2.02-2.56	4-5	Apr-Jul (1990, 91) May-Jun(1992)	15-25	11
	Kogoshima Bay, Japan	-	-	23	1.38	6	Fev-Jul (1994)	15-28	12
	Mikawa Bay, Japan	-	0.91	32.6	0.96	4	Mar-Jul (2008)	11.6-22	13
Mediterranean Sea	Thau lagoon, France	2.00	3.30	11.33	2.45	4	Apr-May (2010)	13-16	14
	Thau lagoon, France	14.70	3.30	22.38	0.59-2.35	2-4	Apr-May (2010,11) Apr-Jun (2013) Mar-May (2014)	13-20	Ts
	Berre lagoon, France	0.43	0.18	17.00	1.68 - 2.22	2-3	Mar-May (2010) Apr (2011)	7-17	Ud
	Bages-Sigean lagoon, France	-	0.07	31.90	2.66-2.67	2	May-Jun (2010) Jun-Jul (2011)	14-28	Ud

DISCUSSION

Environmental conditions

The environmental conditions in Thau lagoon are typical from temperate regions, with clear seasonality. Temperature fluctuates between high values in summer and low values in winter, while salinity appears to be highly influenced by precipitation, rivers flow and water masses residence time, with lower values during spring season (De Casabianca et al., 1997; Fiandrino et al., 2012). Chlorophyll *a* was the only environmental variables with spatial variability in the lagoon, revealing lower values in Station 3, which is characterized by shallower waters and higher residence time of water masses. However, since *Aurelia* sp. abundance did not show significant differences between stations, it suggests that the population is not influenced by the particular characteristics of each station and spatial variability was not further explored. Although zooplankton abundance was monitored in just one station, we therefore consider that the annual trend in St1 is representative of the entire lagoon. Inter-annual variability was however recorded for salinity and zooplankton abundance (>200 µm), which might have affected the dynamics of *Aurelia* sp. population.

Environmental windows

Overall, the temperature window differed between life-stages. While ephyrae was present between 8-13°C, medusae occurred within a higher range of temperatures (13-19.5°C). Such results underline the important influence of this variable in the life-cycle of this gelatinous species. Salinity however, appears to cause little influence, since both life-stages were present within similar window (33.5 – 37). Outside of Mediterranean Sea, comparable temperature and salinity windows with Thau lagoon were reported in Southampton Water (Lucas and Williams, 1994). Though, the ecological niche of *Aurelia* spp. in different habitats around the world should take into account its biogeography (Dawson and Martin, 2001). For instance, the Northestern Atlantic appears to be inhabited by *Aurelia aurita* (Dawson et al., 2005; Ki et al., 2008), while in the Mediterranean, several cryptic species are present under similar climate variability, such as *Aurelia sp.1* in France (Dawson, 2003; Dawson et al., 2005), *Aurelia sp.5* in Veliko Jezero (Dawson and Jacobs, 2001; Dawson et al., 2005; Ki et al., 2008), *Aurelia sp.8* (Dawson et al., 2005; Malej et al., 2012) as well as *Aurelia sp. 7* (Ki et al., 2008) in the Adriatic Sea. Therefore, differences of ecological niches used by different

populations of *Aurelia* spp. might reflect genotypic variation among them, which should be considered in further ecological studies.

Occurrence and annual cycle

The annual cycle of *Aurelia* sp. in the Northwestern Mediterranean coastal lagoons appear to follow the typical pattern of temperate populations. *Aurelia* sp. is univoltine in Thau lagoon, with a life-span ranging from 7 to 8 months. Ephyrae first appear in early winter giving rise to adult individuals in the beginning of spring, when temperature rises. Medusae remain in the lagoon until late spring disappearing during summer and autumn. Such life-span and annual cycle was previously reported by Bonnet *et al.* (2012) and confirmed during our study. Univoltine populations with similar annual cycle have been reported elsewhere (reviewed in Lo and Chen, 2008). For instance, as in Thau, strobilation begin in November in Northern Adriatic (Malej et al., 2012) and in Kiel Bight (Germany), entering in the medusae stage in April (Moller, 1980). Strobilation is later in Wadden Sea (Netherlands; Van der Veer and Oorthuysen, 1985), in Southampton Water (UK; Lucas and Williams, 1994) and in Inland Sea (Japan; Uye and Shimauchi, 2005), realeasing ephyrae in January-March but growing to adult stage in similar periods. In all the latter reports medusae reach their maximum size in June-August and disappear in late Summer/Autumn, suggesting a life-span concurrent with our results. Though, a great variability of population dynamics of *Aurelia* spp. is recognized. For instance, medusae might be present all year round in an Adriatic lagoon (Veliko Jezero, Kogovsek *et al.*, 2012) as well as in Tokyo Bay (Japan), Black Sea or Tapong Bay (Taiwan) (Omori et al., 1995; Mutlu, 2001; Lo and Chen, 2008) and different generation may overlap (Lucas, 1996; Lo and Chen, 2008).

Abundance

Abundance appears to be generally higher in enclosed and semi-enclosed ecosystems than in open environments (Ishii and Bamstedt, 1998). During our study, maximum abundances recorded in Thau lagoon were higher than in open water environments, such as Kiel Bight (Moller, 1980; Schneider, 1989) and Tokyo Bay (Toyokawa et al., 2000), but did not reach the high values reported for other semi-enclosed habitats, such as in Denmark and Taiwan (Olesen et al., 1994; Lo and Chen, 2008), where the values overpass 300 ind.m^{-3} (**Table III.7**). Still, Thau lagoon sustains an abundant pelagic population of *Aurelia* sp. (**Fig. III.3**).

When assessing the environmental influence on *Aurelia* sp. abundance, significant correlations were only recorded for medusa stage, indicating that higher temperature and food availability promote the increasing numbers of medusae in the water column. The abundance of *Aurelia* spp. medusae is totally reliant on the ephyrae production by polyps and its recruitment success (Hernroth and Grondahl, 1985; Lucas et al., 2012). Although temperature and food availability appear to have a fundamental role in the survivorship of medusae, the abundance of the adult stages depends primarily on the benthic population dynamics and the environmental drivers acting at this stage (Boero et al., 2008). Very few investigations have focused on this issue (e.g. Miyake *et al.*, 2002; Toyokawa *et al.*, 2011; Willcox *et al.*, 2008), though, increasing coastal constructions were showed to promote the development of polyps population (Holst & Jarms, 2007; Hoover & Purcell, 2009; Makabe et al., 2014). This appears to be the case in Thau lagoon, where polyps are well established (see Chapter II) and high abundances of *Aurelia* sp. were recorded.

The recruitment success was assessed by opposing the abundance of both life stages in each year. Differences were only noticed in 2014, where ephyrae appear to be more abundante than medusae. Such results may be explained by physical or ecological processes. Advection was previously pointed as a source of dispersion or agglomeration of gelatinous organisms, as a consequence of their low swimming capacity (Ishii and Bamstedt, 1998; Riisgård *et al.*, 2012; Toyokawa *et al.*, 2000; Van der Veer and Oorthuysen, 1985; reviwed in Graham *et al.*, 2001). Bearing in mind that in 2014 only one station was monitored, we hypothesize that the highly influential wind events in the lagoon (Fiandrino et al., 2012), might explain the absence of medusae in St1 during this year. However, such results can be also an outcome of mortality. Recent evidence suggest that predation in early life stages of *Aurelia* spp. have been underestimated and might have an important role in shaping medusae populations (Takao et al., 2014), which is further supported by our investigations (see Chapter IV).

Growth

Growth is one of the most important indicators of population dynamics and is usually assessed in *Aurelia* spp. studies (*e.g.* Olesen *et al.*, 1994; Uye and Shimauchi, 2005; Van der Veer and Oorthuysen, 1985). However, in jellyfish literature growth rate calculations are heterogeneous hampering accurate comparisons within species and even less between species. Many researchers calculate growth based on changes in bell

diameter over time (Moller, 1980; Lucas and Williams, 1994; Palomares & Pauly, 2009), on wet weight (Hansson, 1997; Toyokawa et al., 2000; Uye and Shimauchi, 2005; Aoki et al., 2012) or dry weight (Lucas, 1996; Ishii and Bamstedt, 1998). Jellyfish growth assessments must be carefully designed, since their characteristic high water content might conceal the real growth. Body weight has been showed as variable depending on salinity conditions, biasing growth estimations performed on the basis of weight (Hirst and Lucas, 1998). Instantaneous specific growth rates, commonly applied in the literature, based on average body size within a population over time does not give independent values (Hansson, 1997). According to the latter author, the accuracy of a size estimate at one sampling occasion affects the estimate of instantaneous specific growth rate for both the preceding and the succeeding time interval. Therefore, our growth rates were estimated based on a regression line fitted to bell diameter from all the individuals, in order to avoid such sources of error. The same should have been performed with data from the literature. However, the entire data set of bell diameter is typically not available. Therefore, data sets of mean bell diameter of SM phase over time were assembled and calculations were performed uniformly, allowing accurate comparisons (**Table III.7**).

The growth pattern of *Aurelia* sp. in Thau lagoon follows what was previously reported for *Aurelia* spp. by other authors (e.g. Lucas and Williams, 1994; Olesen *et al.*, 1994; Schneider, 1989; Uye and Shimauchi, 2005). After ephyrae release, growth remains low until the growth onset, after which bell diameter increases dramatically until attaining their maximum size. Afterwards, a reduction of the bell diameter was observed for some years (2010 and 2013). In line with the previously described pattern, growth rates were calculated for three different periods: Ephyrae, SM and LM (**Table III.5**).

Ephyrae growth did not overpass 0.08 $mm.day^{-1}$, which is in accordance with previous reports (0.1 $mm.d^{-1}$) (Lucas and Williams, 1994). The growth of ephyrae was shown to be dependent not only on food availability, but also on prey size, quality and behaviour (Sullivan et al., 1997; Båmstedt et al., 2001; Riisgård and Madsen, 2011) which together with temperature are the main drivers of ephyrae growth (Båmstedt et al., 2001).

The transition between ephyrae to young medusa stage, *i.e.* growth onset, occurred in the beginning of the spring season (*i.e.* April). However, the reason of the specific timing for the growth onset remains unclear. It would be expected to register earlier growth onset in years of higher winter temperature, but such relationship was not

obtained in our results, suggesting that other factors, in addition to temperature, regulate the timing of the growth onset.

When considering the SM inter-annual variability was recorded, with higher growth rates promoting bigger individuals (**Table III.5**). Such inter-annual variability was also previously reported elsewhere for different populations of *Aurelia* spp. (*e.g.* Moller, 1980; Schneider, 1989; Van der Veer and Oorthuysen, 1985). Among different habitats, comparable values of growth with Thau were obtained for Southampton Water, Wadden Sea and Berre lagoon (**Table III.7**). It is worth noticing that Bages-Sigean, a geographically near semi-enclosed lagoon, under similar climate variability, presented much higher growth rate values and one of the biggest individuals of *Aurelia* spp. ever reported. Overall, in the Northeastern Atlantic, *Aurelia* spp. appears to attain smaller sizes than in the Northwestern Pacific, with Mediterranean populations lying in between. However, this is not straightforward. For instance, the largest *Aurelia* spp. individual reported in the literature was found in Mljet Island (Mediterranean Sea) reaching 55 cm diameter (Benovic et al., 2000). Furthermore, extreme values of maximum individual size may occur in a very small geographic area, *e.g.* in Kertinge Nor (5.4cm) (Olesen et al., 1994) and Kiel Bight (44cm) (Moller, 1980), suggesting that general climate variability does not explain solely the difference in the growth of *Aurelia* spp..

In our study, temperature was considered the main driver of growth. Matching results were previously reported for *A. aurita*, when food availability is not limited (Hansson, 1997). As for all zooplanktonic organisms though, temperature alone does not explain the different growth rates observed in different habitats, as further demonstrated by the lack of significant correlation between temperature and growth calculated from the values in **Table III.7**. Ishii and Bamstedt (Ishii and Bamstedt, 1998) showed for the first time that food availability can explain the growth and the maximum size of *Aurelia aurita*, as also suggested by several authors (Olesen et al., 1994; Lucas, 1996). Still, further importance must also be given to food quality. According to Båmstedt *et al.* (2001) *A. aurita* may achieve proper development based on the most abundant prey, but bigger size range and higher nutritional quality promotes higher growth rate and larger individuals. Since diversity of zooplankton community was not assessed during our study and significant correlations between food availability and SM growth were not obtained, the role of prey abundance and quality is speculative. However, we conjecture that this variable has an important role in shaping the size spectrum of *Aurelia* sp.

pelagic populations in Thau lagoon. Consequently the pressure of bigger medusae predation on the zooplankton community might result in a great reduction of their abundance (*ca.* 80% Bonnet et al 2012), generating a cascade effect, defining the ecological quality of the lagoon.

The decreasing of bell diameter during LM stage is well known (Moller, 1980; Van der Veer and Oorthuysen, 1985; Lucas and Williams, 1994), but usually not quantified. The shrinkage of *Aurelia* spp. is largely associated with releasing of planulae, representing the input of energy in the production of gametes and in the spawning process, which results in morphological degradation and death of adult medusae (Lucas, 1996; Hansson, 1997; Aoki et al., 2012). In Thau lagoon, larger individuals appear to shrink faster, which is in accordance with values registered in Bages-Sigean lagoon, where individuals with *ca*. 32 cm shrank with a rate of up to 5 $mm.d^{-1}$ (Bonnet, D; unpulished data). Nevertheless, the reasons for such relationship remain unclear.

Further research

As previously discussed, jellyfish growth rates have been calculated by direct measurement methodology. However, new techniques are now available offering potential suitable methods for jellyfish growth assessments, potentially overcoming the flaws of the currently used methods. For instance, RNA:DNA ratios and RNA content were previously used for zooplankton growth quantification (*e.g.* Wagner et al., 2001), but is the methods based on enzyme activities that are attracting the attention of researchers as potential indices of growth, since some enzyme activities are directly related to protein synthesis (*e.g.* Berges et al., 1990). For example, evaluating Aminoacyl-tRNA synthetases (AARS) activity is pointed as proper index of growth in zooplankton (Yebra and Hernández-León, 2004) and has been successfully employed with some organisms (e.g. *Calanus helgolandicus* and *Calanus finmarchicus*) (Yebra et al., 2005, 2006). To our knowledge, biochemical methods were rarely used to assess growth of gelatinous organisms (*e.g.* Morais et al., 2015), but could provide promising methodologies capable of great use in jellyfish research field.

CONCLUDING REMARKS

Here we provide new insights on the pelagic population dynamics of *Aurelia* sp. in Thau lagoon, presenting evidences of the impact of environmental condition on reproductive strategy, growth and life histories. Our results highlight the plasticity of

Aurelia spp., able to inhabit a great variety of habitats culminating in assorted life histories. However, further local to regional investigations, complemented with molecular phylogenetic studies, at a global scale, are still required. Such investigations will allow to understand the relative role of phenotypic plasticity and genotype in governing population response to the ambient environments, and consequently to environmental changes. Considering the current future climate predictions, the lack of diagnosis regarding the environmental drivers of pelagic ecology, limit the understanding of jellyfish blooms development and particularly their eventual forecast.

CHAPTER IV: FISH PREDATION ON *AURELIA* SP. IN THAU LAGOON

INTRODUCTION

To a large extend, research on jellyfish dynamics have focused on factors driving blooms, while little is known in regard to drivers of jellyfish mortality, which is fundamental to understand their population dynamics. In particular, mortality during the early life stages may have a major effect on recruitment and abundance of the adult population (Lucas, 2001). For instance, predation on polyps and ephyrae is suggested as a control mechanism of adult jellyfish (*e.g.* Ishii *et al.*, 2004; Takao *et al.*, 2014).

Jellyfish were long described as 'dead ends' of the food webs, as a result of their high water content and low nutritional value (*e.g.* 2.3-3.6 KJ.g.dry mass^{-1} of *A. aurita* compared with 20.71±0.95 KJ.g.dry mass^{-1} of squids; Doyle et al., 2007 and references herein). However, jellyfish are an important source of food for several organisms and, although sparse, some fish species prey strictly on jellyfish (e.g. moon fish, *Mola mola* and butterfish, *Peprilus triacanthus*) (Arai, 1988, 2005; Ates, 1988; Mianzan et al., 1996; Purcell & Arai, 2001). Several organisms were also reported as important jellyfish consumers, such as mollusks, arthropods, birds and reptiles (Arai, 2005), and omnivorous fishes, commonly present in coastal lagoons (Blanco et al., 2003), benefit from the most available prey type, as for *Sparus aurata* (Pita et al., 2002; Escalas et al., 2015). Indeed, predation by a large number of fish species with broad diets was pointed as more ecologically important than the predation by the relatively few specialized fishes with primarily gelatinous diets (Purcell & Arai, 2001; Arai, 2005). In addition, reports indicate that in periods of massive proliferation, jellyfish are a non-negligible source of energy (Arai et al., 2003; Arai, 2005; Cardona et al., 2012), although accurate estimations on the impact of fish predation on jellyfish are still scarce. The above examples suggest that the trophic relationship between fish and jellyfish is likely more important than previously though. Hence, in this context the removal of large biomass of competitors and predators through overfishing may greatly promote favorable conditions for the increase of jellyfish abundance (Purcell et al., 2007; Richardson et al., 2009; Purcell, 2012) and consequently the more specialized predators might be favoured from the increasing food availability (**Fig. IV.1**; Boero, 2013).

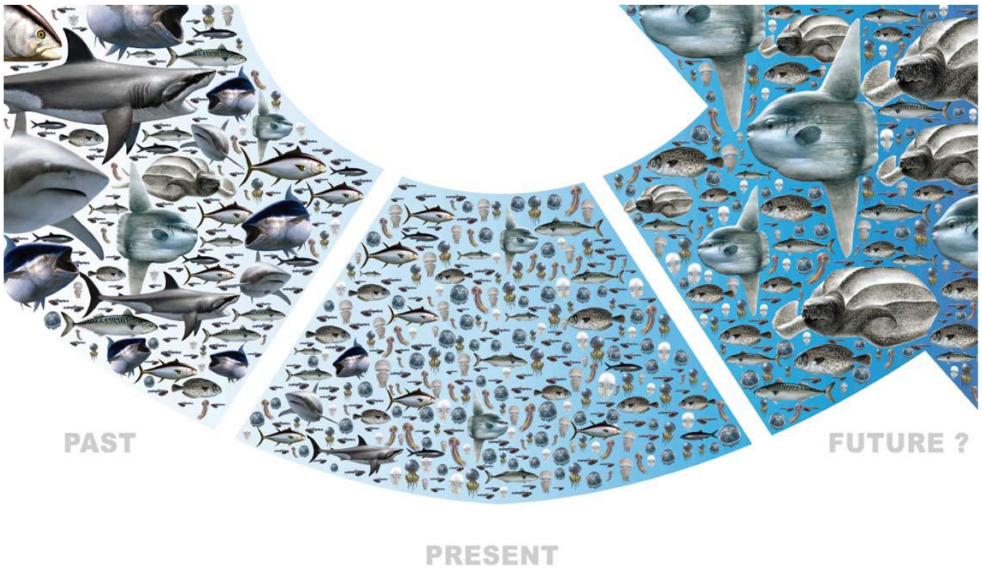

Fig. IV.1: Past, present and future scenario of global ecosystem state, highlighting the decrease of large fish, which release jellyfish from predation and competition, favouring jellyfish populations, which in return may benefit their predators in the future (After Boero, 2013).

Resolving trophic relationships is fundamental to understand pathways of energy transfer in food webs. A method commonly used is stomach content analysis, which may provide biased information giving excessive importance to hard preys that are more resistant to digestion (Hyslop, 1980) and therefore novel methodologies are required. Among these are the molecular techniques, which are capable of identifying digested preys, thereby improving the accuracy of predators diet assessments (Symondson, 2002). For instance, jellyfish are digested rapidly and preservative methods often destroy or shrink the gelatinous material, which results in underestimations of jellyfish consumptions and trophic relationships. Therefore, jellyfish predator's diversity might be higher than acknowledged (Ates, 1988; Purcell & Arai, 2001; Arai, 2005).

In this chapter a combined approach of molecular field measurements and laboratory experiments were used to assess fish predation on jellyfish. Molecular techniques were used to identify diversity of wild fish predators in Thau lagoon. In addition, laboratory experiments were run to assess direct predation impact of *Sparus aurata* on different food sources, to assess the effect of prey concentration and the selectivity of *S. aurata* between gelatinous and non-gelatinous prey item.

METHODS

The potential fish predation on *Aurelia* sp. was investigated through a dual approach, involving both *in situ* investigations and experimental studies. Molecular techniques were used to detect the potential ingestion of this jellyfish by various fish species in Thau lagoon (**Fig. IV.4**). In addition, laboratory experiments were carried out using a Mediterranean fish of high economical value (the gilthead seabream *Sparus aurata*) to assess the potential level of predation by its juveniles and adults on different life stages of *Aurelia* sp., and the respective attractiveness of these latter as food sources, when compared to various other potential types of aquaculture feeds (pellets and live prey of suppose different nutritional quality).

Natural fish predators of *Aurelia* sp. in Thau Lagoon

Sampling

Potential fish predators were sampled with the help of professional fishermen, between May and July of 2012 and 2013, which corresponds to the season of *Aurelia* sp. bloom events (Bonnet et al 2012, see also Chapter III). Immediately after collection, individuals were measured (TL in mm), weighted (W in g) and placed in separate plastic bags filled with ethanol 70%, in order to avoid possible loss (or mixing) of fish gut contents during sampling. Bags were stored in individual containers. Once in the laboratory, fishes were dissected and their gut contents were collected and preserved at -30°C until DNA extraction. In addition, to obtain *Aurelia* sp. genetic signature for control and to verify if the amplified gene is present in all life stages, polyps, ephyrae, juvenile and adult medusae of *Aurelia* sp. were collected. Samples were preserved in absolute ethanol and the medusa stage was additionally preserved in sea water, as a precautionary measure, in the case of the ethanol preservation was unsuccessful.

DNA extraction and amplification

Before DNA extraction, ethanol was removed from *Aurelia* sp. samples by washing them with pure molecular grade water. After thawing, all samples were mechanically grinded in a mixer mill MM400 (Retsch). Genomic DNA was extracted from 25 mg (wet weight) each sample of *Aurelia* sp. and 25 mg of each sample of fish gut contents. DNA extraction followed the protocol of Stopar *et al.* (2010), using *DNeasy blood and tissue kit* (QIAGEN). The extracted DNA was quantified under *Nanodrop* (NanoDrop ND-1000 Spectrophotometer, Thermo Scientific). Two gene fragments were amplified

by PCR reaction: partial sequences of mt-16S rDNA (primer pair AS3) and mt-COI (primer pair AC3) (Wang et al., 2013). **Table IV.1** presents the primers pairs for each gene fragment.

Table IV.1: Sequence and fragment length of primer pairs used for PCR amplification.

Gene	Primer name	Sequence (5' - 3')	Fragment length (bp)
mt-16S rDNA	AS3-F AS3-R	ATTGGTGACTGGAATGAATG TATGACAGCCCTTAGAGTTC	245
mt-COI	AC3-F AC3-R	TCTCTGCTATGGTAGGAACT CCAGTACGGATCATACGAAT	495

All PCR amplifications were performed using *PCR kit illustra puretaq ready to go* (GE Healthcare), with 50 to 100 ng of genomic DNA, 0.5µM of each forward and reverse primers (Eurofins Genomics) and 22.5µl of molecular grade PCR water (5 Prime). The thermal profile for all PCR reactions was composed by 3min at 95°C, 35 cycles of 1min at 95°C, 1min at 48°C and 90sec at 72°C, followed by 1min at 72°C (Stopar et al., 2010; Ramšak et al., 2012). The products of PCR reactions were analyzed through electrophoresis (Mupid-exU; Advance) at 100V for 30min. An aliquot of 5-7µl samples were load on Agarose gel 1.5%, using loading buffer 6X (Euromedex), *SybrgreenI* staining (Invitrogen) and 1Kb DNA ladder (Euromedex). Gels were visualized and photographed on UV table using GelDoc XR system (Biorad).

Laboratory investigations of fish predation on *Aurelia* sp.

Sparus aurata as biological model

The gilthead seabream (*Spaurus aurata*) is a prominent species in Thau lagoon and the main target of the commercial fishery in the lagoon, representing at times up to 56.6% of total annual 'capéchade' catches (the most common fishing gear in Thau lagoon) (Crespi, 2002). Irrespective of age, most of gilthead seabream spend winter months at sea, where spawning occurs, and return to coastal lagoons around April (Audouin, 1962; Mercier et al., 2012). Although some adults spend significant amount of time in coastal lagoons (Mercier et al., 2012), in Thau Lagoon the population of *S. aurata* is composed mainly by juveniles under maturity age (2-3 years, 27-33cm length; Lasserre, 1974 *in* Crespi, 2002). Moreover, gilthead seabream has been raised for decades in aquaculture farms worldwide and its life history is therefore well known (*e.g.* Moretti et al 1999).

All these criteria made it a particularly good candidate to assess the potential role of jellyfish as, at least, occasional food source for opportunistic fish species.

Laboratory experiments

The experiments were performed at the IFREMER institute (Institut Français de Recherche pour l'Exploitation de la MER) in the research station of Palavas-les-Flots (France), from April to June 2014. A total of 433 *S. aurata* were obtained from the 'Les Poissons du Soleil' aquaculture farm. Two age classes were used in the experiments: 370 individuals with about 1 year old (W of ca. 70g; TL= 14-19 cm), hereafter named generation 1 (G1) and 63 individuals with 2 years old (W of ca. 200g; TL= 20-25cm) hereafter named generation 2 (G2). Fishes were acclimated in three 1500L tanks filled with filtered sea water at 20-22°C. Every two days, fishes were fed with commercial dry pellet food for sea bream (B-Nature, Le Gouessant) at 1% of the fish biomass to meet their food requirements.

All prey items were kept at 20±0.5ºC. Live *Artemia* with about 1cm length were collected in soft flats in Le Grau-du-Roi and maintained in 60L tank with air supply. Live *Aurelia* sp. ephyrae and small medusae (Ø 1cm) were obtained from 'Jellyfish Concept' company (Cherbourg, France) and maintained in 15L containers with air supply, fed with newly hatched *Artemia*. Medium and large *Aurelia* sp. medusae (Ø 4 and 7-8cm, respectively) and colonies of *Aurelia* sp. polyps were collected in Thau Lagoon. The first ones were collected with hand nets, while polyps, fixed on oyster or mussel shells, were collected by SCUBA divers. They were all maintained in 60L tanks with air supply.

The experiment set up was composed by 24 separate tanks of 60L (40L of sea water) with shared water and air supply, which ensure identical temperature and salinity in all tanks (**Fig. IV.2**).

Fig. IV.2: Experimental set up, showing the tanks, water and air supply, at IFREMER facilities.

Treatments were performed at 20±0.5ºC, since it is within the optimum temperature range for *S. aurata*, avoiding thermal biochemical stress (Feidantsis et al., 2009), and it also corresponds to temperatures at which blooms of *Aurelia* sp. occur in Thau lagoon (Bonnet et al., 2012). Photoperiod was determined according to field conditions at the time of experiment (13h of light and 11h of dark period). As the maximum fish biomass recommended in *S. aurata* aquaculture farms is of 7 $kg.m^{-3}$ (C. Pryzbyla, personal communication), all experiments were performed with three G1 fishes or two G2 fishes per tank. Before each experiment, fishes were acclimated for four days in experimental tanks and maintained in starvation to ensure that all individuals empty their stomachs.

Three experiments were performed, using three replicates for each treatment: monospecific diets, gradient of concentration and selectivity experiments (**Table IV.2**).

Table IV.2: Initial concentration of prey ($tank^{-1}$ and L^{-1}) provided in the tanks during the different experiments, for G1 and G2 fishes. Concentration of polyps represents the mean concentration of three replicates except for some treatments where one replicate was eliminated, as a result of polyps counting errors (*).

		G1 (n= 3 per tank)		**G2 (n= 2 per tank)**	
Experiment	**Prey type**	**prey item.tank^{-1}**	**prey item.L^{-1}**	**prey item.tank^{-1}**	**prey item.L^{-1}**
Monospecific diets	Dry pellets	44	1.10	80	2.00
	Artemia	44	1.10	80	2.00
	Polyps*	390	9.75	448	11.20
	Ephyrae	44	1.10	80	2.00
	Small Medusae (Ø1cm)	50	1.25	50	1.25
	Medium Medusae (Ø4cm)	1	0.03	1	0.03
	Large Medusae (Ø7-8cm)	1	0.03	1	0.03

Gradient of concentration	Medusae (Ø1cm)	5	0.13	5	0.13
	Medusae (Ø1cm)	10	0.25	10	0.25
	Medusae (Ø1cm)	15	0.38	15	0.38
	Medusae (Ø1cm)	30	0.75	30	0.75
	Medusae (Ø1cm)	40	1.00	40	1.00
	Medusae (Ø1cm)	50	1.25	50	1.25
Selectivity	Ephyrae + Artemia	22 + 22	0.55 + 0.55	40 + 40	1+ 1
	Polyps* + Artemia	215 + 22	5.38 + 0.55	400 + 40	10 + 1

Monospecific diets

The main goal of the monospecific diets experiment was to compare *S. aurata* ingestion rates for the various life stages of *Aurelia* sp. (polyps, ephyrae and different sizes of medusae) with those for other high quality types of food, live or not. As *S. aurata* were acquired from aquaculture farms, dry pellets were used as control, since fishes were previously reared with this diet. The comparison of the different prey items was performed according to the concentration of prey per tank. Additionally, small, medium and large medusae were also compared according to their weight.

The concentration of prey items in each treatment was calculated in order to meet 1% of fish biomass in each tank, calculated based on the weight of control food type: in the treatments with 3 G1 and 2 G2 fishes in each tank, the correspondent weight of dry pellets was 2.1g and 4g, respectively, which match with 44 and 80 pellets.tank^{-1}, respectively. The same concentrations were then used for *Artemia* and ephyrae, since they have equivalent dimensions (0.7-1cm).

Small, medium and large medusae were provided at different concentrations, ensuring a possible comparison in terms of wet weight (WW): 50 and 1 prey.tank^{-1} of small and medium medusae, respectively, ensure WW of ca. 4g of medusa per tank. Although with higher WW (ca. 24g), large medusa was provided at the minimum possible concentration (1 prey.tank^{-1}).

In order to mimic field conditions, one colony of polyps settled on oyster shell was provided. Consequently, the initial concentration of this prey item was not artificially fixed.

Gradient of concentration

The goal of the gradient of concentration experiment was to assess whether *Aurelia* sp. ingestion could be proportional to its availability in the field. Based on the results of the first feeding experiment, small medusae (Ø 1 cm) were used for this test, using six

different treatments (with initial concentrations of 5, 10, 15, 30, 40 and 50 items.tank^{-1}) for both life stages of *S. aurata*.

Selectivity experiment

Selectivity experiments allowed the assessment of the influence of simultaneous availability of high nutritional quality prey (crustacean) and low quality prey (*i.e. Aurelia* sp.) on the potential ingestion of early life stages of *Aurelia* sp.. Therefore, two composite diets were supplied for both G1 and G2 fishes. Diet 1 was composed by ephyrae and *Artemia* with equal initial concentrations, while diet 2 was composed by polyps and *Artemia* (**Table IV.2**). Total prey concentrations were determined as previously described in monospecific diets experiment section and equally distributed by the prey items (*i.e.* ephyrae and *Artemia* representing each 0.5% of fish biomass).

The experiments were run for 2h for all treatments, which is in accordance with previous laboratory experiments on fish predation on jellyfish (Arai et al., 2003) and allow an accurate estimation of ingestion rates, as further confirmed by a preliminary test. Since the experiment time was short, control experiment to determine prey mortality was not required.

Prey items in each case were counted before and after the experiments, either by direct visual observation (for larger medusae) or under a dissecting microscope after filtering all the water content of each tank on a 200µm mesh sieve. Polyps attached to oyster or mussel shells were photographed with a Cannon PowerShot G16 camera at the beginning and end of the experiment. Photographs were pre-treated with Adobe Photoshop CS2 Version 9.0, in order to improve contrast and polyps were counted by eye observation.

Fishes used in *Aurelia* sp. treatments which show positive results of ingestion rates were killed, measured and weighted. The weight of the remaining fishes was estimated according to the mean of the fishes from the same generation tested on the same day or on the previous experiment date.

Calculations

Ingestion rate per gram of fish (*I*; prey.g^{-1}.h^{-1}) was calculated according to the equation:

$$I = ((Ci - Cf)/t.n)/m$$

Where *Ci* and *Cf* are the initial and final concentration (prey.tank^{-1}) of prey in the water, respectively, *t* is the experiment duration (h), *n* is the number of fish in each experimental tank and *m* is the weight of each fish (g). The results are presented as the mean *I* of each treatment (*i.e.* three replicates).

Ingestion rates of small, medium and large medusae were assessed according to their wet weight. Medusa biomass (wet weight; *WW*) was calculated based on medusa bell diameter (*BD;* cm), according to the equation of Uye & Shimauchi (2005):

$$WW = 0.0748\ BD^{2.86}$$

Corresponding ingestion rates in biomass (*Im*; g prey. g fish^{-1}.h^{-1}) were calculated, according to the equation:

$$Im = I * WW$$

The results are presented as mean *I* and mean *Im* for each treatment (*i.e.* based on three replicates).

Statistical analysis

In monospecific diets experiments, differences in *S. aurata's* ingestion rates among prey types were tested by Kruskal-Wallis test, followed by *post hoc* multiple comparison performed by Dunn's test, with no correction. The test of hypotheses explaining the *S. aurata*'s ingestion rates during the gradient of concentration experiment was done using a General Linear Model (GLM), including the initial prey concentration as predictor. Mann-Whitney-Wilcoxon test was performed to identify significant differences of ingestion rate between treatments in selectivity experiments. All statistical analysis was performed using the software R 3.1.1 (The R Project for Statistical Computing 2014) and taking $\alpha<0.05$ as the limit for statistical significance. The experimental design, including objectives, treatments and the statistical tests used for each experiment are summarized in **Fig. IV.3**.

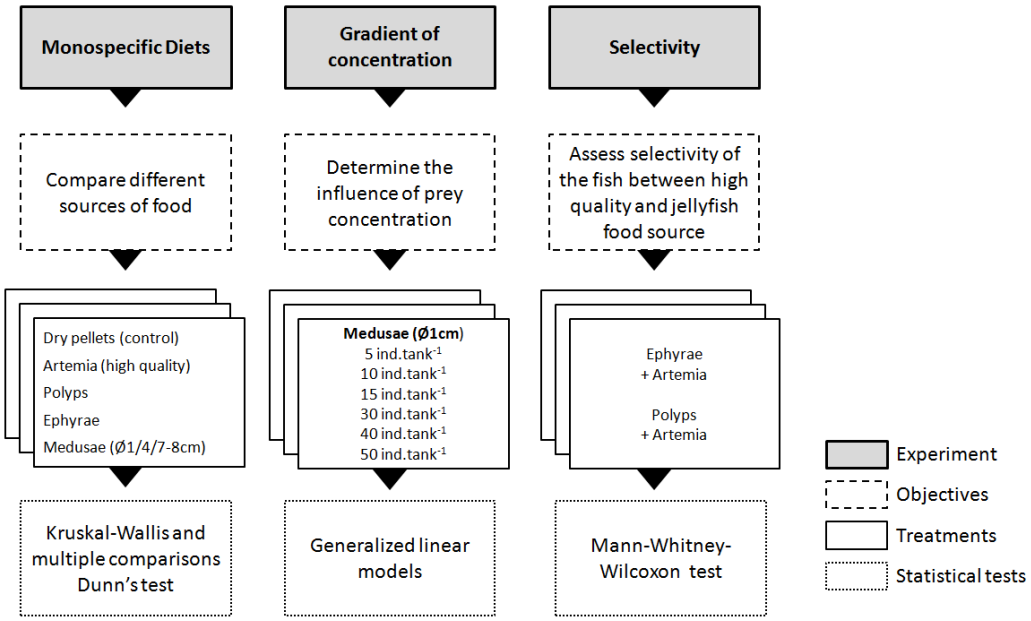

Fig. IV.3: Summary of the experimental design including the type, the specific objectives, the treatments (with three replicates) and the statistical analysis performed in each experiment.

RESULTS

Natural fish predators of *Aurelia* sp. in Thau Lagoon

During the field sampling in Thau lagoon, 36 individuals of five different species of fish were collected for further molecular analysis of their gut contents (**Table IV.3**).

Table IV.3: Species, quantity, weight and capture date of fishes collected in Thau lagoon for molecular gut content analysis.

Species	Quantity	Weight (g)	Capture date
Anguilla anguilla	10	-	17-06-2013
Atherina sp.	5	-	10-04-2013
Mugilidae	9	750	27-03-2012
		700	27-03-2012
		600	27-03-2012
		700	27-03-2012
		650	27-03-2012
		800	07-04-2013
		900	07-04-2013
		700	08-04-2013
		700	14-04-2013
Sarpa salpa	2	650	06-05-2013
		400	07-05-2013
Sparus aurata	10	300	26-03-2012
		250	27-03-2012
		250	27-03-2012

	250	27-03-2012
	250	27-03-2012
	250	14-04-2013
	300	14-04-2013
	200	14-04-2013
	300	14-04-2013
	250	14-04-2013

After observation under UV light, the amplification of the target gene fragment is possible to observe by the presence of different bands, as in the example presented in **Fig. IV.4**.

DNA samples of pelagic stages (ephyra and medusa) of *Aurelia* sp. were positively amplified by the primer pairs, indicating the presence of the target gene fragment in these life stages and therefore their suitability as positive control for *Aurelia* sp. presence in fish gut contents. However, DNA amplification was consistently negative for polyps, suggesting that the extraction procedure failed, since Wang et. al (2013) reported positive amplifications for this life-cycle stage.

Among the ensemble of fish species analyzed (**Table IV.3**), only those of *Sarpa salpa* were clearly positive for *Aurelia* sp. DNA (**Fig. IV.4**). Unfortunately, only two individuals of this species were collected. However, both of them contained DNA that was amplified by the two primer pairs used, suggesting that the ingestion of *Aurelia* sp. by this fish species might be regular in the Thau lagoon, at least during the period of its annual bloom.

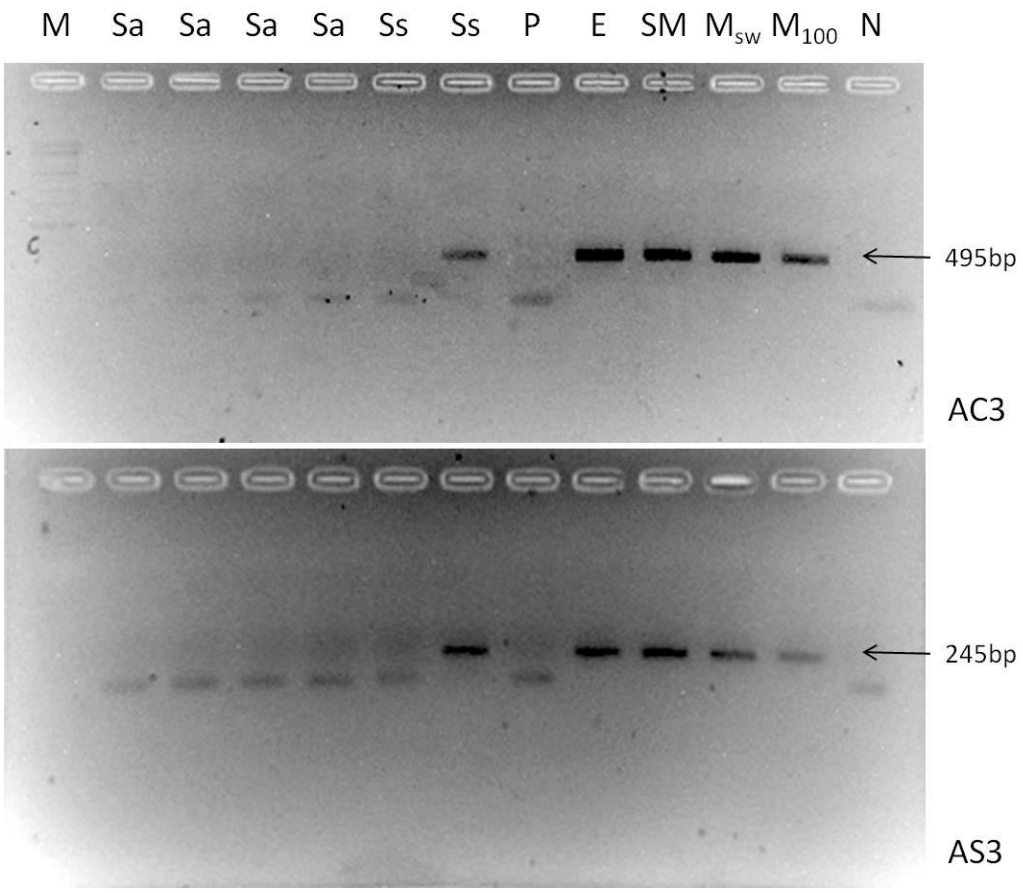

Fig. IV.4: Results of PCR amplifications (in agarose gel, observed under UV light) with the primer pairs AC3 (mt 16S rDNA; top) and AS3 (mt COI; bottom) of DNA samples from all the life stages of *Aurelia* sp. and the gut contents of 6 fish from two contrasted species present in the Thau lagoon: P = polyps, E = ephyrae, SM = medusae (Ø 1cm), Msw = large medusa (Ø 7-8 cm) preserved in seawater, M100 = large medusae (Ø 7-8 cm) preserved in ethanol 100%, Sa = *Sparus aurata*, Ss = *Sarpa salpa*. The identification for each vertical band is given at the top of the picture (M = molecular weight markers, N = negative control).

Laboratory experiments on predation impact

Predation activity was systematically observed within the two hours of the feeding experiments and occurred irrespectively of the life stage of *S. aurata* tested, and the tank. Furthermore, in several treatments, all the provided preys were consumed within the 2h of experiment, indicating the suitability of the experiment time.

Monospecific diets experiments

Predation activity on dry pellets (*i.e.* control) was consistently observed within the two hours of the experiment and occurred for both life stage of *S. aurata*, showing their suitability as control.

The two fish generations displayed predatory activity over both benthic and pelagic stages of *Aurelia* sp. life cycle (**Fig. IV.5**) and, in some cases, the provided prey was totally consumed (*e.g.* small medusa). Large medusae were not totally consumed by any

generation of fish, but bites on the edge of umbrellas were consistently observed (**Fig. IV.6**). In these cases though, the biomass of *Aurelia* sp. consumed by the fish was not quantifiable with our protocol.

Ingestion rates for monospecific diets varied significantly according to the type of prey offered, both for the G1 (Kruskal-Wallis test, chi-squared = 57.65, df = 7, *p-value* = $4.45e^{-10}$) and for the G2 fishes (Kruskal-Wallis test, chi-squared = 41.79, df = 7, *p-value* = $5.71e^{-7}$). Significant differences (Kruskal-Wallis test, chi-squared = 5.48, df = 1, *p-value* = 0.02) have been observed between the two life stages of *S. aurata*. The highest mean ingestion rates for G1 was obtained for small medusa (0.15±0.03 prey item.g^{-1}.h^{-1}), polyps (0.14±0.07 prey item.g^{-1}.h^{-1}) and *Artemia* (0.11±0.00 prey item.g^{-1}.h^{-1}), while ephyrae (0.05±0.02 prey item.g^{-1}.h^{-1}), medium (0.003±0.001 prey item.g^{-1}.h^{-1}) and large medusa (no consumption) presented lower values (**Fig. IV.5**). The multiple comparison performed by Dunn's test though, revealed that only small medusa and *Artemia* presented similar values as control (Dunn's *post hoc* test P = 0.23, P = 0.15, respectively), while the remaining were significantly different (P< 0.05). For G2 fishes, *Artemia* (0.11±0.00 prey item.g^{-1}.h^{-1}) and small medusa (0.07±0.00 prey item.g^{-1}.h^{-1}) were the most consumed preys with no differences from control (Dunn's *post hoc* test P = 0.35, P = 0.29, respectively), followed by polyps (0.04±0.02 prey item.g^{-1}.h^{-1}), ephyrae (0.03±0.02 prey item.g^{-1}.h^{-1}), medium (0.001±0.001 prey item.g^{-1}.h^{-1}) and large medusa (no consumption) with significant lower values than dry pellets (P< 0.05) (**Fig. IV.5**). Fish predation on medium medusae of *Aurelia* sp. was limited, when considering the amount of individuals consumed, irrespective of fish life stage (**Fig. IV.5**). However, when considering ingestion rates in terms of biomass, *Aurelia* sp. medusae of intermediate size (Ø = 4 cm) proved to be at least as important as small ones as a source of food for *S. aurata* (**Fig. IV.7**). Indeed, because one medusae of 4 cm bell diameter provides approximately the same wet weight of food (3.94g) than 50 individuals with bell diameters of 1 cm (3.74 g), ingestion rates in terms of biomass were *in fine* similar between the two size classes of medusae, irrespectively of the life stage (Dunn's *post hoc* test, P = 0.46; P = 0.36, for G1 and G2 respectively). Surprisingly however, the ingestion of *Aurelia* sp. small and medium medusae was consistently higher (Kruskal-Wallis test, chi-squared = 7.78, df = 1, *p-value* = 0.005) in G1 fishes (0.0112 ± 0.002 and 0.0116 ± 0.003 g.g^{-1} fish.h^{-1}, respectively) than G2 (0.005 ± 0.000 and 0.004 ± 0.003 g.g^{-1} fish.h^{-1}, respectively), further suggesting different food preferences for this species according to the life stage (**Fig. IV.7**).

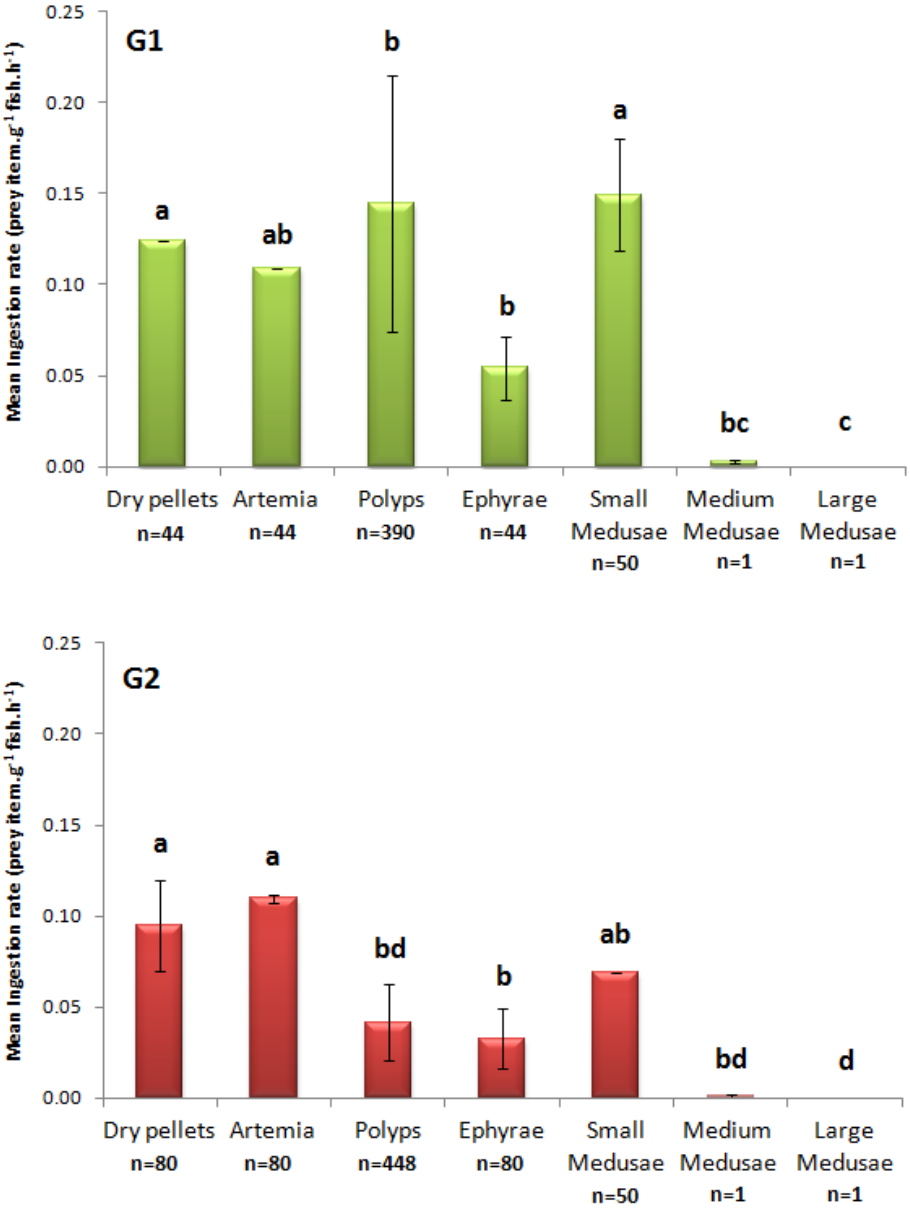

Fig. IV.5: Mean ingestion rates (in number of prey item) observed in G1 (top) and G2 (bottom) *S. aurata* for all prey types during the monospecific diets experiments. Error bars represent standard deviations in each case. Letters indicate significant differences between groups ($P<0.05$), when present. Note that the initial number of prey item offered (n) varied according to prey type (for more details, refer to **Table XIII**).

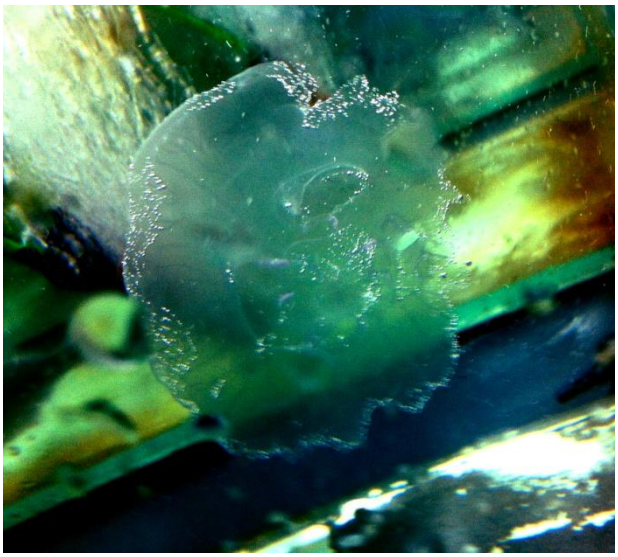

Fig. IV.6: Large medusa showing bites on the edge of the umbrella.

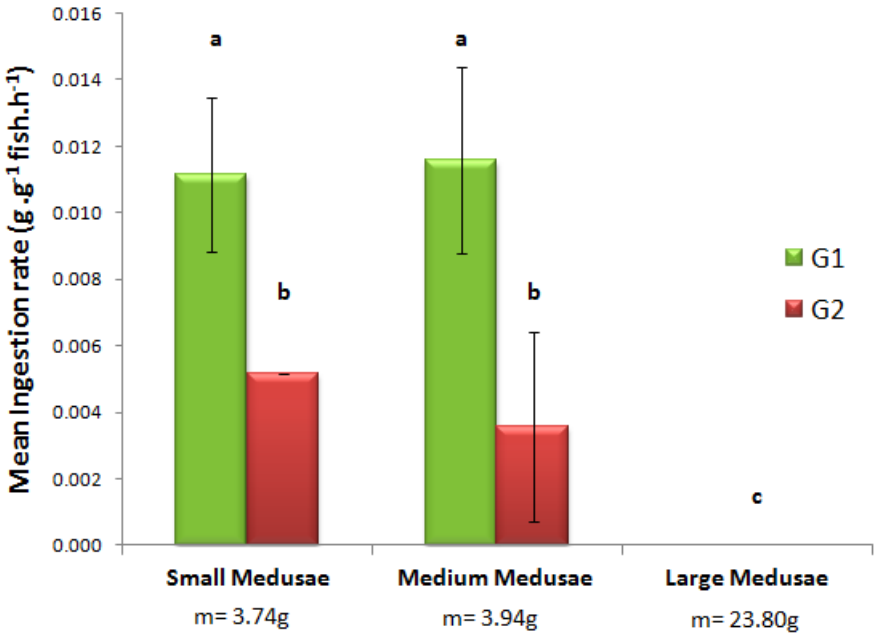

Fig. IV.7: Estimates of mean ingestion rates (in weight) of small, medium and large medusae of *Aurelia* sp. by G1 and G2 *S. aurata* during the monospecific diets experiments. Error bars represent standard deviations in each case. Note that the initial biomasses (m in g) provided were comparable for small and medium medusae. Letters indicate significant differences between groups ($P<0.05$), when present.

Gradient of concentration experiments

The results of ingestion rate obtained from the gradient of concentrations experiment suggested that the predation of *S. aurata* on *Aurelia* sp. in the wild could be proportional to their availability, at least for the small medusae **(Fig. IV.8)**. Ingestion rate increased with increasing concentration supply, for both age classes, showing a

significant correlation (t value= 18.53, *p-value*= < $2.00e^{-16}$; t value= 115.26, *p-value*= < $2.00e^{-16}$ for G1 and G2, respectively). Ingestion rate reached the maximum value of 0.15±0.03 prey.g^{-1}.h^{-1} for G1 fishes and 0.07±0.00 prey.g^{-1}.h^{-1} for G2 fishes, which represents the ingestion of all available prey in the tank. Therefore, maximum ingestion rates in the wild could even be higher than those reported here if prey availability is greater.

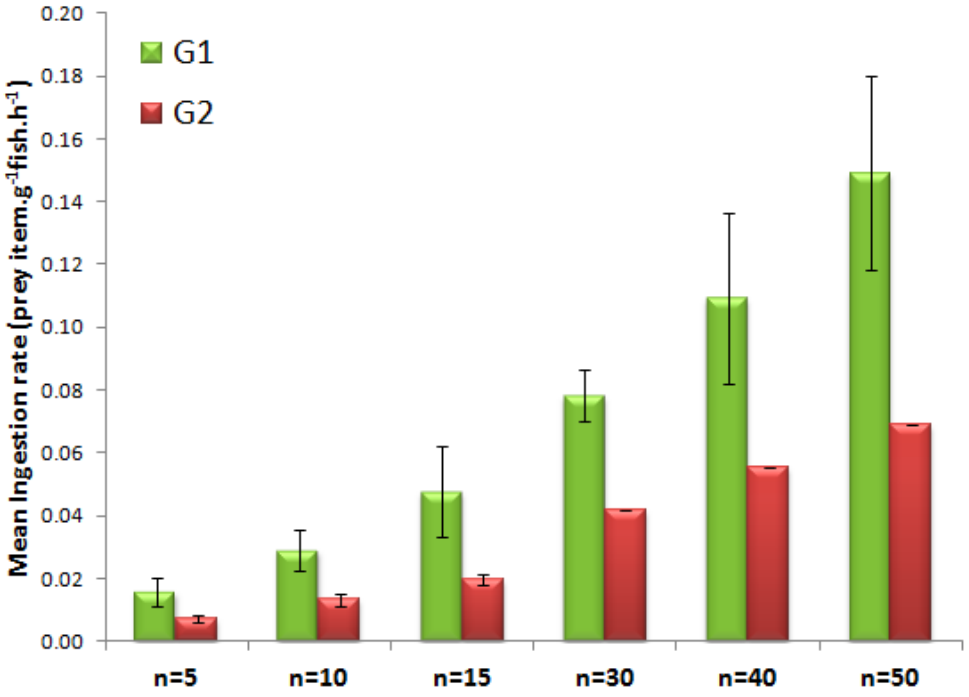

Fig. IV.8: Mean ingestion rates (in number of prey items) observed for G1 and G2 *S. aurata* for increasing initial abundances (n) of small medusae (Ø = 1cm) of *Aurelia* sp. in the tanks. Error bars represent standard deviations in each case.

Selectivity experiments

Selectivity experiments showed that the attractiveness of *Aurelia* sp. as prey depended on *Aurelis* sp. life stage (benthic or pelagic) and that of the fish (juvenile or adult) (**Fig. IV.9**). Although the values indicate higher ingestion rates for *Artemia* in both diets for G1 fishes, significant differences were obtained only when provided together with polyps (Wilcoxon test, W= 68, p-value= 0.02). For G2 fishes, *Artemia* was significantly selected when compared with ephyrae (Wilcoxon test, W= 36, p-value= 0.002). In diet 2, however, the reverse situation was observed with the polyps presenting higher ingestion values (Wilcoxon test, W= 2.5, p-value= 0.02), despite the high variability in polyp ingestion rates among replicate tanks.

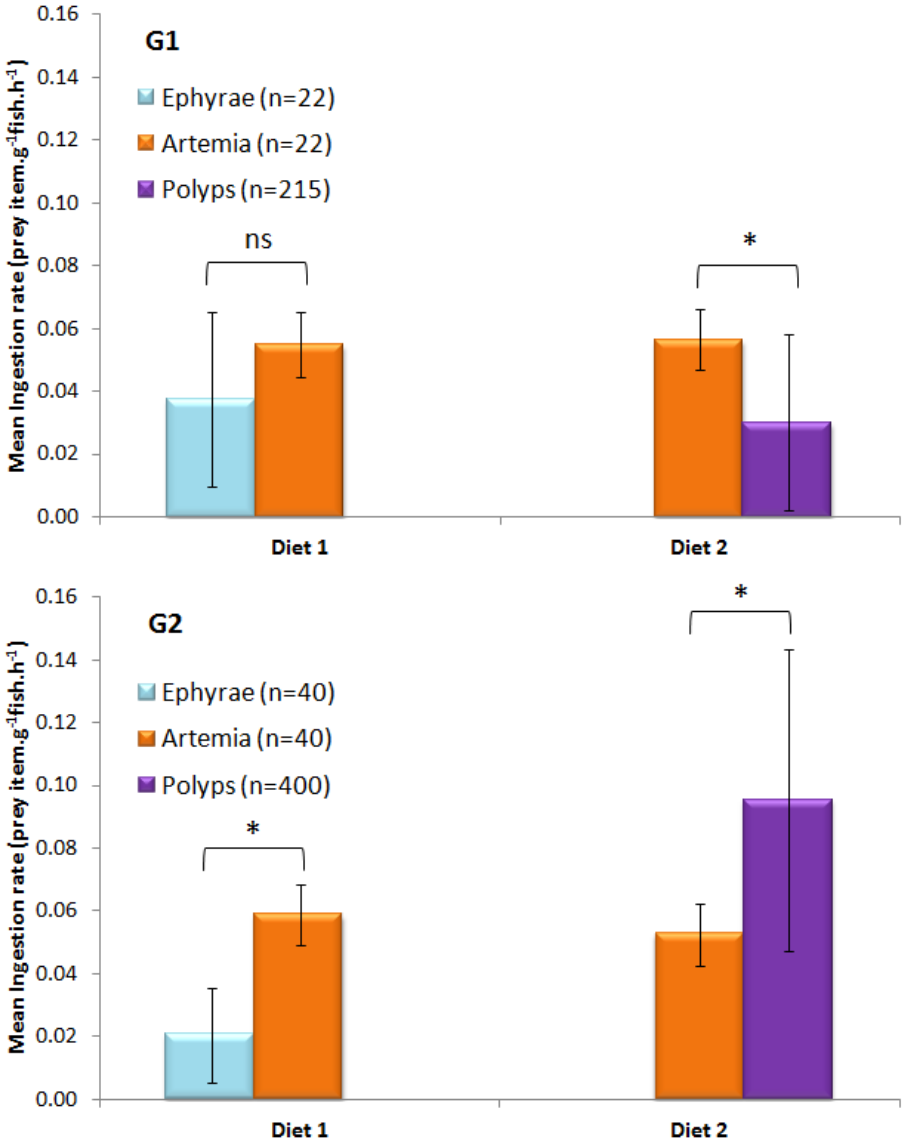

Fig. IV.9: Mean ingestion rates (in number of prey items) observed in G1 (top) and G2 (bottom) *S. aurata* for the various types of live prey (*Artemia* and ephyrae or polyps of *Aurelia* sp.) included in the two mixed diets used for the prey selectivity experiments. Error bars represent standard deviations in each case. Symbols above horizontal bars indicate when differences among groups were significant (*) or not (ns) at the risk level $\alpha = 0.05$.

DISCUSSION

Natural fish predators of *Aurelia* sp. in Thau lagoon

To our knowledge, this is the first assessment of the trophic interactions between fish (predator) and jellyfish (prey) using both field and laboratory complementary approaches.

The use of molecular techniques (*i.e.* PCR, Polymerase Chain Reaction), allowed identifying the presence or absence of *Aurelia* sp. genomic DNA in gut contents of wild

fish. When assessing dietary composition of vertebrates based on numerical gut content analysis, soft bodied organisms are usually underestimated. This is particularly frequent with jellyfish prey items, since they are digested very rapidly and preservative methods may destroy or shrink gelatinous material (Arai, 2005). The development of PCR-based techniques allow the prey remains to be identified, even those partially digested (Symondson, 2002). This technique has been also applied to uncover trophic relationships in other organisms such as cod (*Gadus morhua*) or plaice (*Pleuronectes platessa*) (Albaina *et al.*, 2012; Rosel and Kocher, 2002; see also King *et al.*, 2008), but it has never been used for identification of gelatinous organisms. Here, we show that PCR-based techniques allowed unrevealing positive trophic interactions between fish predators and jellyfish.

Different *Aurelia* sp. life stages were used as positive control, among which only the pelagic stages showed positive results. The consistent negative amplification of polyps DNA samples suggests a deficient methodological procedure. Wang et al. (2013), reported that the developed primers used in this study were able to amplify the gene fragment from all *Aurelia* sp. life stages, including planulae, polyps, ephyrae and medusae. Therefore, we believe that this result may be a consequence of a deficient extraction procedure. The characteristic consistency and elasticity of polyps tissues clogged the extraction equipment and might have hampered the successful extraction of the target gene fragment from polyps samples. DNA samples from the pelagic life stages though, were positively amplified by the specific primer pairs (AS3 and AC3), indicating that our procedure successfully identified the presence of *Aurelia* sp. in fish gut contents, including ephyrae, medusae and possibly polyps.

Results suggested that at least *Sarpa salpa* is a confirmed predator of *Aurelia* sp. in the field and the ingestion by this fish species might be more important than acknowledge in the Thau lagoon, at least during the period of *Aurelia* sp. annual bloom. It is worth noticing that those results constitute the first report of jellyfish consumption by *Sarpa salpa,* which has been described so far as a true herbivore, with a diet largely based on seagrass leaves (Havelange et al., 1997). Therefore, the presence of *Aurelia* sp. in its gut contents was not expected. It is possible that *Aurelia* sp. was accidentally consumed since medusa were often seen entangled in the seagrass leaves (R. Marques, personal observation during field survey of polyps distribution – Chapter II). Moreover, assuming that our primers successfully amplified *Aurelia* sp. polyps from fish gut contents, the benthic stage might have been accidently consumed. Indeed, previous

studies have demonstrated the significant impact of incident mortality on post-settled corals and consequent recruitment, caused by herbivorous predators (Penin et al., 2010, 2011). Nevertheless, *S. salpa* is a highly selective browser (Verlaque 1990), which suggest that *Aurelia* sp. might have been intentionally predated. Furthermore, it is important to highlight that only two individuals of *S. salpa* were collected in the field, in different sampling dates, and both revealed positive amplifications. Such results underline the possible regular and deliberate feeding activity on *Aurelia* sp. by this predator. It is therefore likely that the list of species preying on jellyfish is larger than acknowledged, but so far unnoticed due to biased methodologies to identify such trophic interactions. Such scenario has wide ecological implications in pathways of energy in the food web. Indeed, although the negative results obtained for the remaining fish species investigated suggest that they are not regular predators of *Aurelia* sp. in Thau lagoon, it cannot be fully excluded that they occasionally feed on this jellyfish. Opportunistic fish species, such as *S. aurata* (Pita et al., 2002; Escalas et al., 2015), might take advantage of local peaks in *Aurelia* sp. densities to partially sustain their growth, as further suggested by the results of our laboratory experiments.

Laboratory experiments on predation impact

Although *Aurelia* sp. genomic DNA was not identified in wild individuals of *Sparus aurata* gut contents, in laboratory conditions, both generations of *S. aurata* feed on all life stages of *Aurelia* sp. offered as prey. As mentioned above, this is not particularly surprising since *S. aurata* is an opportunistic feeder which commonly adapt its diet to available food resources and therefore occupying a broad feeding niche (Wassef & Eisawy, 1985; Pita et al., 2002). Furthermore several species of Sparidae family were already reported as jellyfish consumers (Ates, 1988; Mianzan et al., 1996), stressing the aptness of *S. aurata* as jellyfish predator. Our results highlight the potential of *S. aurata* as a substantial jellyfish predator, in contrast with earlier descriptions of the feeding preferences of this species (Pita et al., 2002; Escalas et al., 2015).

Monospecific experiments

The ingestion rates of both generations of *S. aurata* varied significantly according to the type of prey offered. Small medusae appeared to be preferred by both generations of *S. aurata*, which showed ingestion rates as high as those observed with the control pelleted food or with live adult *Artemia*. Additionally, polyps were also favored by the younger

fishes. In contrast, large medusae with 7-8 cm bell diameter were bitten but never fully consumed, while predation on medium sized medusae (Ø 4cm) was intermediate. Therefore, our results showed that the intensity of *S. aurata* predation, at least on the pelagic stages, depends on prey size-range. Prey vulnerability can be expressed as the product of encounter probability and susceptibility to capture, and size is the dominant factor determining vulnerability (Houde, 2001). *Aurelia* sp. might be considered highly vulnerable to capture by fish as they are large organisms with lower evasion capacity (Houde, 2001). However, food particle size consumed by *S. aurata* is also constrained by its mouth size (Wassef & Eisawy, 1985; Goldan et al., 1997; Russo et al., 2007). Therefore, our results suggest that ephyrae (Ø <1cm) appear to be too small to support high consumption rates, while the predation of large medusae is likely limited by *S. aurata* mouth size. Still, fish bites were observed in the edge of their umbrella, suggesting that the quality of prey is not the hamper factor of its ingestion. Indeed, Linde et al. (2004) stated that the small mouth size of *S. aurata* is overcome by increased strong jaws, adapted to biting feeding strategy, allowing them to feed on small pieces of large preys. Here we show that although just partially consumed, large jellyfish may provide a potential source of food for *S. aurata* and in turn, its bites might damage the umbrellas of this jellyfish.

It is worth to notice though, the high consumption of polyps by G1 fishes. In the wild, juveniles of *S. aurata* prey mainly on epibenthic polychaetes, small fishes, epibenthic crustaceans and gastropods (Tancioni et al., 2003; Escalas et al., 2015), but with a clear dominance of bivalves in some habitats (Pita et al., 2002). As the polyps of *Aurelia* sp. in our experiments were provided fixed on the shells of living bivalves (oyster and mussel), it is possible that the actual target of fish predation was the settling substrate, rather than on the polyps themselves. Therefore, although methodological constraints cannot be totally discarded, indirect predation might have had a great impact on the results. In any case, bivalves are commonly colonized by polyps in the wild and particularly in Thau lagoon, where bivalves play a crucial role in the development of *Aurelia* sp. benthic population (see Chapter II). Thus *S. aurata* has probably a non negligible impact on the benthic population of *Aurelia* sp. in the lagoon, either by direct predation on its polyps or by indirect predation on the bivalves.

Although our results suggest that the small medusa (Ø 1cm) is the most consumed *Aurelia* sp. stage by *S. aurata*, when considering the contributions according to prey biomass, both small and medium medusae play a parallel role as food source,

highlighting their vulnerability to fish predation. Indeed, *Aurelia* sp. within this size range occurs in Thau lagoon during spring months (March - May; Bonnet *et al.*, 2012, see Chapter III), overlapping with the migration of *S. aurata* sub-adults from Mediterranean Sea to Thau lagoon (Crespi, 2002; Mercier et al., 2012), where this jellyfish species might provide a highly available and accessible source of food. Nevertheless, medium and especially small medusae remain in the water column for a very short time, as a result of their exponential growth rates (0.57 – 2.53 $mm.d^{-1}$; see Chapter III). This life history adjustment allow jellyfish to undergo peaks of biomass, benefitting from high resources availability (Boero et al., 2008), and simultaneously reducing the vulnerability to predation.

Gradient of concentration experiments

Our results provide evidence that increasing prey availability boosts ingestion rates of *S. aurata* and since all the provided prey were consumed, maximum ingestion rates in the wild could even be higher than those reported here if prey availability is greater. *Aurelia* spp. is mainly composed by water (ca. 96% of total wet weight) with very low nutritional value (Lucas, 1994; Doyle et al., 2007). To meet the energetic requirements, higher volumes of this organism must be ingested (Cardona et al., 2012). However, the high rates of jellyfish digestion (and presumably assimilation) reported by Arai *et al.* (2003), might support a continuous high ingestion rate of jellyfish. Indeed, in rearing conditions, *S. aurata* was shown to be able of increase voluntary feed intake, when the food levels of proteins and lipids are low (Santinha et al., 1999). Furthermore, energy content of jellyfish increases during the period of gonad maturation (Milisenda et al., 2014), which occurs during bloom events in Thau lagoon (Bonnet et al., 2012). Thus, high concentrations of jellyfish in blooms, associated with high ingestion rates of the fish, may compensate for the low nutritional value, since the fishes may satisfy their energy requirements within a very small area, preventing energy waste in foraging and capture behavior (Mianzan et al., 1996; Cardona et al., 2012). Here we provide empirical evidence which supports the hypothesis that at times the relative importance of jellyfish, as food source, may be higher than acknowledge. Moreover, important amounts of *Aurelia* sp. medusae might be consumed punctually by *S. aurata* each spring, contributing to structure the population of this jellyfish species in Thau lagoon.

Selectivity experiments

In the field, environmental conditions usually offer a great variety of prey items. The intensity of fish predation is probably dependent on the relative densities of the various preys available in the lagoon during jellyfish bloom events. Indeed, the results of our prey selectivity experiments pointed out that jellyfish are not preferred by *S. aurata* individuals when a type of prey with higher nutritional quality is equally available in the tank. Parallel results were reported for the threadsail filefish (*Stephanolepis cirrhifer*) (Miyajima et al., 2011), who described that selectivity was an outcome of prey accessibility and also reported for *S. aurata* in the field (Pita et al., 2002). However, during blooms events, jellyfish dominance occurs as an outcome of their predation impact on zooplankton communities (Purcell & Sturdevant, 2001; Hansson et al., 2005; Bonnet et al., 2012; McNamara et al., 2013; Pereira et al., 2014), shifting from high energetically zooplankton community to low quality jellyfish dominating population. Therefore, during bloom events, the high availability and accessibility of jellyfish, might promote their consumption by *S. surata*.

Theoretical assessment of *S. aurata* grazing impact on wild *Aurelia* sp. populations

In order to estimate the grazing impact of a predator, maximum daily ingestion rates are required, as well as the quantification of predator's abundance in the field. Here, such data were not available. However, theoretical estimations were calculated, based on our results and literature information.

The requirement of protein (*RP*) to maintain one individual of *S. aurata* can be calculated according to the equation $RP = 0.61g/ BW^{0.70}/day$, where BW represent fish body weight (Kg) (Lupatsch 2005). Therefore, one *S. aurata* of 200 g (similar to G2 fish weight), requires 0.20 g of digestible protein per day. The wet weight (WW) of one small and one medium *Aurelia* sp. was firstly calculated based on Uye & Shimauchi (2005) equation (see Methods section). The wet weight of individuals with 1 and 4cm bell diameter is 0.07g and 3.94g, respectively. The associated dry weight (DW) corresponds to 3.91% of its WW for small medusa (*i.e.* DW = 2.9 mg) and 3.12% WW for medium medusae (*i.e.* DW = 123 mg) (Lucas, 1994). Protein content of medusa (P) can then be calculated according to the equation $Log\ P\ (mg) = 1.7483 + 1.1253\ log\ DW\ (g)$ (Lucas, 1994). Therefore, one medusa with 1cm bell diameter contains 3.305e-4 g of protein, while medusa with 4cm contains 2.063e-3 g of protein. Assuming an exclusive

diet of *Aurelia* sp., 605 individuals with 1cm or 97 with 4cm bell diameter would be necessary to satisfy the daily nutritional requirements of one individual of *S. aurata*.

Based on *S. aurata* biomass from capéchades fishing gear in 1997 (3 104 kg) and in 1998 (86.40 kg) we calculated the mean collection per year (1 595.2 $kg.y^{-1}$). Assuming a homogeneous population of individuals with 200g, the abundance of *S. aurata* in Thau lagoon would be 7 976 $ind.year^{-1}$ (Crespi, 2002). Therefore, 4.83e6 small medusae or 7.74e5 medium medusae of *Aurelia* sp., would be necessary to satisfy the daily nutritional requirements of *S. aurata* and maintain its population in Thau lagoon. Assuming an equal horizontal distribution and considering the mean abundance of medusa (Ø > 1cm) between April and June, (*i.e.* when present: 12.1 $ind.100m^{-3}$), such numbers represent 13.3% of a population composed by small medusa or 2.13% of a population composed by medium medusa of *Aurelia* sp..

These results stress the potential predation impact of *S. aurata* on the population of *Aurelia* sp. in Thau lagoon. However, it is important to underline the theoretical quality of this estimation, implying that the real grazing impact might be miscalculated.

Nevertheless, this could be a potential source of food to avoid starvation in individuals that have little energy to spare on foraging, which frequently occurs in the early spring since feeding activity decreases during cold winter months in *S. aurata* (Gallardo et al., 2003; Mercier et al., 2012).

CONCLUDING REMARKS

Our results suggest that jellyfish like *Aurelia* sp. can be a source of food for fish species, with potentially important consequences on energy fluxes within food webs and on the population dynamics of jellyfish. Here we show that even fish species listed as true herbivores might prey on gelatinous organisms, which points towards a possible underestimation so far of jellyfish predators diversity. Such findings supports the hypothesis that not only fishes with primarily gelatinous diets might benefit from the increasing jellyfish blooms, but also the more opportunistic predators might, at least sporadically, take advantage from this highly accessible and available source of food. Furthermore, our laboratory experiments revealed that *Sparus aurata* could prey on benthic (by direct or indirect predation) and pelagic stages of *Aurelia* sp., and therefore might have an important grazing impact on the species by increasing its predation rates, when the availability of prey with higher nutritional quality is reduced in the wild. The DNA amplification approach used here has proved valuable for assessing fish-jellyfish

trophic interactions and can have further applications in other areas and species. Accurate quantification of the exact intensity of fish predation in the wild is still required, but here we show that fish have the potential to control jellyfish blooms, through top-down regulations of jellyfish biomass, at different stages of their life cycle. Thus, here we provide supporting evidences to the hypothesis that overfishing may greatly promote favorable conditions for jellyfish outbreaks, not only by reducing food competition but also by removing their predators. On the light of the predicted increase of frequency and magnitude of jellyfish blooms, fish predation by generalist and opportunistic predators may gain importance as natural control of jellyfish outbreaks.

CHAPTER V: GENERAL CONCLUSIONS

The current world population overcomes 7 billion humans, living and exploring the earth's resources, and is expected to continue to rise. Therefore, the human impact on the natural ecosystems is large with global scale consequences. Halpern et. al. (2008) assessed the anthropogenic impacts on marine ecosystems and concluded that no area is unaffected by human impact. Mediterranean Sea was identified as one of the most highly impacted areas, under a high level of human mediated changes.

Although multiple drivers act synergistically and affect a large portion of the world's marine environment, climate change and overfishing were pointed as the strongest forcing factors.

Jellyfish play an important role in the ecosystems and their outbreaks appear to be increasing, at least in some coastal areas of the world. Many human mediated changes in the environment have been pointed as the main precursors of jellyfish outbreaks, but the combined effects are the most likely scenario. The recognized synanthropic condition of jellyfishes, led to an increasing research focused on jellyfish ecology, but many of their features are still overlooked. For instance, despite their acknowledge fundamental role in the development of jellyfish blooms, the polyps population of jellyfish life-cycle is still poorly scrutinized. Furthermore, in addition to identifying the sources of bottom-up control of jellyfish populations, top-down processes were largely ignored and urgently need further investigation, as they might be more important than previously thought.

Here we provide a deep study of the ecology of *Aurelia* sp. in a coastal lagoon, assessing process of top-down and bottom-up control. We addressed the whole life cycle of this jellyfish species, revealing evidences of the process contributing to their production and mortality. To our knowledge, such comprehensive study embracing an ecosystem based approach and ecology a species of jellyfish was never reported. A summary of the main conclusions of this study is presented in **Fig. V.1**.

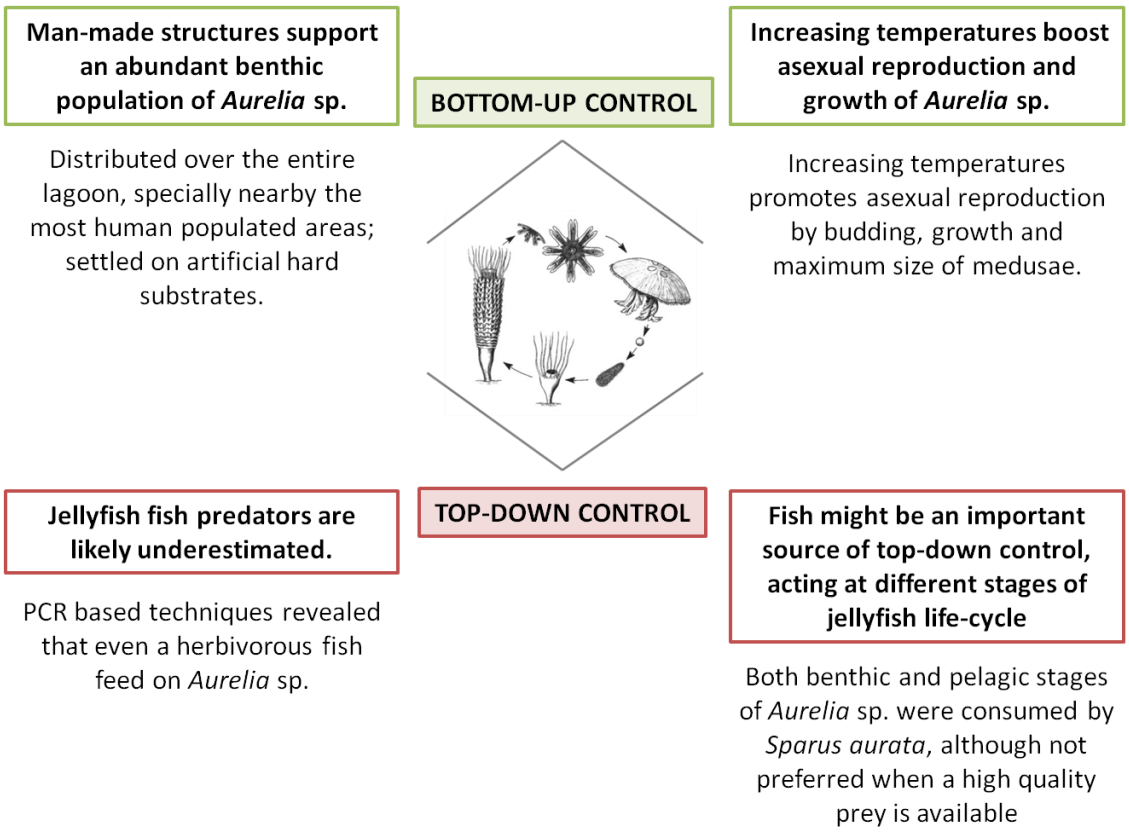

Fig. V.1: Resume of the most important findings of the thesis.

Human mediated processes of bottom-up control

Climate change is one of the biggest future challenges that humanity faces. The most pronounced consequence is the increasing atmospheric temperatures and consequently, warming of the oceans.

One of the most important expected outcomes of increasing temperatures is sea level rising, which is projected to persist throughout the 21st century and beyond, exposing the coastal areas to adverse impacts, such as submergence, coastal flooding and coastal erosion (Field et al., 2014). To face such scenario, coastal adaptation will be required, implying the augmentation of coastal constructions. The proliferation of artificial structures in the marine environment is therefore pointed as one of the main drivers of the global increase of jellyfish blooms, by providing suitable habitats for polyps fixation. Here, we provide new supporting evidences of this hypothesis.

Thau lagoon can be perceived as a large-scale mesocosm, capable of providing clues to what might happen in larger seas and oceans. This ecosystem is characterized by very few hard natural substrates, but a resident and isolated population of *Aurelia* sp. is well established, performing consistent annual blooms. Therefore, the survivorship of this

population must rely on a stable population of polyps. Such hypothesis was here confirmed by the identification of a large population of *Aurelia* sp. polyps, distributed over the entire lagoon and settled on the available artificial substrates. Moreover, the presence of the most conspicuous populations of polyps nearby the most human populated areas, jointly with the evident impact of oyster culture rafts, further express the positive impact of human made substrates on the expansion of the *Aurelia* sp. benthic population in Thau lagoon, and perhaps in many other coastal areas of the world.

Warming has caused and will continue to cause shifts in the abundance, geographic distribution, migration patterns, and timing of seasonal activities of marine species, paralleled by community structure modifications. This has resulted and will further result in changing interactions between species, including competition and predator-prey dynamics (Field et al., 2014). However, such changes are highly dependent on the species and their physiology.

In the case of jellyfish, ocean warming appears to have positive effects on their abundance and promotes the development of jellyfish blooms. Our results, revealed that both benthic and pelagic stages of *Aurelia* sp. seem to be highly influenced by increasing temperatures, enhancing their production. The asexual reproduction of polyps seems to be endorsed by higher temperatures, which consequently, augment the population of polyps in the lagoon. In its turn, pelagic stages revealed higher growth rates and maximum body size correlated with warming waters and higher food availability.

However, the assemblage of jellyfish species might respond differently to the expected climate change. Even within the same genus, a great variety of life history strategies was previously reported, as for *Aurelia* spp., implying a continue investigation of their population dynamics.

Human mediated processes of top-down control

Overfishing has been claimed as one of the most important drivers of jellyfish outbreaks. Overfishing means that we are removing more biomass from the marine system, than what the system can naturally restore. After depleting the biomass of the top predators, we are now ‘fishing down marine food webs’, which is promoting favourable condition for jellyfish (Pauly et al., 2009).

The reduction of the fish stock released the competitive pressure of fish on jellyfish for the available food. However, we focused on the least studied consequence of the overfishing: the reduction of predators.

Here we report the first complementary field and laboratory approaches of fish predation on jellyfish, revealing the most likely underestimation of fish predators diversity and their impact on jellyfish populations. Our results, revealed that even an herbivorous fish, *e.g. Sarpa salpa*, might feed on *Aurelia* sp. in Thau lagoon. Furthermore, our laboratory experiments, allow concluding that opportunistic fish species, such as *Sparus aurata*, might have a larger grazing impact on *Aurelia* sp. population in Thau lagoon, than what was previously thought. Polyps were shown to be vulnerable to fish predation, whether by incident mortality, deliberate or indirect predation. The vulnerability of pelagic stages though, appears to be size-dependent. Nevertheless, jellyfish were shown as a potential source of food for fishes, at least during bloom events, and the fishes might benefit from the highly available and accessible source of food. In turn, such predation supports an important process of top-down control of jellyfish populations.

Here we raise an important question regarding the role of jellyfish in the food webs. In the past, jellyfish were perceived as 'dead ends' of the food webs contributing little to transferring energy to higher trophic levels. Nowadays, recent techniques are uncovering the real weight of jellyfish in fish predators diets, especially for the most opportunistic species, as here highlighted. Once, Purcell & Arai (2001) and Arai (2005) stated that predation by a large number of fish species with broad diets is more ecologically important than the predation by the relatively few specialized fishes with primarily gelatinous diets. Therefore, in the predicted future scenario proposed by Boero (2013) generalists species may also be included as the most fostered by the increasing jellyfish blooms. On the other hand, their role as top-down control might be more important than acknowledge.

Further research

Bearing in mind the future predictions of the rising anthropogenic impact on the marine ecosystems, further research on jellyfish ecology and their adaptation to the changing environment, is of crucial importance. The processes of bottom-up and top-down control are highly influenced by several human mediated factors, which require additional research effort on their study. In particular, we identify three main key areas

requiring further investigations. (i) First, field studies focusing on jellyfish benthic population dynamics are particularly required and essential to comprehend its driving factors and ultimately achieving a potential forecast of jellyfish blooms. Trophic interactions of polyps are nearly unknown and the importance of inter- and intra-specific competition is poorly assessed. (ii) Second, there is an urgent need to perform local to regional observations of the pelagic population dynamics, complemented with phylogenetic studies. Large spatial and temporal scales should be covered, in order to match and compare the different populations, within and among jellyfish species. Understanding the role of phenotypic plasticity and genotype is crucial to uncover the driving factors of jellyfish blooms. (iii) Third, in view of the predicted climate change, further investigations of the impact of ocean warming, acidification, freshwater impulses, pollution, changes in food quality and availability, among others, should be performed for each life-stage of different jellyfish species. (iv) Finally, assessing the sources of jellyfish mortality is of paramount importance. Fish predation impact is clearly underestimated and requires accurate estimates, in order to include it in ecosystem based models.

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ANNEX

Chapter III: Dynamics of *Aurelia* sp. pelagic population

Table 1: Different temperature indices potentially influencing growth onset (GO): Mean, difference between the first date and the last date (ΔT) and cumulative sum (Cum. Sum) of the associated period.

Year	GO	Two sample dates before GO			January-February			January-March		
	(Julian days)	Mean	ΔT	Cum. Sum	Mean	ΔT	Cum. Sum	Mean	ΔT	Cum. Sum
2010	98	9.61	5.06	19.21	7.43	1.53	22.30	8.30	4.93	41.51
2011	110	6.82	0.90	13.63	7.53	1.47	30.10	8.80	4.73	52.80
2013	107	8.95	0.70	17.90	7.70	0.90	30.80	8.02	1.60	40.10
2014	77	11.40	4.00	22.80	9.60	0.30	38.40	10.66	3.10	74.60

Table 2: Results of ANOVA analysis of the correlation between growth onset (GO) and different temperature indices. Mean, difference between the first date and the last date (ΔT) and cumulative sum (Cum. Sum) of the associated period.

Period of time	Temperature indices	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Two sample dates before GO	**Mean**	1	552.45	552.45	9.73	0.09
	Residuals	2	113.55	56.77		
	ΔT	1	308.78	308.78	1.73	0.32
	Residuals	2	357.22	178.61		
	Cum. Sum	1	552.31	552.31	9.72	0.09
	Residuals	2	113.69	56.85		
January-February	**Mean**	1	553.32	553.32	9.82	0.09
	Residuals	2	112.68	56.34		
	ΔT	1	381.04	381.04	2.67	0.24
	Residuals	2	284.96	142.48		

	Cum. Sum	1	217.34	217.34	0.97	0.43
	Residuals	2	448.66	224.33		
January-March	**Mean**	1	501.52	501.52	6.10	0.13
	Residuals	2	164.48	82.24		
	ΔT	1	5.03	5.03	0.02	0.91
	Residuals	2	660.97	330.48		
	Cum. Sum	1	429.08	429.08	3.62	0.20
	Residuals	2	236.92	118.46		

Chapter IV: Fish predation on *Aurelia* sp..

Table 3: Results of the multiple comparison analysis, performed by Dunn's test, in order to assess differences of ingestion rate of prey items used in the monospecific diets experiments by fish generation. In bold: Significantly different ($P < 0.05$).

		G1						**G2**					
		Artemia	**Ephyrae**	**Small medusae**	**Medium medusae**	**Large medusae**	**Pellet**	***Artemia***	**Ephyrae**	**Small medusae**	**Medium medusae**	**Large medusae**	**Pellet**
Ephyrae	z	1.18						2.28					
	P	0.12						0.01					
Small medusae	z	-1.77	-2.95					0.93	-1.35				
	P	**0.04**	**0.00**					0.18	0.09				
Medium medusae	z	2.16	0.98	3.93				3.50	1.22	2.57			
	P	**0.02**	0.16	**0.00**				0.00	0.11	**0.01**			
Large medusae	z	3.70	2.53	5.47	1.54			4.34	2.07	3.42	0.84		
	P	**0.00**	**0.01**	**0.00**	0.06			0.00	**0.02**	**0.00**	0.20		
Pellet	z	-1.03	-2.21	0.74	-3.19	-4.73		0.38	-1.90	-0.55	-3.12	-3.96	

	P	0.15	**0.01**	0.23	**0.00**	**0.00**		0.35	**0.03**	0.29	**0.00**	**0.00**	
Polyps	z	0.70	-0.48	2.47	-1.46	-3.01	1.73	2.78	0.51	1.86	-0.72	-1.56	2.40
	P	0.24	0.32	**0.01**	0.07	**0.00**	**0.04**	**0.00**	0.31	**0.03**	0.24	0.06	**0.01**

Table 4: Results of the multiple comparison analysis, performed by Dunn's test, in order to assess differences of ingestion rate of prey items used in the monospecific diets experiments, according to biomass, by fish generation. In bold: Significantly different ($P < 0.05$).

		G1		G2	
		Medium medusae	**Large medusae**	**Medium medusae**	**Large medusae**
Medium medusae	z	-0.09		0.35	
	P	0.46		0.36	
Large medusae	z	3.63	3.72	2.77	2.42
	P	**0.00**	**0.00**	**0.00**	**0.01**

Table 5: Results of Generalized Linear Models, which assessed correlations between concentration of small medusae (Ø1cm) and ingestion rate during gradient of concentration experiments, by fish generation. In bold: Significant correlation (*p-value* < 0.05).

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	5.57E-04	4.58E-03	1.22E-01	9.04E-01
G1	2.84E-03	1.53E-04	1.85E+01	**<2e-16**
(Intercept)	-5.02E-04	3.61E-04	-1.39E+00	1.73E-01
G2	1.39E-03	1.21E-05	1.15E+02	**<2e-16**

Table 6: Results of Mann-Whitney-Wilcoxon Test to assess differences of the mean ingestion rate of the prey types of each diet, during selectivity experiments, for G1 and G2 *S. aurata*.

	G1		G2	
	W	**p-value**	**W**	**p-value**
Diet 1	55.50	0.20	36.00	**0.00**
Diet 2	68.00	**0.02**	2.50	**0.02**