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Nursery function of coastal lagoons: implications of habitat connectivity for the management of lagoon habitats

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ABSTRACT

Coastal lagoons and their habitats perform an important nursery function for marine migrant (MM) fish species, which enter in these ecosystems to exploit the abundant trophic resources and the best biotic and abiotic conditions. The aim of this work is to study the nursery function carried out by shallow water habitats of the Venice lagoon, i) studying the sea-lagoon connectivity, ii) studying and characterizing the habitat preferences, iii) studying the trophic ecology of a target species (*Sparus aurata*).

In this work: i) analyzing the distribution of eggs, larvae and juveniles, collected with a bongo net and a seine net in the whole Venice lagoon, it was possible to observe that the north sub-basin is the one where MM are more concentrated, ii) developing predictive models on distribution of juveniles MM in different habitats of the north sub-basin, it was possible to observe how preferences towards environmental parameters and habitats change with ontogeny but in general saltmarshes were positively selected iii) analyzing diet, head morphology and stable isotope of *S. aurata* during ontogeny it was possible to observe the importance of tidal creek for the trophic ecology of this MM species.

Only through the integration of these methods it is possible to evaluate the complex nursery function of the lagoons, to direct the actions of restoration and management.

GENERAL INTRODUCTION

Nursery role of the transitional water ecosystems for fish

Transitional water ecosystems, located at the interface between land and sea, are highly productive and valuable areas which support fundamental ecological links with other environments (Able, 2005; Beck et al., 2001; Costanza et al., 1997; Vasconcelos et al., 2010). Transitional water ecosystems provide to human a wide range of valuable ecosystem services and goods (Newton, 2018; Rova et al., 2015, 2019). Ecosystem services are defined as the contribution of ecosystem structure and function to human well-being (Burkard et al., 2012) and results from the interactions between the ecological and social components of integrated social-ecological systems (Reyers et al., 2013). Among the different ecosystem services that transitional water ecosystems provide (e.g. climate regulation, waste treatment, erosion prevention, tourism, hunting, education), the maintenance of fisheries is extremely important (Barbier et al., 2011). These areas indeed are highly productive and provide numerous habitats for fish (Able, 2005; Beck et al., 2001; Vasconcelos et al., 2010). Generally, the maintenance of fisheries in these ecosystems is governed by the provision of suitable reproductive habitats and nursery grounds, or shelter living space with high habitat quality, food sources and good hydrodynamic conditions (Barbier et al., 2011; Newton, 2018).

An important component of biodiversity in transitional water ecosystems is represented by ichthyofauna (Elliott and Hemingway, 2002; Vasconcelos et al., 2015). Generally, even if specialist species are still present inside these ecosystems, the high variability in environmental conditions favors presence of generalist species. In transitional water ecosystems, fish species can be divided into different categories (Franzoi et al., 2010; Elliott et al., 2007; Potter et al., 2015). Categories can be attributes according to the different physiological tolerance of fish to the environmental variability, to the types of migratory and reproductive behavior of species and to the different ways species of the assemblage use estuarine environment (Elliott et al., 2007; Franco et al., 2008; Vasconcelos et al., 2011). Nevertheless, transitional water ecosystem fishes are mainly classified according to their migratory behavior (Perez-Ruzafa et al., 2006), based on life-history and reproductive strategy. Fish can be divided in guilds, where a guild is defined as a group of species which exploits in the same way the same environmental resources (Elliott et al., 2007; Root, 1967). Following the guild approach, reviewed in Elliott et al. (2007), Franco et al. (2008) and Potter et al. (2015), fish can be generally divided in: 1) Lagoon/Estuarine Residents (ES), fish able to spend their whole life cycle (or most of it) within the lagoon, including reproduction, and have wide tolerance to variation in environmental conditions; 2) Marine migrant (MM), species that spawn at sea and often enter estuaries in large numbers and particularly as juveniles. This category can be subdivided into i) marine estuarine-opportunist, marine species that regularly enter estuaries in substantial numbers, particularly as juveniles, but use nearshore

marine water as an alternative habitat, ii) marine estuarine dependent, marine species that require sheltered estuarine habitats as juvenile but live along coasts where there are no such habitats and these species are thus dependent on the habitats of that type that are present in estuaries. Moreover, some fish species, the juvenile marine migrant, are found into transitional water ecosystems especially during the first period of their life stages (Franzoi et al., 2010). 3) Marine stragglers (MS), species that spawn at sea and enter estuaries only in low numbers and sporadically; 4) Diadromous, species that migrate between the sea and the freshwater. They could be Anadromous (AN), when most growth is at sea before migration into rivers to spawn, or Catadromous (CA), when they spend their trophic life in fresh water and subsequently migrate out to sea to spawn. 5) Freshwater species (FW), fish that spawn in freshwater and which are present occasionally in transitional waters and can be found in oligohaline zone of estuaries and lagoons.

Among fish which inhabit transitional water ecosystems, strong relevance must be given to “marine migrant” fish species, which completely colonize shallow water habitats to exploit the large availability of spatial and trophic niches present in these ecosystems (Beck et al., 2001; Elliott and Hemingway, 2002; Elliott et al., 2007; Rossi, 1986; Vasconcelos et al., 2010). Therefore, transitional water ecosystems represent essential habitats for juvenile marine migrant fish species, performing the function of elective nursery areas for their juvenile stages (Beck et al., 2001; Boesh and Turner, 1984; Cabral et al., 2007; Dahlgren et al., 2006; Deegan et al., 2000; Elliott and Hemingway, 2002; McLusky and Elliott, 2004; Mendes et al., 2014; Vasconcelos et al., 2007, 2008; Whitfield and Pattrick, 2015).

After spawning at sea, large numbers of eggs and larvae of “marine migrant” fish arrive near the coasts transported through the sea currents without any parental assistance (Cowen et al., 2000, 2006; Elliott and Hemingway, 2002; Legget et al., 1984; Miller et al., 1984; Miller, 1988; Vasconcelos et al., 2008, 2010). The entrance of marine migrant fish inside transitional water ecosystems can take place both actively or can be linked to a tidal flow (Chiappa-Carrara et al., 2003; Crawford and Carey, 1985; Das et al., 2000; Ferrari et al., 1985; Forward et al., 1998; Henri et al., 1985; Pattrick and Strydom, 2014; Ricardo et al., 2014; Smith and Stoner, 1993), even if it is not purely passive. In fact, the larvae have both endogenous rhythms of behavior and functional sensory systems to perceive environmental signals. Increases in body size and swimming capabilities allow individuals to perform active movements as vertical migrations for the selection of different water masses (Boehlert and Mundy, 1988; Islam et al., 2007; Pattrick and Strydom, 2014; Rijnsdrop et al., 1985; Schultz et al., 2003; Vasconcelos et al., 2011).

Even if some habitats could perform an important nursery function even if they were not placed within the transitional water ecosystems (Able, 2005; Able et al., 2006), upon entering in transitional environments, juvenile migrant fish quickly settle in shallow-water habitats and many works hypothize and agree that in these habitats they probably benefit from higher food abundance and lower predation risks (Beck et al., 2001; Cabral et al., 2007; Dahlgren et al., 2006; Elliott and Hemingway, 2002; McLusky and Elliott, 2004; Turnois et

al., 2013; Vasconcelos et al., 2010, 2011; Whitfield and Pattrick, 2015). During the period within the transitional water ecosystem, juveniles use a mosaic of habitats and only few species are confined to a single nursery habitat (Bostrom et al., 2011; Nagelkerken, 2007; Nagelkerken et al., 2015; Sheaves et al., 2015). In general, fish move among different habitats, according to several abiotic and biotic factors (e.g. salinity, water temperature, food availability, sediment type, presence of vegetation, hydrodynamic, position regard sea-lagoon gradient) and to ontogenetic stage of different species (Able, 2005; Adams et al., 2006; Brown et al., 2016; Cabral et al., 2007; Elliott and Hemingway , 2002; Herzka, 2005; Nagelkerken et al., 2015; Stoner et al., 2001; Vasconcelos et al., 2010, 2011; Whitfield and Pattrick, 2015). After a variable period of growth ranging from months to years depending on species, individuals migrate back to the sea to recruit into the adult population (Able, 2005; Beck et al., 2001; Gibson, 1973, 1994; Gillanders et al., 2003; Miller et al., 1985; Nagelkerken et al., 2000; Reis-Santos et al., 2015; Vasconcelos et al., 2008).

Inside transitional water ecosystems, juvenile marine migrant fish find more suitable condition for metabolic growth, namely high food availability, refuge from predators, favorable water temperature and low biotic stress (e.g. less predation) (Beck et al., 2001; Blaber and Blaber, 1980; Cabral et al., 2007; Dahlgren et al., 2006; Elliott and Hemingway, 2002; Gibson, 1994; Gillanders et al., 2003; McLusky and Elliott, 2004; Miller et al., 1985; Tournois et al., 2013; Vasconcelos et al., 2010, 2011; Whitfield and Pattrick, 2015).

Evolution of nursery function concept

The presence of transient species inside the transitional water ecosystems and their use as nursery areas has been long known (Beck et al., 2001; Gunter, 1967; Vasconcelos et al., 2011). Even if they were carried out in large estuaries, first observations, concerning the blue crab in the Atlantic coast of United States of America, were made by Hay in 1905 (Beck et al., 2001). Gunter and Deegan, respectively in 1945 and in 1993, affirmed that “the young of many animals usually thought of as marine, require areas of low salinity for nursery grounds” (Able, 2005) and “estuarine fish faunas around the world are dominated in numbers and abundance by species which move into estuary as larvae, accumulate biomass and then move offshore” (Beck et al., 2001). Subsequently, the marine-estuarine life-history was considered a general law for many fish species (Gunter, 1967) and it has been established that these species were found inside transitional water ecosystems and colonized them during the juvenile life stages.

The “nursery” term and concept remained rather vague and undefined until the work of Beck et al. (2001) according to which, “a habitat is considered as a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult population is greater, on average, than production from other habitats in which juveniles occur”. Initially, the entire transitional water ecosystem (lagoon or estuary) was considered as a nursery area (Beck et al., 2001; Deegan, 1993). Subsequently, the

attention gradually shifted towards specific habitats within coastal transitional and marine ecosystems, especially mudflats, salt marshes, mangrove forests and seagrass beds (Beck et al., 2001).

Following the Beck et al. (2001) definition, to maximize the contribution of a habitat to adult recruitment, must be great the combination of four factors: 1) density, 2) growth rate, 3) survival of juveniles, and 4) movement to adult habitats. Consequently, not all the areas occupied by juveniles can be considered a nursery (Beck et al., 2001). These concepts definitively clarify the difference between juvenile and nursery areas (Able, 2005). But Dahlgren et al. (2006) comments the Beck et al. (2001) approach because it does not consider the importance of habitat size. For example, two habitats used by juveniles, the first covers 90% of an estuary and contributes 85% of juveniles to adult population, the second covers 10% and contributes 15%. The contribution per unit area is therefore respectively 0.94 (85/90) and 1.5 (15/10) (Dahlgren et al., 2006). Using the Beck et al. (2001) approach, only the second will be considered nursery because it has a greater contribution, however the first provides 5 times more individuals to the population (Dahlgren et al., 2006).

To overcome this error related to habitat size, Dahlgren et al. (2006) define a new term, the Estuarine Juvenile Habitats (EJH) to describe juvenile habitats that contribute a greater overall proportion of individuals to adult populations (Dahlgren et al., 2006). Therefore, it becomes essential to directly measure and track the movement of individuals from juvenile habitats to the adult population, using natural or artificial marker (otolith chemical composition, genetic markers, stable isotopes, artificial tags) (Avigliano et al., 2017; Brown, 2006; Campana, 1999; Fuji et al., 2016; Gillanders, 2002; Hart et al., 2015; Kerr and Campana, 2013; Reis-Santos et al., 2013; Thorrison et al., 2011; Thorrold et al., 2001; Trueman et al., 2012).

However, over time, the criteria to identify a nursery habitat is evolving (Vasconcelos et al., 2007). Despite the new useful definition of Dahlgen et al. (2006) many aspects of nursery value of transitional water ecosystem (e.g. the presence of food/resources, the connectivity between habitats within the lagoon, the sea-lagoon connectivity, the presence of shelter zone) are still underestimated (Nagelkerken et al., 2015; Sheaves, 2009; Sheaves et al., 2006, 2015). Indeed, due to the high complexity, many useful approaches to estimate the nursery value of a transitional water ecosystem, individually, are not able to provide a complete view of the problem (Sheaves et al., 2015).

To identify the true nursery value of a habitat it is therefore essential to understand and consider all the complex dynamics that support nursery function (Sheaves et al., 2015), combining various approaches and techniques. Even if density of individuals could help understand the role of different transitional water ecosystem habitats, many other factors must be considered to identify the true nursery habitats. To provide a rich and exhaustive knowledge of the nursery value of a transitional water ecosystem, different ecological components, that vary from situation to situation, must be considered: the connectivity between habitat and the marine environment, the characteristics and presence of habitats on the near seascape, the population

dynamics of the investigated species (survival and growth in nursery habitats), the ontogenetic migrations, the influence of physicochemical conditions, the interspecific interactions (in particular the role played by predation), the eco-physiological factors, the diet shifts, the availability, distribution and the dynamics of the resources/prey used and the presence of shelter zones (Sheaves et al., 2015). In particular, the trophic function of the different habitats within the lagoon, influencing the individual's growth rates, appears essential to determine the lagoon nursery role (Able, 2005; Beck et al., 2001; Phelan et al., 2000; Ross, 2003; Vasconcelos et al., 2011).

Management importance of transitional waters

It is well known that transitional water ecosystems, occupying highly prized locations, are some of the most heavily used and threatened natural systems in the planet (Barbier et al., 2011; Bassett et al., 2013; Lotze et al., 2006; Sheaves et al., 2015; Worm et al., 2006). This deterioration due to human activities (e.g. habitat destruction, pollution) is intense and could alter the composition and diversity of natural communities, as well as their capabilities to support good and services (Cossarini et al., 2008; Newton, 2018). Is known that these changes affect the number of viable fisheries (33% decline) and the provision of nursery habitats (69% decline) (Barbier et al., 2011).

Transitional water ecosystems are also strongly threatened by climate change (e.g. rise in sea level, temperature increase, changes in river flows) (Bassett et al., 2013; Sheaves et al., 2015; Vasconcelos et al., 2007; Tagliapietra et al., 2011). According to global population projections, by the year 2025, 75% of the world's population may reside in coastal areas (Hinrichsen, 1998; Adams et al., 2006). Unfortunately, ecological needs and human demands can conflict sharply (Borde et al., 2003; Chittaro et al., 2009) and some habitats used by juvenile fish, (e.g. saltmarsh) are extremely vulnerable to degradation or loss (Brown, 2006) and can easily alter, reduce or disappear (Tagliapietra et al., 2011).

Concerning the importance and fragility of transitional water ecosystems, it becomes essential to deepen the study about the transitional water ecosystems and the nursery role of the coastal lagoon for marine migrant fish. The increasing difficulty of protecting an entire ecosystem, due to limited time and funds (Mohan et al., 2015), has prioritized the conservation of specific and higher quality habitats. Consequently, it appears extremely important to identify and protect the most threatened and true nursery habitats that provide the most recruits to adult populations (Mohan et al., 2015; Sheaves et al., 2015). The identification of nursery areas is indeed a very important tool to generate strategies for the maintenance of fishery resources (Avigliano et al., 2017; Beck et al., 2001) and thus for the maintenance and conservation of the precious ecosystem services provided by transition water ecosystems (Newton, 2018).

AIM OF THE STUDY

In the lagoon of Venice, till now, numerous studies have been conducted concerning fish fauna (Cavraro et al., 2011, 2013, 2014, 2017a, 2017b, 2018; Fiorin et al., 2007; Franco et al., 2002, 2003, 2006a, 2006b, 2006c, 2009, 2010, 2012a, 2012b; Franzoi et al., 2002, 2010; Franzoi and Pellizzato, 2002; Mainardi et al., 2002, 2004, 2005; Malavasi et al., 2004, 2005, 2007; Pranovi et al., 2013; Riccato et al., 2003; Scapin et al., 2018a, 2018b; Zucchetta et al. 2009, 2010, 2012, 2016). However, regarding juvenile marine migrant fish, few studies to determine nursery values, combining various approaches (e.g. food or environmental preferences, sea-lagoon connectivity), have been conducted.

The general aim of this PhD project was to deepen the knowledge about the nursery function played by coastal lagoons, using the Venice lagoon as a study area. Indeed, the identification of nursery habitats is an extremely important tool for the maintenance and conservation of the ecosystem services provided by transitional water ecosystems (Newton, 2018) and to generate strategies for the maintenance of fishery resources (Avigliano et al., 2017; Beck et al., 2001). Moreover, identify nursery habitats could help to prioritize the management actions towards specific and valuable habitats or portions of the lagoon, due to the increasing difficulty in protecting an entire ecosystem (Mohan et al., 2015). Indeed, juvenile marine migrant fish use a mosaic of habitats, even daily, for different purpose, and only few species are confined to a single nursery habitat. In this study, an integrated approach was used, considering as many factors as possible (e.g. the sea-lagoon connectivity, the use of lagoon habitats during ontogenetic growth, the response of individuals to abiotic conditions, the trophic relations within the habitats).

The PhD project and thus the thesis is organized in different parts and chapters each of which focuses on a different spatial scale and consider different factors in order to explore the nursery function of the study area. The research activity addressed the following aspects:

- The study of sea-lagoon connectivity, investigated at the level of the entire lagoon basin. Was evaluated the distribution of eggs, larvae, post-larvae and juveniles of marine migrant species along three ideal sea-lagoon transects, identified in the three main sub-basins (north, central and south) in which the Venice lagoon can be divided. Along these transects, sampling of ichthyoplankton, larvae and juvenile were performed during the peak of fry migration within the lagoon for two years.

  CHAPTER 1: <u>ENTRANCE AND DISTRIBUTION INTO THE VENICE LAGOON OF EGGS, LARVAE AND JUVENILE OF MARINE MIGRANT FISH: SEA-LAGOON CONNECTIVITY</u>

- The study of the use of shallow lagoon habitats by most abundant juvenile marine migrants, evaluating the role of different types of shallow water lagoon habitat as nursery areas and elucidating the influence and importance, during ontogenetic growth within the lagoon, of the different abiotic

and biotic characteristics of habitat in determining the distribution and abundance of individuals. Seven sampling campaigns has been conducted in the northern sub-basin of the Venice lagoon during the period of entry and growth of many marine migrant species, from late winter to the end of spring 2016.

CHAPTER 2: MARINE MIGRANT JUVENILES DISTRIBUTION DYNAMIC IN THE NORTHERN VENICE LAGOON

- The study of growth, condition and trophic ecology of a target juvenile marine migrant species abundant, highly prized and with high economic importance, the sea bream *Sparus aurata*, in a narrow spatial scale, the saltmarsh habitats. For this purpose, in 2016 the post-larvae and juveniles of this species were sampled in tidal saltmarsh habitats located at the two ends of the sea-lagoon gradient, thus having different biotic and abiotic characteristics. When possible, for each station, two saltmarsh habitat types were considered: tidal creek and saltmarsh edge. The study of the ontogenetic changes in diet has envisaged the use of various methodological approaches: the direct analysis of the stomach contents, the analysis of the stable isotopes of carbon and nitrogen, the morphometric analysis of the changes in shape of the head and the analysis of secondary production.

CHAPTER 3: FEEDING ECOLOGY AND SECONDARY PRODUCTION OF GILTHEAD SEABREAM'S JUVENILES IN A SALTMARSH HABITAT OF VENICE LAGOON

The information collected in this study will allow us to better understand the dynamics and the importance of the different lagoon habitats, evaluating the factors that increase the total true nursery value of a lagoon, to direct the actions of restoration and management. Only through the integration of these different approaches and the overall interpretation of the results it will be possible to evaluate the complex nursery function carried out by the lagoon of Venice.

STUDY SITE

The Venice lagoon (45° 26' N, 12° 20' E) (fig. 1), located in the north-east of Italy with a longitudinal axis in the north-south direction, is about 50 km long and 10 km wide (Gacic et al., 2004). With a total surface area of about 550 km^2, of which around 400 km^2 is open water surface (Brigolin et al., 2014), the Venice lagoon is the largest Italian and Mediterranean (Rapaglia et al., 2011, 2015; Franco et al., 2006a) lagoon. It is a shallow coastal lagoon ecosystem with an average depth of 0.8 m (Rapaglia et al., 2015) and only 5% of the lagoon deeper the 5 m (Brigolin et al., 2014). The large shallow areas, covering about 75% of the total surface (Molinaroli et al., 2009) are connected by a network of natural and man-made channels whose depth is less than 2 m (Solidoro et al., 2002). Three deeper shipping channels (500-1000 m wide and 25-50 m deep, Brigolin et al., 2014, Gacic et al., 2004) connect the lagoon with the Adriatic Sea through three wide mouths (Lido, Malamocco and Chioggia) and allow the exchange of water with the sea with an average total tidal discharge of 6500 $m^3 s^{-1}$ (Cucco and Umgiesser, 2006; Gacic et al., 2002; Rapaglia et al., 2015). Through these inlets the exchange of water in each tidal cycle is about a third of the total volume of the lagoon (Gacic and Solidoro, 2004) and water renewal occurs in few days in the areas closest to the inlets and up to 30 days in the inner part (Cucco and Umgiesser, 2006). Tidal flows enter the lagoon with a range of ±30 cm during neap tide and ±110 cm during spring tide (Rapaglia et al., 2011). In addition to tidal phases, water exchange is strongly affected by weather conditions (Cucco and Umgiesser, 2006). The Venice lagoon is subjected to Bora and Scirocco, the northeasterly and the southeasterly wind systems of the Adriatic Sea (Umgiesser et al., 2004). During the autumn period, when low atmospheric pressure is present in this area, Scirocco wind systems, that blow from the South East to the central part of the Adriatic Sea cause the biggest events of flooding in the city of Venice and other supra-idal areas (Gacic et al., 2004; Umgiesser et al., 2004). Conversely, freshwater inputs are relatively low in Venice lagoon, with an average annual river discharge of amounts 35.5 $m^3 s^{-1}$ (Gacic et al., 2004; Zuliani et al., 2001, 2005).

Having more than one inlet, the Venice lagoon can be considered a "systems of lagoons" rather than a single lagoon and can be divided in four sub-basins (Molinaroli et al., 2009; Tagliapietra and Ghirardini, 2006): Treporti, Lido, Malamocco, and Chioggia. However, traditionally, according to its hydrology, the Venice lagoon is divided in three main sub-basins: Northern, Central and Southern connected with the sea respectively by Lido, Malamocco and Chioggia inlets (Avanzi et al., 1979). The northern sub-basin is the widest (about 260 km^2 wide) and includes the cities of Venice, Murano, Burano and other inhabited islands (Franco et al., 2006a). Compared to the other two sub-basins, the northern is the one with the lowest salinity due to the main freshwater tributaries in the lagoon (Dese, Vela, Osellino and Lusore) which flow in more than the 50% of freshwater of the whole inputs (Zonta et al., 2005; Zuliani et al., 2005). The central sub-basin (about 186 km^2 wide) is characterized by a large canal (Canale Malamocco Marghera) which connects Malamocco's

sea inlet to the industrial harbor of Marghera producing the highest water exchange with the sea (10718 m^3 s^{-1}) (Cucco and Umgiesser, 2002). The Southern sub-basin (about 105 km^2 wide), which hosts the town of Chioggia is the one with the lowest exchange of water with the sea and lowest freshwater inputs (Franco et al., 2006a; Cucco and Umgiesser, 2002).

As many transitional water ecosystems, typically characterized by high level of spatial heterogeneity and physico-chemical gradient (Elliott and Hemingway, 2002; McLusky and Elliott, 2004), the Venice lagoon is characterized by a variety of ecological shallow habitats interconnected which have different functional roles (Franzoi et al., 2010). Among the most valuable habitats emerge seagrass beds, sand flats, mud flats, salt marshes and tidal creeks inside salt marshes (Franco et al., 2006a; Franzoi et al., 2010; Malavasi et al., 2005; Molinaroli et al., 2009). The Venice lagoon indeed constitutes a representative and complex example of social-ecological system (Rova and Pranovi, 2017; Rova et al., 2019) being characterized by various economic activities as tourism, fishing, acquaculture, industrial activities, maritime shipping and port and agriculture (Rova et al., 2015, 2019). Among the different provisioning services of the Venice lagoon, most of the products consist in seafood (Rova et al., 2015), making fishing and aquaculture extremely important.

The Venice lagoon is included in different national and international protection plans (e.g. Birds Directive 79/409/CEE). Considering the Habitat Directive (92/43/CEE), the Venice lagoon is designated as a Special Protection Area (SPA, IT3250046) and the Northern and Central-Southern sub-basin are considered as Sites of Community Importance (SCI, respectively IT3250030 and IT3250031).

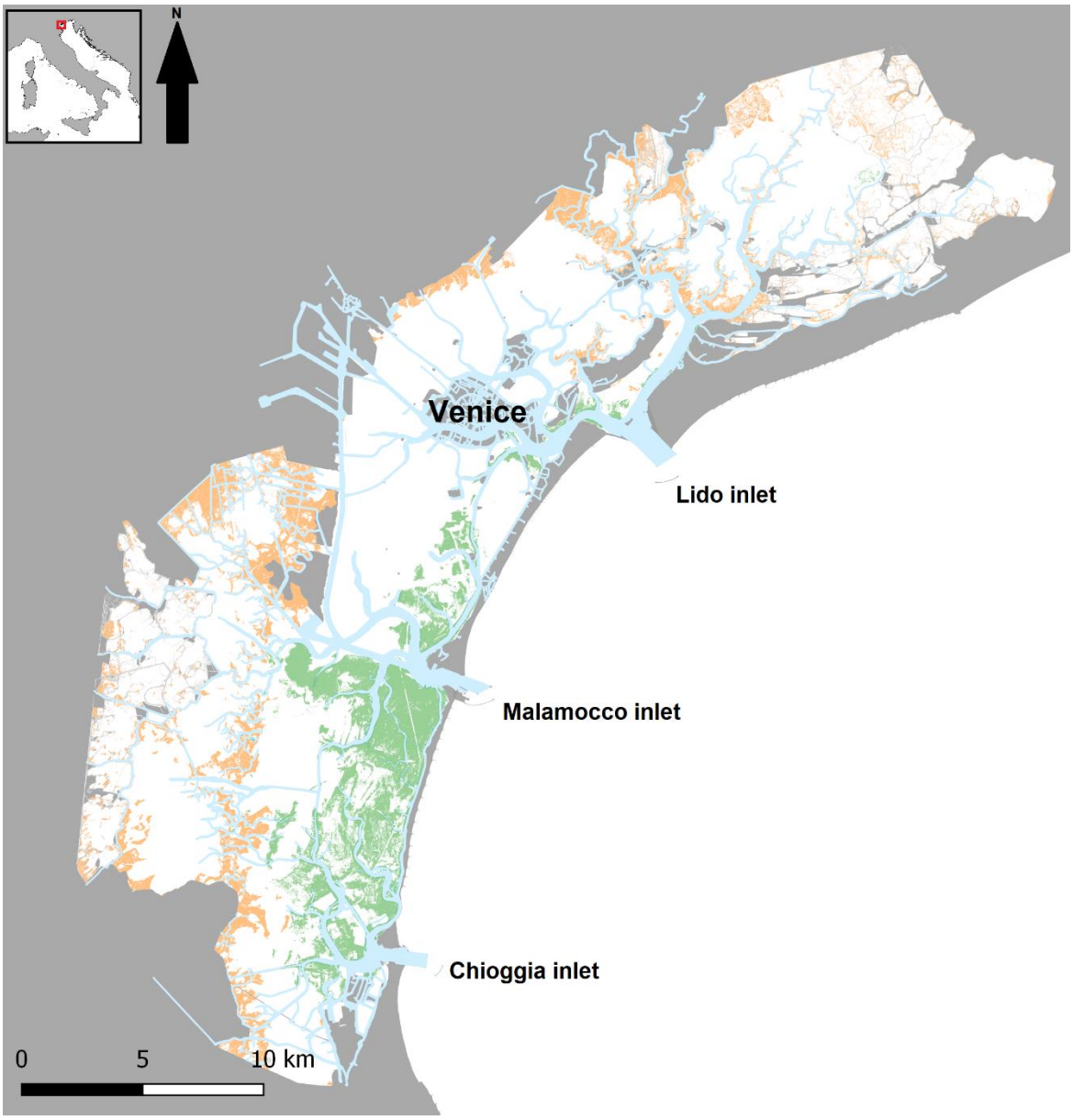


Figure 1 – Location of the study site, the Venice lagoon. In grey the land, in light blue the channels, in dark blue the marsh creeks, in green the seagrass beds and in light orange the saltmarshes.

CHAPTER 1

ENTRANCE AND DISTRIBUTION INTO THE VENICE LAGOON OF EGGS, LARVAE AND JUVENILE OF MARINE MIGRANT FISH: SEA-LAGOON CONNECTIVITY

1.1 Introduction

Sea-lagoon connectivity, and thus the maintenance of organism's flow from the sea to the lagoon and vice versa, represents a crucial aspect of the ecological functionality of coastal lagoons (Able, 2005; Able and Fahay, 2010; Gillanders, 2002; Gillanders et al., 2003; Herzka, 2005; Reis-Santos et al., 2015; Sheaves, 2005; Sheaves et al., 2015; Vasconcelos et al., 2012). Connectivity, defined as the rate of exchange of individuals of the same species among spatial units (Herzka, 2005; Polis et al., 1997; Sheaves, 2009), is a key feature of ecosystem functioning (Engelhard et al., 2017; Levin and Lubchenco, 2008) and is essential for population persistence and productivity (Olds et al., 2012). In the marine environment, connectivity among ecosystems and habitats is maintained by the movements of larvae, juveniles and adult fish (Engelhard et al., 2017; Hamilton et al., 2012; Welsh and Bellwood, 2014). This ecological connectivity is of particular importance for "marine migrant" fish (Franco et al., 2008), species which reproduce and spawn in the sea and perform periodic migrations between the marine environment and the transitional water habitats in order to exploit the large availability of trophic resources present in these coastal ecosystems. Estimating connectivity for estuaries and lagoons is relevant since these ecosystems perform an important nursery function for marine fishes (Gillanders, 2005; Vasconcelos et al., 2008).

After spawning at sea, eggs and larvae of juvenile marine migrant fish arrive near the coast moving through the sea currents (Cowen et al., 2000, 2006; Elliott and Hemingway, 2002; Legget et al., 1984; Miller et al., 1984; Miller, 1988; Vasconcelos et al., 2008, 2010). The entrance of juvenile migrant fish inside transitional water ecosystems is a critical part for the successful completion of a species' life cycle (Pattrick and Strydom, 2014) and is generally linked to the tidal flow, even if it is not purely passive (Chiappa-Carrara et al., 2003; Crawford and Carey, 1985; Ferrari et al., 1985; Forward et al., 1998; Henri et al., 1985; Pattrick and Strydom, 2014; Ricardo et al., 2014; Robins et al., 2012; Smith and Stoner, 1993). In fact, fish larvae have both endogenous rhythms of behavior and functional sensory systems to perceive environmental signals. Growth and increase in body size and swimming capabilities allow individuals to perform active movements (e.g.

vertical migrations) for the selection of different water masses (Boehlert and Mundy, 1988; Islam et al., 2007; Pattrick and Strydom, 2014; Rijnsdrop et al., 1985; Schultz et al., 2003; Vasconcelos et al., 2011). After entering in the transitional water environment, juvenile migrant fish quickly settle in shallow-water habitats, where tidal currents are weaker, reducing the risk of being transported back into the sea with the ebb tide (Bohelert and Mundy, 1987, 1988; Creutzberg et al., 1978; Elliott and Hemingway, 2002; Pattrick and Strydom, 2014; Perez-Ruzafa et al., 2004; Vasconcelos et al., 2011). Even the shape of the coastline, the morphology of the estuary and the presence or absence of available settlement habitats can affect the retention of fish larvae (Gillanders et al., 2011). After a period of growth within transitional water habitats, different in time depending on species, individuals migrate back to the sea to recruit into the adult populations (Able, 2005; Beck et al., 2001; Gibson, 1973, 1994; Gillanders et al., 2003; Miller et al., 1985; Nagelkerken et al., 2000; Vasconcelos et al., 2008).

To exploit the favorable conditions inside transitional water ecosystems (e.g. trophic and hydrodynamic), the entrance and the distribution within the transitional water ecosystem is a crucial step for marine migrant fish species. The distribution and abundance of eggs, larvae and post-larvae of marine migrant species in transitional water ecosystems is closely linked to the hydraulic circulation and to the chemical-physical conditions of the water (Chiappa-Carrara et al., 2003; Perez-Ruzafa et al., 2004). For this reason, this biotic component could represent an adequate bio-monitor of the sea-lagoon connectivity. The study of the entrance into the transitional water ecosystems of juvenile marine migrant fish and the identification, characterization and localization of elective habitats for juveniles represent important elements supporting the management of lagoon ecosystems (Avigliano et al., 2017; Beck et al., 2001; Colloca et al., 2009; Sheaves et al., 2015).

In the upper Adriatic Sea, many species with marine reproduction, which represent important stocks exploited for fishing, at the juvenile stages are concentrated in shallow water habitats of coastal transition environments. This group includes sea bream *Sparus aurata*, sea bass *Dicentrarchus labrax*, flounder *Platichthys flesus*, sole *Solea solea*, mullets *Chelon ramada*, *C. auratus*, *C. saliens*, *C. labrosus* and *Mugil cephalus*. The first arrivals of these species are characterized by early life history stages with standard length generally lower than 20 mm (Franzoi et al., 1989, 2005; Franzoi and Trisolini, 1991; Rossi 1986). Even the larval and juvenile stages of anchovy *Engraulis encrasicolus*, sardine *Sardina pilchardus* and sprat *Sprattus sprattus* are seasonally abundant in coastal marine environment and within the lagoon ecosystems. In the lagoon environment of the upper Adriatic Sea, the main peak of young marine migrant presence is recorded in late winter - early spring, even if a second peak of fry migration is observable at the end of summer - early autumn (Rossi, 1986). These species then migrate back to the sea during the late autumn months, in correspondence with the abrupt decrease of lagoon water temperature (Franzoi et al., 1989; Rossi, 1986). Numerous studies of the fish fauna of shallow water lagoon habitats of the Venice lagoon have been

conducted (Franco et al., 2003, 2006a, 2006b, 2006c, 2012a, 2012b; Franzoi et al., 2010; Mainardi et al., 2002, 2004, 2005; Malavasi et al., 2004, 2005, 2007; Riccato et al., 2003) highlighting the importance of these habitats as potential nursery areas for marine migrant fish species (Franco et al., 2006a, 2010; Franzoi et al., 2005; Franzoi and Pellizzato, 2002; Zucchetta et al., 2009, 2010). Unfortunately, until now, the information about the ichthoplanktonic component is very limited (Cavraro et al., 2017a; Spartà, 1942, Varagnolo 1964, 1971, Ziraldo, 1996).

The juvenile individuals which every year are distributed in shallow water lagoon habitats enter in the Venice lagoon through the inlets of Lido, Malamocco and Chioggia. These three sub-basins differ markedly in terms of hydrodynamic, morphological, biological characteristic and in relation to the anthropic pressures that insist on them (Molinaroli et al., 2009; Solidoro et al., 2004, 2010).

From December to May, during the peak of fry migration in Venice lagoon, the sampling of both ichthyoplankton and juveniles fish fauna was conducted in pre-set stations distributed along three ideal sea-lagoon edge transects, in marine and lagoon areas. In this two-year study, from 2015 to 2017, the entire Venice lagoon was considered. The aim of the study was to assess the sea-lagoon connectivity, which is an important component for the evaluation of potential nursery role of the different parts of the Venice lagoon, highlighting any differences between sub-basins and between marine migrant fish life stages. The hypothesis tested in this chapter is that some portions of the lagoon and some sub-basins, differing from each other in winds, currents, water exchange, morphology of the sea inlet and habitat complexity, play a different role in attracting the marine migrant fish.

1.2 Materials and Methods

1.2.1 Field and laboratory activities

Samplings took place between 2015 and 2017 in all the three lagoon sub-basins (Ghezzo et al., 2010), influenced by the Lido inlet (North sub-basin), the Malamocco inlet (Central sub-basin) and the Chioggia inlet (South sub-basin) (fig. 2, 3, 4). In each sub-basin, two different sampling activities were carried out: the collection of samples of ichthyoplankton (fish eggs and larvae) in marine areas and in the main channels within the lagoon, and the collection of fish fauna at the post-larval and juvenile stages in marine areas and lagoon shallow waters (depth < 1.5 m). The sampling methods have been standardized to guarantee spatial and temporal comparisons.

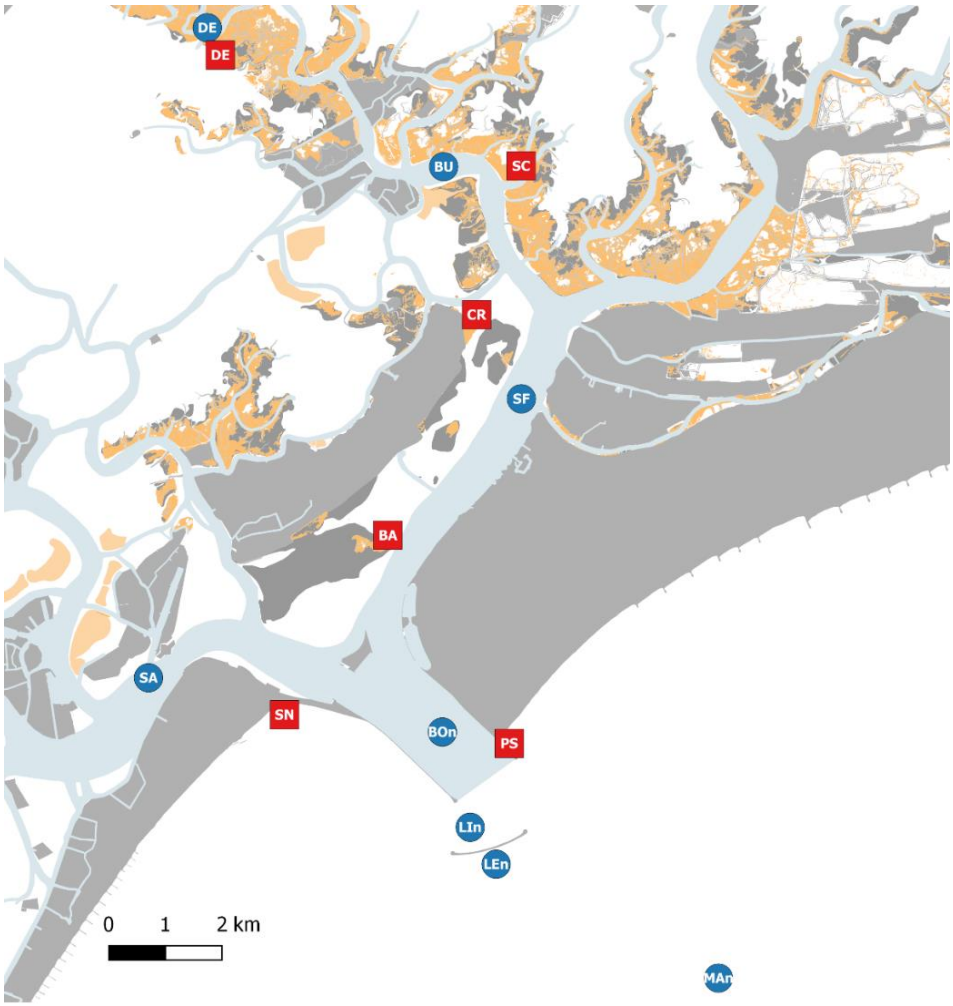


Figure 2 – Sampling sites in northern sub-basin of Venice lagoon, Lido inlet. blue = bongo net sampling, red = seine net.

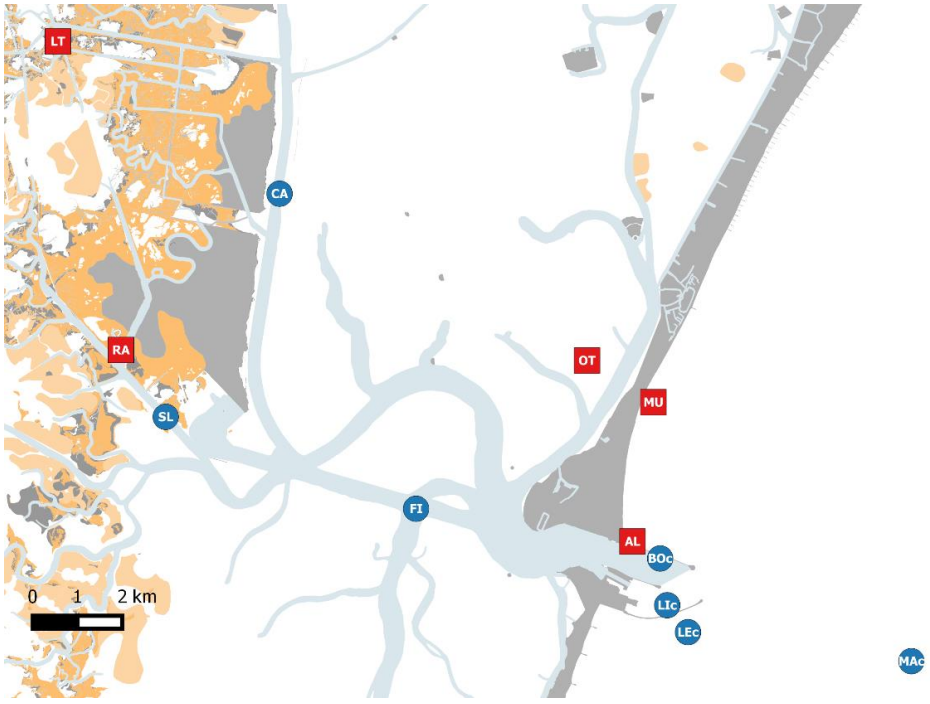


Figure 3 - Sampling sites in central sub-basin of Venice lagoon, Malamocco inlet. blue = bongo net sampling, red = seine net.

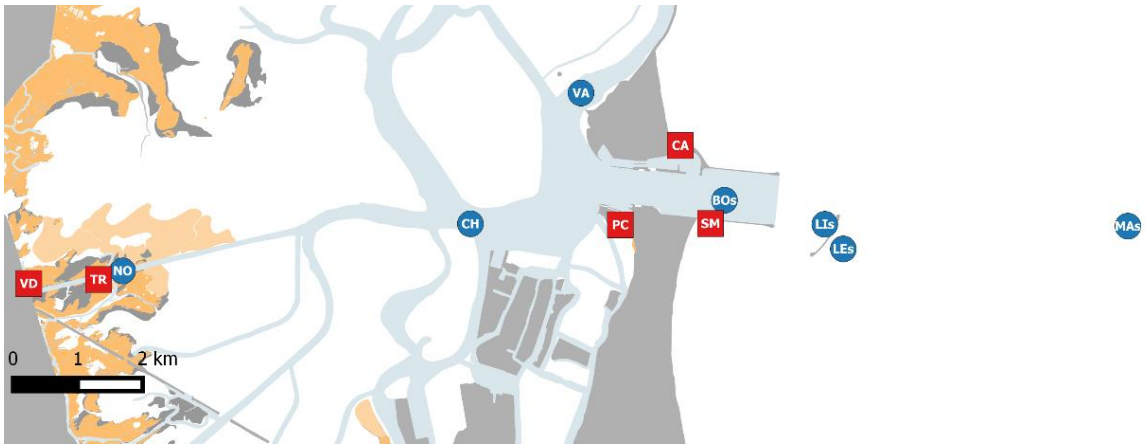


Figure 4 - Sampling sites in south sub-basin of Venice lagoon, Chioggia inlet. blue = bongo net sampling, red = seine net.

Ichthyoplankton

Three sea-lagoon transects have been identified through the inlets and along each transect seven sampling stations were chosen: three in the sea, one inside the inlet and three along the lagoon channels directly influenced by the water entering from the sea (fig. 2, 3, 4). To explore the entire transect from the sea to the lagoon edge, one more confined sampling station was added in the North sub-basin (fig. 2). To carry out these activities, eight daily sampling campaigns were carried out: four from November 2015 to April 2016 and four from November 2016 to March 2017 (tab. 1).

Table 1 - Sampling dates for the collection of eggs and larvae with bongo net.

Campaign	Sampling year	Lido inlet	Malamocco inlet	Chioggia inlet
I	I	25/11/2015	30/11/2015	01/12/2015
II	I	28/01/2016	26/01/2016	27/01/2016
III	I	10/03/2016	11/03/2016	11/03/2016
IV	I	05/04/2016	06/04/2016	04/04/2016
I	II	02/12/2016	30/11/2016	01/12/2016
II	II	30/01/2017	27/01/2017	28/01/2017
III	II	27/02/2017	28/02/2017	01/03/2017
IV	II	29/03/2017	30/03/2017	28/03/2017

During each sampling campaign, in each station, samples of ichthyoplankton were collected using two coupled nets called “bongo net”, respectively with 350 and 500 µm mesh size, according to the FAO standards (fig. 5): each net is 250 cm long and has a 60 cm mouth (internal diameter). Each net is equipped with a flowmeter, placed at the entrance, for the measurement of the towing length. This information was then used to calculate the theoretical volume explored and the ichthyoplankton density. Ichthyoplankton samplings were always conducted during the flood phase of spring tide. At each station, a five-minute oblique

haul was made, allowing the exploration of the entire water column (Società Italiana di Biologia Marina – Ministero Dell'Ambiente, 1990). The haul was carried out opposite to the current direction, at a speed of one-two nodes. Each sample was immediately fixed in neutralized 5% formalin.

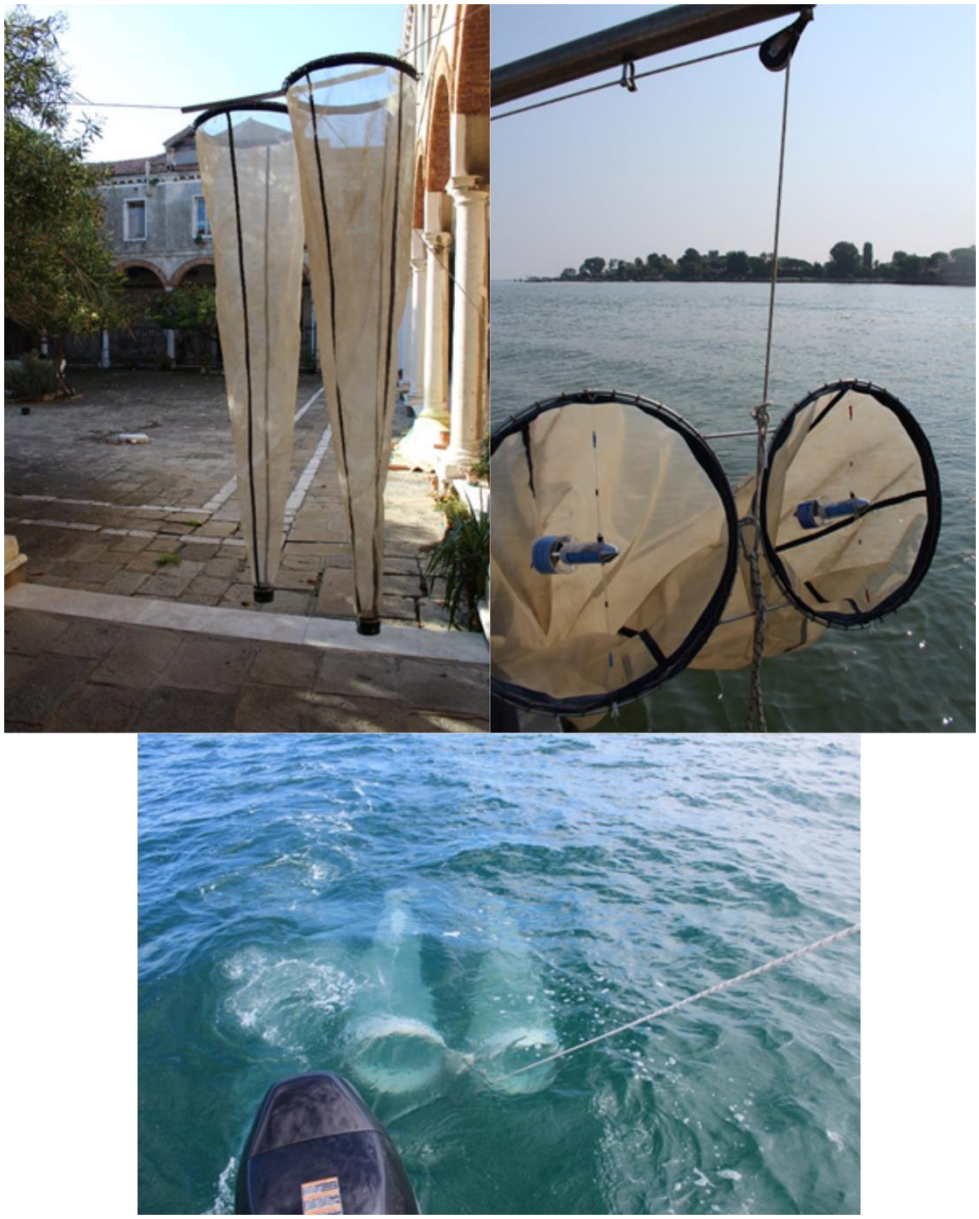


Figure 5 - Work phases with bongo net.

In the laboratory, ichthyoplankton samples were filtered and rinsed to remove any formalin residue. Each sample was observed in full under the stereomicroscope (6.3x-80x magnification), to isolate the eggs and larvae of fish, which were then individually identified to the lowest possible taxonomic level (fig. 6). In the case of the eggs, identification was possible at family level and, only in a few cases, up to the level of genus or species. In the case of larvae, it was possible to identify individuals, with few exceptions, up to genus or species level. A large quantity of bibliographic material was used to identify the ichthyoplanktonic forms found in the samples (Aboussouan, 1964; Arbault and Boutin, 1968; Cunningham, 1889; D'Ancona and Lo Bianco, 1931-33; FAO, 1987; Fraser and Thorson, 1976; Lee, 1966; Marinaro, 1971, 1991a, 1991b; Munk,

2005; Palomera and Rubies, 1977; Raffaele, 1888; Ré and Meneses, 2009; Richards, 2006; Russell, 1976; Saka, 2001; Spartà, 1942; Tsikliras, 2010; Varagnolo, 1964), since a taxonomic key to identify eggs and larvae of Mediterranean Teleost is not yet present in the literature. After the taxonomic identification, each taxon has been assigned to an ecological guild (Franco et al., 2008; Franzoi et al., 2010).

Figure 6 – Left to right. Up: eggs and larvae of Platichthys flesus. *Center: eggs and larvae of* Solea solea. *Down: eggs and larvae of* Sprattus sprattus.

Post-larvae and juveniles

In each of the three sub-basins, along three ideal transects, five fish sampling stations were chosen in shallow water areas (water depth <1.5 m) located both inside (lagoon, three stations) and outside (sea, two stations) the sea inlet (fig. 2, 3, 4). As for ichthyoplankton, one more confined sampling station was added in the North sub-basin (fig. 2) and the sampling stations remained the same both years. In the lagoon stations, except on two occasions when the weather and tidal conditions did not allow it, two hauls were made, one on the saltmarsh edge and one in the tidal creek inside the saltmarsh. For these activities, six daily sampling campaigns were carried out, from February to April 2016 and from March to May 2017 (tab. 2).

Table 2 - Sampling dates for the collection of juveniles with seine net.

Campaign	Sampling year	Lido inlet	Malamocco inlet	Chioggia inlet
I	I	19/02/2016	18/02/2016	22/02/2016
II	I	21/03/2016	19/03/2016	24/03/2016
III	I	15/04/2016	11/04/2016	12/04/2016
I	II	09/03/2017	06/03/2017	08/03/2017
II	II	23/03/2017	20/03/2017	21/03/2017
III	II	03/05/2017	05/05/2017	02/05/2017

To collect fish, in each sampling site and sampling occasion, a beach-seine net (2 mm inter-knot) was trawled on shallow waters over an average area of 480 m^2 (fig. 7). The seine net is appropriate for catching small (<100 mm Total Length) and juvenile fishes inhabiting shallow-water estuarine habitats (Hemingway and Elliott, 2002; Rozas and Minello, 1997). According to the site-specific environmental conditions, the length of the net and the distance covered by fishing action might vary respectively from 8 to 20 m and from 20 to 80 m. The bottom surface explored by net during each sampling was calculated (trawl length x net width) in order to standardize the catches. All fish collected were sacrificed with an excess of 2-phenoxyethanol, preserved refrigerated until the arrival in laboratory and then frozen at -20°C.

Figure 7 - Work phases with seine net.

In the laboratory, the samples to be processed were removed from the freezer and left to thaw for 24 hours in the refrigerator at about 6°C. All individuals were identified by species, counted, measured to the nearest 0.1 mm (Standard Length SL) and weighed (precision 0.01 grams, Total Weight TW). In the case of samples with less than 100 individuals per taxon, the measurements were performed on all fish. In the case of more abundant samples, the measures were limited to a representative subset of at least 100 individuals per taxon. Fish were always identified following the scientific literature: Fisher et al. (1987), Gandolfi et al. (1991), Tortonese (1970, 1975), Whitehead et al. (1984-1986, 1988) and, limited to juvenile forms, Arias and Drake (1990), D'Ancona and Lo Bianco, (1932-1933), Ré and Meneses (2009). In the case of postlarvae and juveniles of Mugilidae, the classification was confirmed after the observation of the pattern of chromatophores (Franzoi et al., 1989; Serventi et al., 1996). The identification of specimens belonging to this Family were validated after leaving them three weeks in 8% buffered formalin. Individuals were then grouped in ecological guild following Franzoi et al. (2010).

Environmental parameters

For both ichthyoplankton and ichthyofauna samplings, in each station the main abiotic parameters were collected. Considering ichthyoplankton sampling, water temperature (±0.1 °C), salinity (±0.01 PSU), dissolved oxygen (±0.1 % saturation) and turbidity (±0.1 FNU) were recorded for the upper and lower layer of the sampled water column with Hanna Instrument 9829. Simultaneously, 200 mL of water were filtered on Whatman GF/F 47 mm diameter filters to determine, in laboratory, the total chlorophyll concentration (µg/L) in the water column following the method proposed by Lorenzen (1966), with a Trylogy Laboratory Fluorometer. Considering post-larvae and juvenile sampling, environmental parameters were recorded for the mid-water column and three cores of sediment (diameter 2 cm) were also collected to determine, in the laboratory, the total chlorophyll concentration of the upper 2 cm sediment (µg/g) following the above-mentioned method. In April, a core of sediment (diameter 3 cm) was collected in each station sampled with the seine net to determine the granulometry (% sand) of the upper 10 cm layer following the methodology reported in Sfriso et al. (2003) and the content of organic matter through loss of ignition method (Heiri et al., 2001) at 550°C.

1.2.2 Data analysis

The environmental data collected simultaneously to ichthyoplankton and juvenile fish samples were analyzed using a principal component analysis (PCA) after having been square root transformed.

Eggs, larvae and juvenile's abundance data were standardized in order to obtain comparable density measures (ichthyoplankton: number of individuals per m^3, juveniles: number of individuals per 100 m^2).

Density data was analyzed to highlight any differences in space and time, separately for eggs, larvae and juveniles. Four factor statistical tests (ANOVA) were performed basing on GLM (Generalized Linear Models) with negative binomial family (chi-square test on deviance; Venables and Ripley, 2002). The considered factors were: sampling campaign (four levels for ichthyoplankton, three levels for juveniles), sub-basin (three levels: North, Central, South), position, after having classified the sampling stations on the basis of their position respect the sea inlets (tab. 3) (two levels: sea, lagoon) and sampling year (two levels).

For the lagoon stations, the two hauls carried out in the two positions of the saltmarsh were considered as a single station and the densities were calculated adding the number of individuals collected during the two hauls and dividing it for the sum of the two sampled areas. Then, to highlight differences between saltmarsh edge and tidal creek, the previous tests were performed considering the two hauls of the lagoon stations as two habitats.

Table 3 - Classification of stations based on position to sea inlet.

Ichthyoplankton					
North sub-basin		**Central sub-basin**		**South sub-basin**	
position	**station**	**Position**	**station**	**position**	**station**
sea	MAn	Sea	MAc	sea	MAs
sea	LEn	Sea	LEc	sea	LEs
sea	LIn	Sea	LIc	sea	LIs
lagoon	BOn	lagoon	BOc	lagoon	BOs
lagoon	SA	lagoon	FI	lagoon	VA
lagoon	SF	lagoon	SL	lagoon	CH
lagoon	BU	lagoon	CA	lagoon	NO
lagoon	DE				

Juvenile					
North sub-basin		**Central sub-basin**		**South sub-basin**	
position	**station**	**position**	**station**	**position**	**station**
sea	PS	sea	AL	sea	CA
sea	SN	sea	MU	sea	SM
lagoon	BA	lagoon	OT	lagoon	PC
lagoon	CR	lagoon	RA	lagoon	TR
lagoon	SC	lagoon	LT	lagoon	VD
lagoon	DE				

To summarize the sea-lagoon degree of connectivity, a colonization index of lagoon waters was used (I_c) (Cavraro et al., 2017a),

$$I_C = \frac{DENS_L}{(DENS_L + DENS_S)}$$

Where $DENS_L$ represent the density of organisms within the lagoon (calculated as the average density recorded in stations classified as position "lagoon") and $DENS_S$ represent the density of organisms at sea (calculated as the average density recorded in the "sea" stations). The index was applied separately to eggs, larvae and juvenile marine migrant fishes. The colonization index can vary, theoretically, between 0, when individuals are present only at sea, and 1, when they are present only within the lagoon. Values above 0.5 can be considered as an indication of an accumulation of organisms within the lagoon environment.

To evaluate the progressive entrance of individuals within the lagoon from the sea, a "center of gravity" (COG) was also calculated. For each campaign and each sub-basin, centers of gravity were calculated on densities of total marine migrant component separated for eggs, larvae and juveniles. The density of each station was used to calculate the center of gravity (COG) of each sea-lagoon transect during each sampling campaign following the formula:

$$COG = \frac{\sum_{i=1}^{N} DENS_i \cdot dist_i}{\sum_{i=1}^{N} DENS_i}$$

where $DENS_i$ is the density at each station *i* of the transect during the considered campaign, $dist_i$ is the standardize distance of the station *i* from the "*sea station*", located 2 miles from the coast. The index was calculated standardizing for each sub-basin the distance from the inner to the further station of the transect, cumulating the bongo net and the seine net sampling stations. Because each transects has a different length, distances have been standardized from 0 to 1. The center of gravity can vary between 0, when individuals are present only at sea, and 1, when they are present only in the inner stations. Because of the standardized distances, for each sub-basin the station located in the sea inlet, which represents the entry point of individuals in the lagoon, has a different value. When the COG has a value lower than the one corresponding to the sea inlet it means that individuals are more concentrated in the sea stations and vice versa.

1.3 Results

1.3.1 Ichthyoplankton sampling

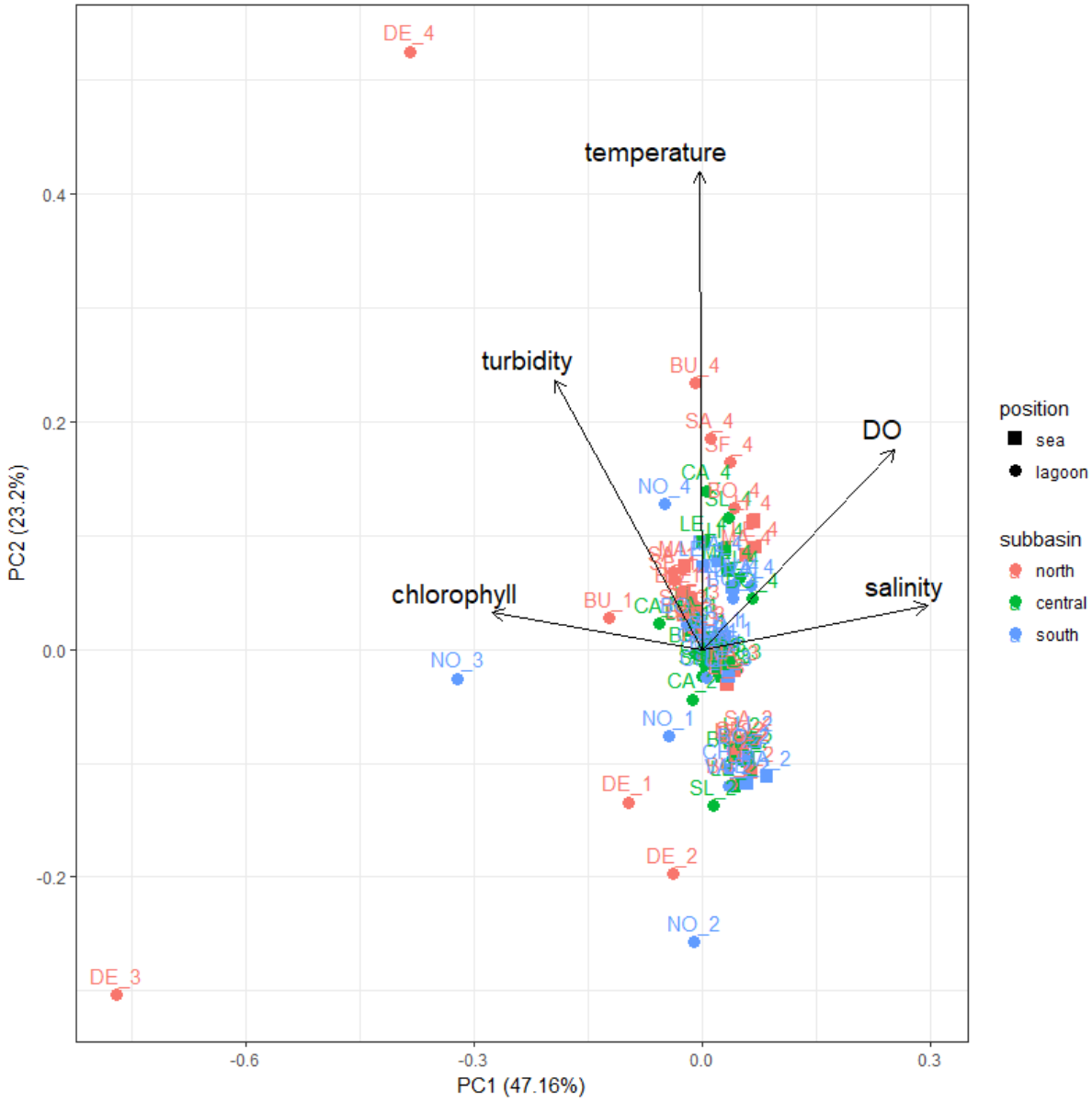
Environmental parameters

Environmental data obtained during the bongo net samplings are reported in Appendix A (tab. A1). Temperature showed the normal seasonal variation, with minimum value observed at the end of January, in

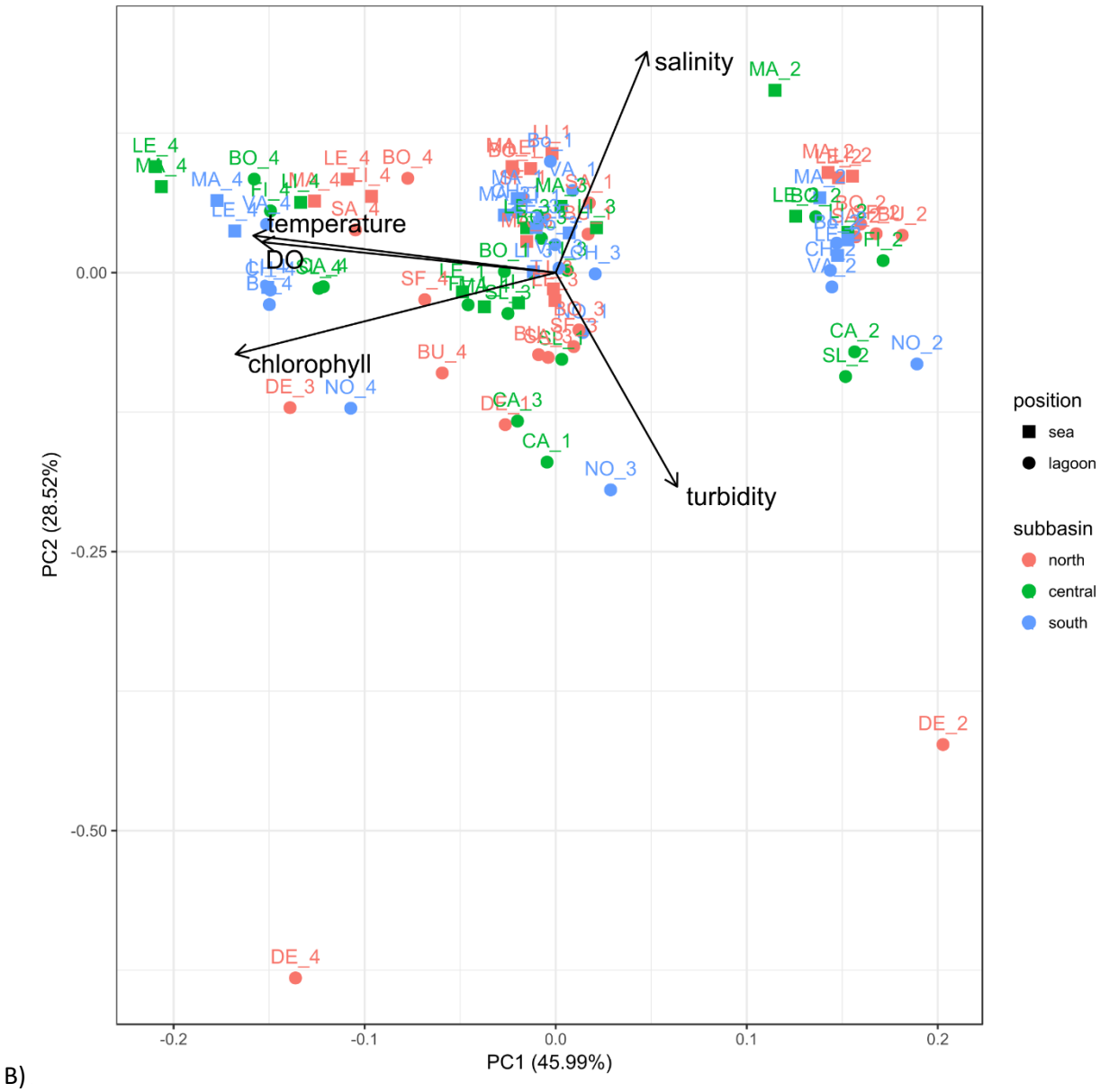
both cycles (II campaign). From a spatial point of view, in each transect, the innermost stations showed lower values than the sea in autumn (I campaign) and at the end of January (II campaign), while at the end of March (IV campaign) showed higher values. The concentration of dissolved oxygen and chlorophyll concentration in the water showed the same seasonal trend, in line with the renewal of primary production both at sea and in the lagoon. Regarding the dissolved oxygen, a decrease in concentration values proceeding from the sea towards the inner areas of the lagoon, especially in the central and south sub-basin, is generally observed. In the innermost station of the north sub-basin, in March 2016, extreme low oxygen and high chlorophyll concentrations were recorded. Salinity generally decreases along the sea-lagoon gradient, with similar values between the three sub-basins, except for the south sub-basin during January 2017, which had a markedly lower salinity than the north and central sub-basins. However, lower values of salinity were always recorded in the inner station of the north and south sub-basin in March 2016. Turbidity, conversely, increases along the sea-lagoon gradient, in particular in the central sub-basin.

The result of the Principal Component Analysis (PCA) showed that the first two components accounted for the 70.36% of the total variance of environmental data in the first sampling cycle and for the 74.50% in the second cycle, without marked differences between sub-basins (fig. 8). During the first sampling cycle, even if some environmental variables (e.g. chlorophyll concentration in water) could be related both to temporal and spatial factor, the first axis could be associated mostly with a temporal gradient while the second axis is influenced mainly by a spatial gradient (fig. 8A), however the graphic results of PCA are influenced by the high value of chlorophyll and turbidity in the inner station of the north sub-basin (DE) during respectively March and April. During the second sampling cycle (fig. 8B) the sampling campaigns are relatively well distinguished along the horizontal axis even if the campaigns of December 2016 and February 2017 are grouped together in the center of the graphs.

In the first cycle (fig. 8A), the first axis (PC1, which explains 47.16% of the variance), shows a sea-lagoon gradient with a decrease in salinity, dissolved oxygen and an increase in chlorophyll. The vertical axis (PC2, which accounts for 23.2% of the variance), on the other hand, is essentially influenced by variations in temperature and turbidity. In the second cycle (fig. 8B), the first axis (PC1, which explains 45.99% of the variance), is therefore associated with seasonal differences between the four sampling campaigns, showing to be influenced by variations in temperature, dissolved oxygen and chlorophyll. The second axis (PC2, which explains 28.52% of the variance) is instead more closely related to the spatial variability between the stations, being essentially influenced by salinity and turbidity and highlighting the sea-lagoon gradient, from top to bottom.


DE_4
temperature
turbidity
BU_4
SA_4
SF_4
DO
CA_4
NO_4
SL_4
LE_4
chlorophyll
BU_1
salinity
NO_3
CA_2
NO_1
DE_1
SL_2
DE_2
NO_2
DE_3
0.4
0.2
0.0
-0.2
-0.6
-0.3
0.0
0.3
PC2 (23.2%)
PC1 (47.16%)
position
sea
lagoon
subbasin
north
central
south

A)



B)

Figure 8 – PCA ordination on environmental data recorded during sampling with bongo net, A = first sampling cycle, B = second sampling cycle. Labels = sample stations and sampling campaign. DO = dissolved oxygen. Data were square root transformed and then standardized (scale values to zero mean and unit variance) in R.

Ichthyoplankton composition

A total of 24 Teleost taxa were caught (21 in the first cycle of sampling, 15 in the second cycle) and about a third belonged to the marine migrant guild (tab. 4). It is possible therefore to observe some differences in composition of ichthyoplanktonic community between the two sampling cycles. These differences concern mostly the eggs (18 taxa found in the first cycle and 12 in the second cycle), rather than the larvae (12 taxa found in the first cycle and 13 in the second cycle) and are caused by marine straggler species. Smaller differences in marine migrant composition between sub-basin were also detected.

Species characterized by greater abundance (*S. sprattus*, *S. pilchardus*, *P. flesus*, *S. solea*) belonged to the guild of marine migrant and have been found in the two cycles, both as eggs and larvae. Regarding *D. labrax*, larvae of this species were only found during the second sampling cycle. The presence of eggs of Mugilidae and larvae of *S. aurata* (only in the first cycle) were occasional.

The eggs component is composed only by marine taxa (MM and MS, tab. 4). Indeed, lagoon resident species does not have pelagic eggs, to prevent the dispersion of early life history stages outside the lagoon environment.

Table 4 - List of taxa caught at eggs and larvae stages during bongo net sampling in both cycles. In bold are highlight marine migrant taxa. ER = estuarine resident, MM = marine migrant, MS = marine straggler. n = found in north sub-basin, c = found in central sub-basin, s = found in south sub-basin.

			Eggs		Larvae	
Family	**Taxon**	**Guild**	**I cycle**	**II cycle**	**I cycle**	**II cycle**
Bothidae	Bothidae n.i.	MS	X,ncs	X,ncs	X,c	X,cs
Callionymidae	Callionymidae n.i.	MS	X,cs	X,nc		
Carangidae	*Trachurus trachurus*	MS	X,s			
Clupeidae	***Sardina pilchardus***	**MM**	**X,ncs**	**X,ncs**	**X,ncs**	**X,ncs**
	Sprattus sprattus	**MM**	**X,ncs**	**X,nc**	**X,ncs**	**X,ncs**
Engraulidae	***Engraulis encrasicolus***	**MM**	**X,cs**	**X,s**	**X,nc**	
Gadidae	Gadidae n.i.	MS	X,ncs	X,ncs		X,ncs
Gobiidae	Gobiidae n.i.	ER			X,ncs	X,ncs
Lotidae	*Gaidropsarus* n.i.	MS	X,s	X,s		X,ns
Merluccidae	*Merluccius merluccius*	MS				X,s
Moronidae	***Dicentrarchus labrax***	**MM**	**X,n**	**X,ncs**		**X,cs**
Mugilidae	***Chelon* n.i.**	**MM**	**X,s**			
Sparidae	Sparidae n.i.	MS	X,ncs			X,s
	Sparus aurata	**MM**			**X,ncs**	
Pleuronectidae	***Platichthys flesus***	**MM**	**X,cs**	**X,ncs**	**X,c**	**X,ncs**
Scophthalmidae	Scophthalmidae n.i.	MS			X,c	X,n
Soleidae	*Buglossidium luteum*	MS	X,ncs	X,ncs	X,cs	X,ns
	Microchirus n.i.	MS	X,ns			
	Pegusa n.i.	MS	X,ncs		X,cs	
	Solea solea	**MM**	**X,ncs**	**X,ncs**	**X,ncs**	**X,ncs**
	Soleidae n.i.	MS	X,ncs			
Trachinidae	*Echiichthys vipera*	MS	X,ncs	X,ncs		
Triglidae	Triglidae n.i.	MS			X,c	

Considering the eggs, analysis of percentage composition (calculated on the mean density by sub-basin and by campaign), show that marine migrant taxa and in particular *S. sprattus*, dominate the assemblage especially during the first two sampling campaigns of both sampling cycles (end of autumn – early winter)

(Appendix A, fig. A1). Among the other marine migrant taxa, especially during the second sampling cycle, significant was the contributions of *S. pilchardus* (all sub-basins, I campaign), *P. flesus* (all sub-basins, II and III campaigns) and *D. labrax* (north and central sub-basins, I campaign and south sub-basins, III campaign). Moreover, marine migrant taxa are plenty present in the lagoon station of all sub-basins (Appendix A, fig. A2) and generally, the innermost station of all three sub-basins are dominated by marine migrant species. The ichthyoplanktonic community at the larval stage (Appendix A, fig. A3, A4), with a similar patter across the three sub-basins, is dominated by Clupeidae. *S. sprattus* dominate the assemblage of the whole three gradients in both cycles especially during second and third sampling campaigns. Considering differences between marine and lagoon stations, only in the north sub-basin there is a progressive decrease in importance of *S. sprattus* larvae from the marine stations to inner lagoon stations. Conversely *S. pilchardus* larvae dominate the assemblage especially during the first sampling campaign of the second cycle. Considering others marine migrant taxa, during the first sampling cycle *E. encrasicolus* was caught during the first sampling campaign in the north sub-basin and *S. aurata* in all the three sub-basins especially in the third sampling campaign. However, these two species disappear from samples during the second sampling cycle while were caught larvae of *S. solea* and *P. flesus* in all three sub-basins.

Differences in egg and larval density were tested on marine migrant fish, considering the following factors: sampling cycle (first or second), the sub-basin (north, central and south), the sampling campaign (I, II, III and IV) and the position (sea and lagoon) (tab. 5).

Table 5 – Results of statistical test (ANOVA, GLM, Negative Binomial Family) on density of eggs and larvae of total marine migrant and Sprattus sprattus. ** = p<0.01; n.s. = not significant.*

Factor	Eggs marine migrant	Larvae marine migrant	Eggs *S. sprattus*	Larvae *S. sprattus*
Sub-basin	n.s.	n.s.	n.s.	n.s.
Campaign	n.s.	*	*	*
Position	n.s.	n.s.	n.s.	n.s.
Cycle	n.s.	*	n.s.	*
Sub-basin x Campaign	n.s.	n.s.	n.s.	n.s.
Sub-basin x Position	n.s.	n.s.	n.s.	n.s.
Sub-basin x Cycle	n.s.	n.s.	n.s.	n.s.
Campaign x Position	n.s.	n.s.	n.s.	n.s.
Campaign x Cycle	n.s.	n.s.	n.s.	n.s.
Position x Cycle	n.s.	n.s.	n.s.	n.s.
Sub-basin x Campaign x Position	n.s.	n.s.	n.s.	n.s.
Sub-basin x Campaign x Cycle	n.s.	n.s.	n.s.	n.s.
Sub-basin x Position x Cycle	n.s.	n.s.	n.s.	n.s.
Campaign x Position x Cycle	n.s.	n.s.	n.s.	n.s.
Sub-basin x Campaign x Position x Cycle	n.s.	n.s.	n.s.	n.s.

Considering the eggs of marine migrant fishes, no significant differences ($p < 0.01$, tab. 5) were found considering all the factors (campaigns, sampling cycle, position, sub-basin) (tab. 5, fig. 9). Instead, differences in the average density value of *S. sprattus* eggs were statistically significant only considering sampling campaigns (tab. 5): in both years a density peak of the eggs of this clupeid during the 2nd campaign were highlighted, especially in southern sub-basin (fig. 10). Regarding the other marine migrant species (fig. 11), they were found in samples rather irregularly and no significant differences were observed. While some species (*E. encrasicolus* and *D. labrax*) were found occasionally along the three transects (fig. 11), for other species (*S. pilchardus, S. sprattus*, *S. solea* and *P. flesus*) in at least one campaign a more regular presence along the sea-lagoon transect was observed, especially during the second sampling cycle (fig. 11); this is true especially in the southern sub-basin.

The larvae of marine migrant species turn out to be influenced by the presence of *Sprattus sprattus* larvae, which dominate the larvae population both years, in all the three sub-basins and in both positions (sea and lagoon). In fact, 88% of larvae belonged to *S. sprattus*. Excluding *S. sprattus* larvae, no significant differences between any factor were found in marine migrant larvae density.

Considering the average density of all marine migrant larvae, significant differences ($p<0.01$, tab. 5) were found with regard to the campaign and sampling cycle (tab. 5, fig 12). Significantly higher mean density (fig. 12) was observed in the second sampling cycle and these were probably related to the high concentration of *S. sprattus* larvae (fig. 13). A similar pattern was also observed for *P. flesus* larvae, although this species was characterized by lower abundances to those observed for *S. sprattus* larvae (fig. 14). From a spatial point of view, no significant difference was observed comparing positions and sub-basins (tab. 5).

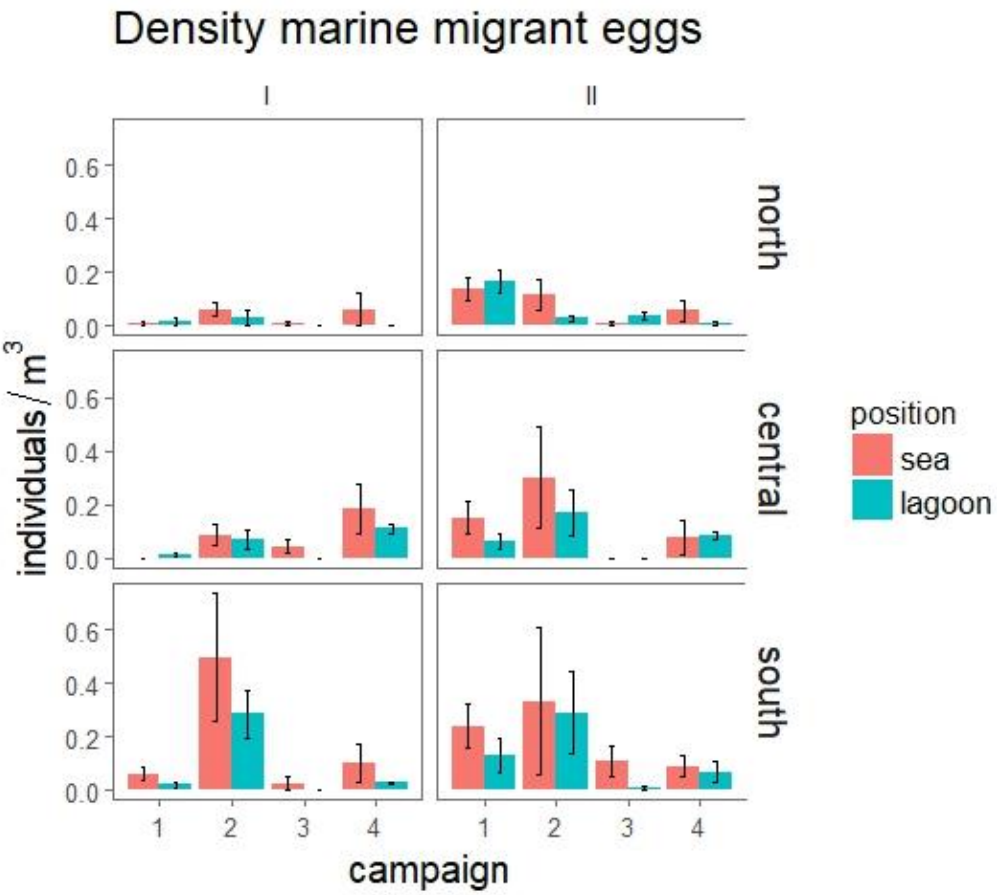


Figure 9 – Mean densities (± St. Err.) of the whole marine migrant eggs community collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.

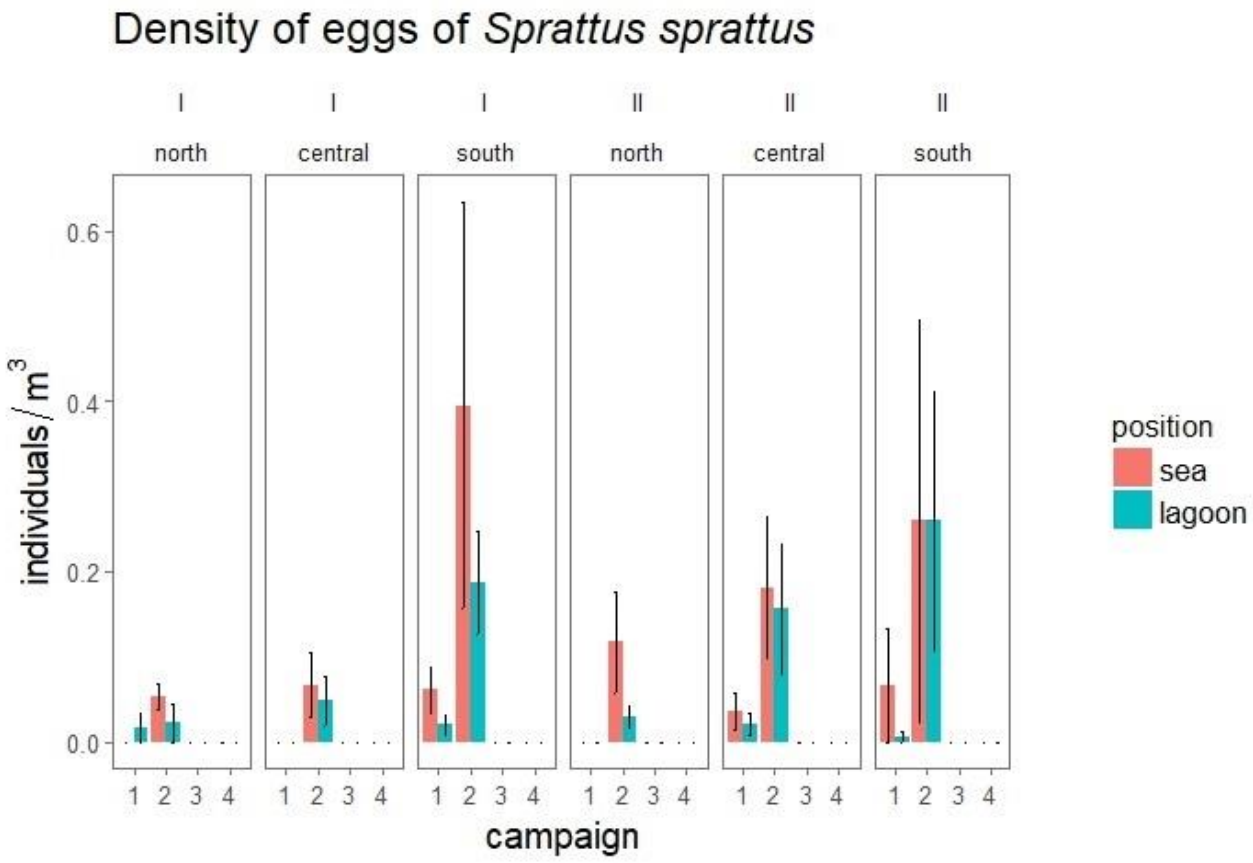


Figure 10 - Mean densities (± St. Err.) of eggs of Sprattus sprattus *collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.*

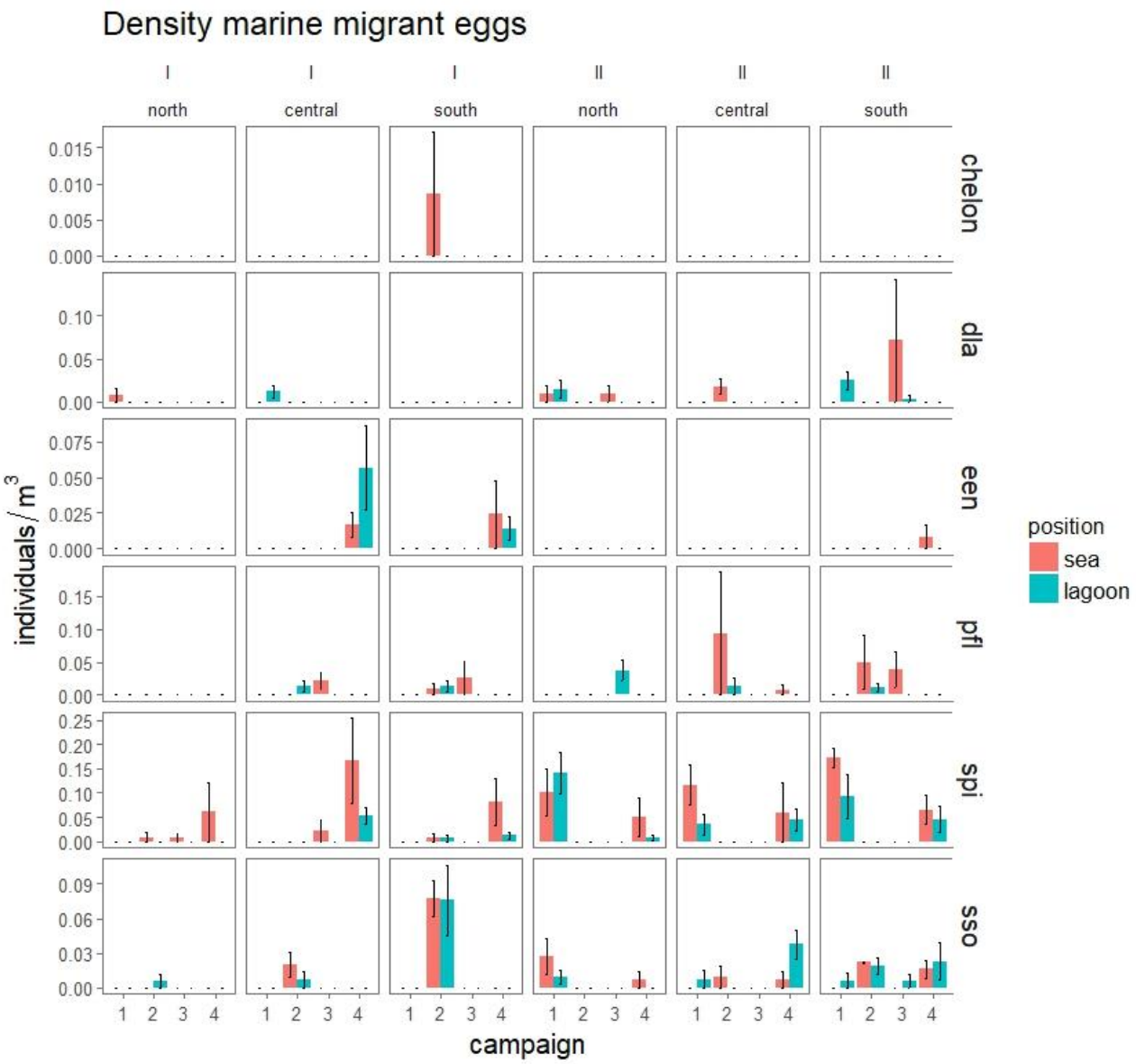


Figure 11 - Mean densities (± St. Err.) of eggs of marine migrant taxa collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). Chelon = Chelon *sp.*, *dla* = Dicentrarchus labrax, *een* = Engraulis encrasicolus, *pfl* = Platichthys flesus, *spi* = Sardina pilchardus, *sso* = Solea solea. *I = first sampling cycle, II = second sampling cycle.*

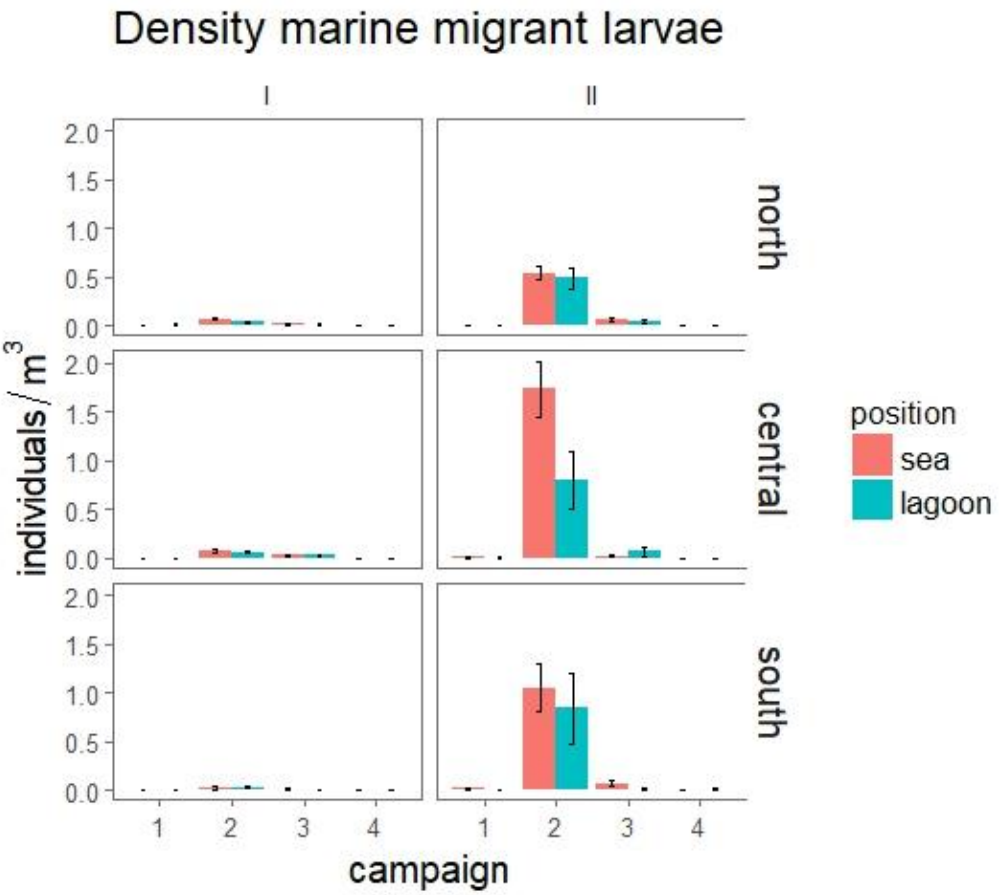


Figure 12 - Mean densities (± St. Err.) of the whole marine migrant larvae community collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.

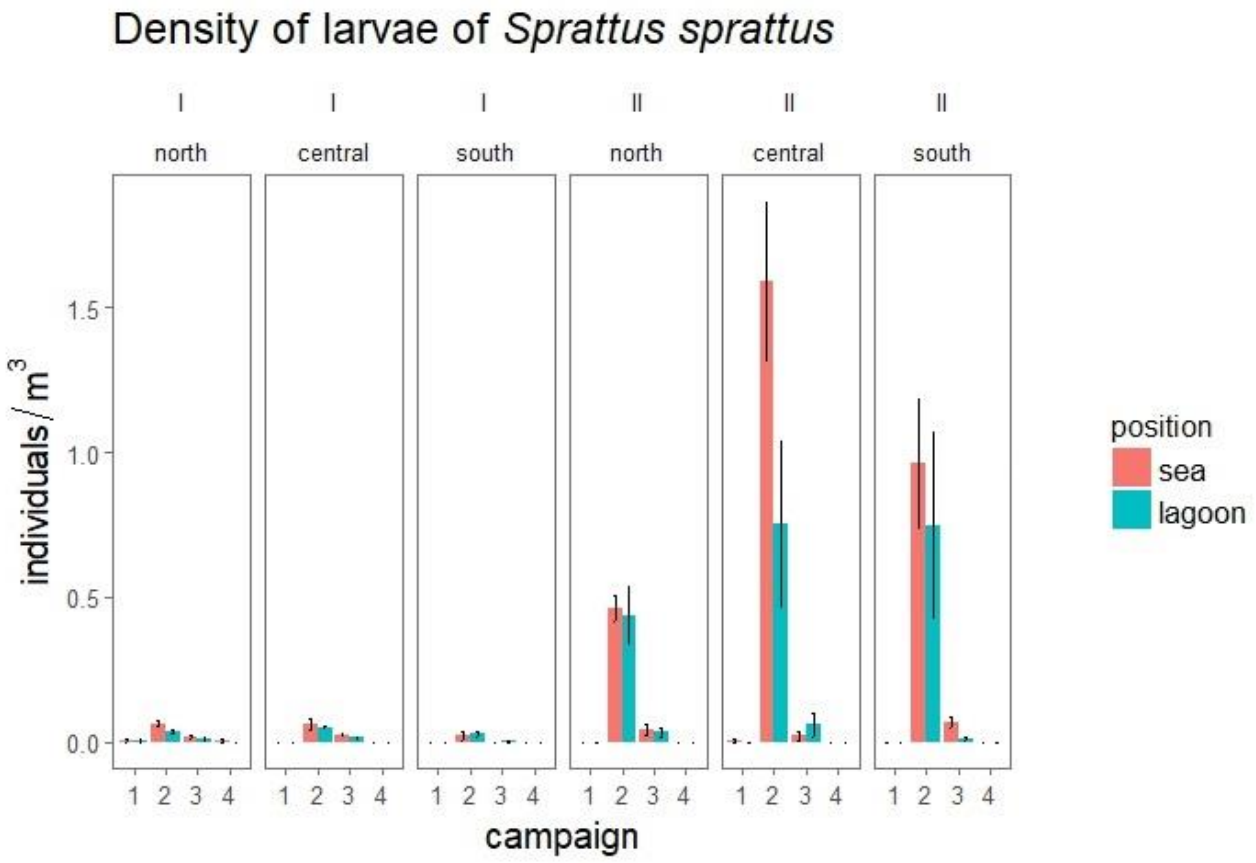


Figure 13 - Mean densities (± St. Err.) of larvae of Sprattus sprattus *collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.*

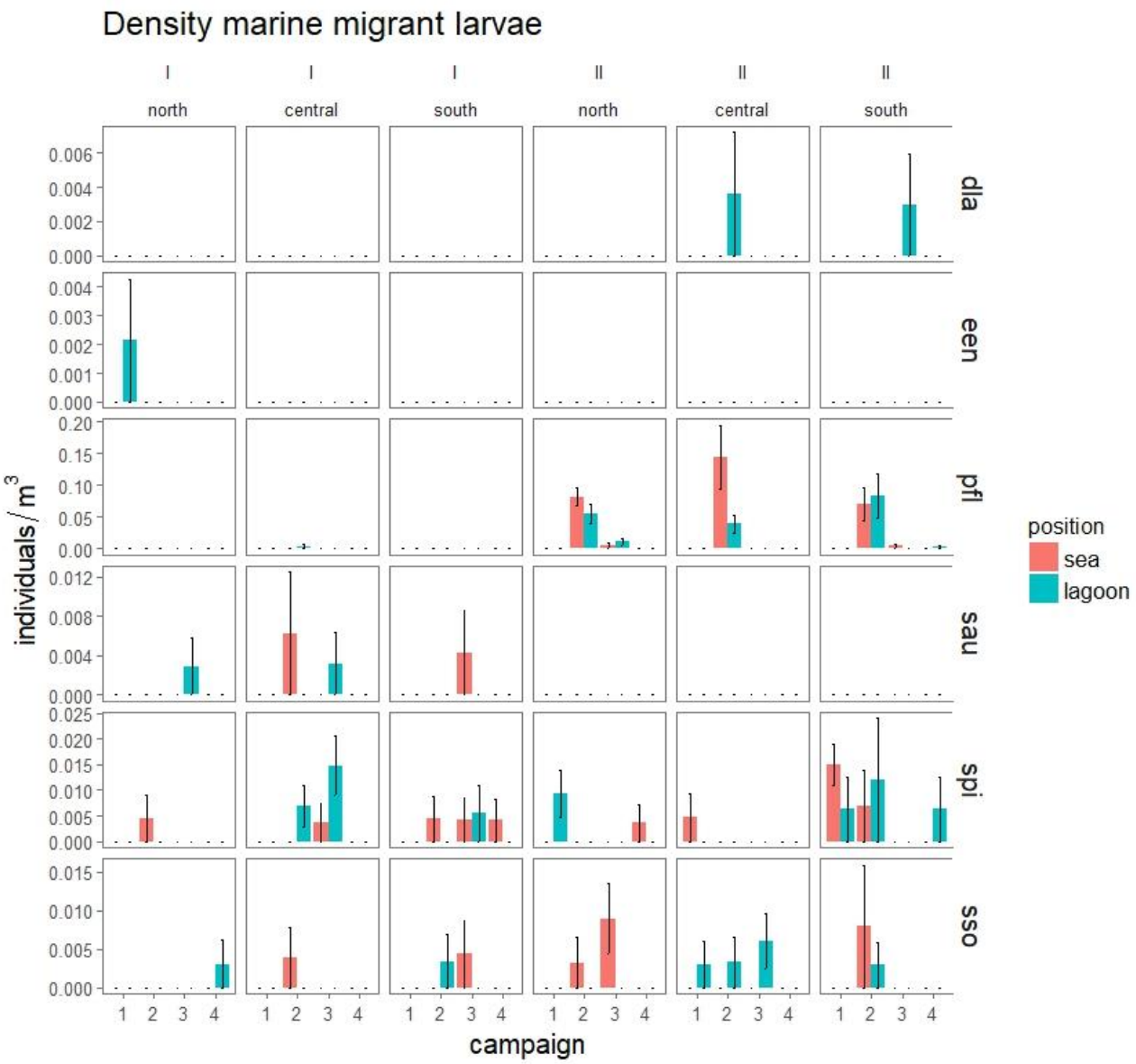


Figure 14 - Mean densities (± St. Err.) of larvae of marine migrant taxa collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). dla = Dicentrarchus labrax, *een* = Engraulis encrasicolus, *pfl* = Platichthys flesus, *sau* = Sparus aurata, *spi* = Sardina pilchardus, *sso* = Solea solea. *I = first sampling cycle, II = second sampling cycle.*

1.3.2 Ichthyofauna sampling

Environmental parameters

Environmental data obtained during the beach seine net samplings are reported in Appendix A (tab. A2). The differences in the sampling dates between the two years, caused by adverse meteorological conditions, do not seem to influence the environmental parameters collected during the campaigns. The environmental parameters collected show similar patterns in both years. The analysis of these environmental data shows the seasonal natural rise in temperatures during the three campaigns of both years, with higher values in the lagoon stations compared to the marine ones, especially during the third sampling campaign.

Regarding salinity, however, differences between sub-basins were found. Generally, in the north sub-basin, a gradual decrease in salinity, proceeding from the sea towards the lagoon was not observed, as found in the other two sub-basins. In the north sub-basin, lower salinity values only in the inner station were always observed. Turbidity values instead showed a more predictable trend, with an increase, in all the sub-basins, proceeding from the sea towards the edge, but a greater variability in values in the central sub-basin was observed.

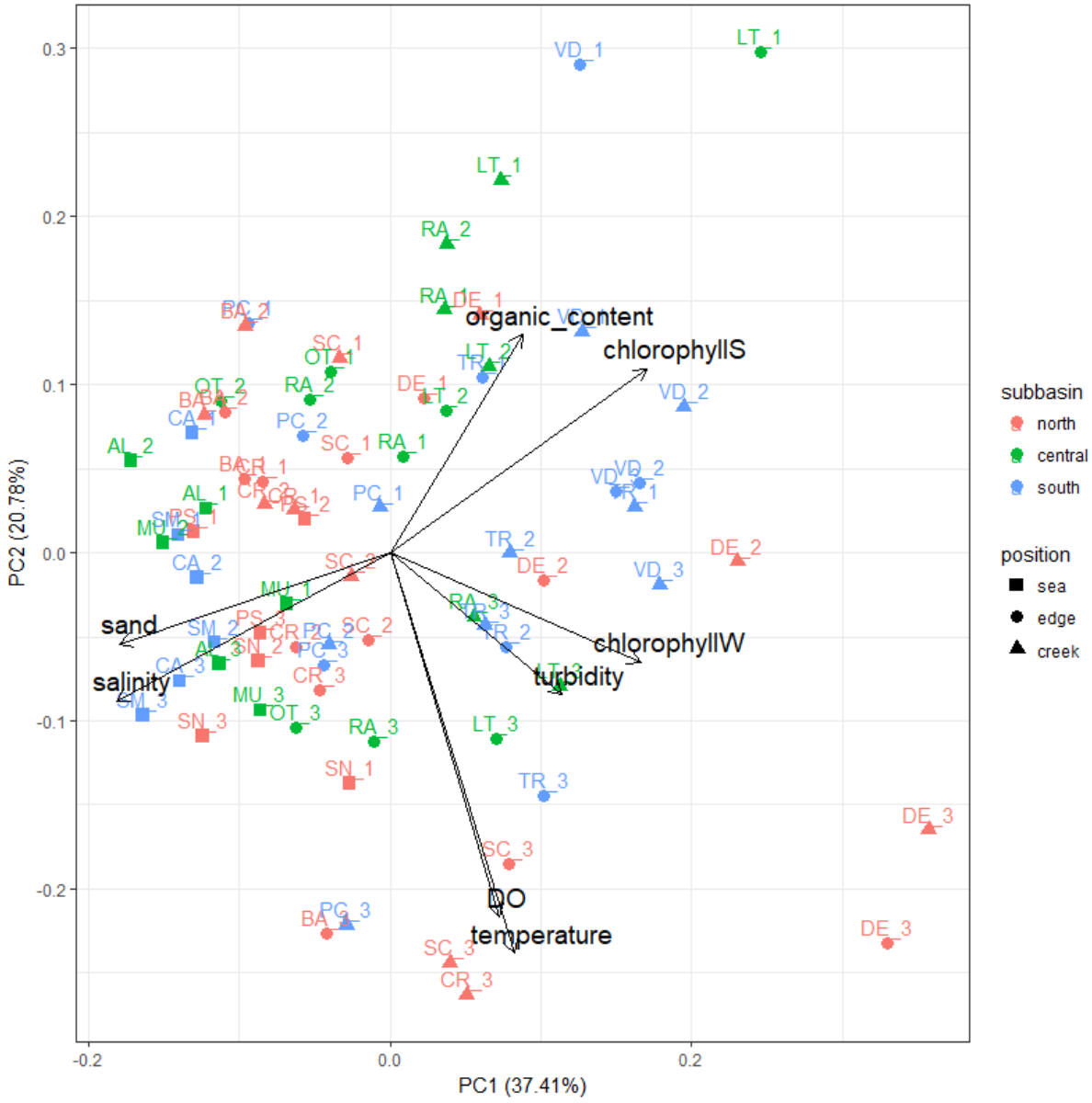
Dissolved oxygen and chlorophyll values didn't show a marked spatial or temporal pattern, but a general homogeneity between sub-basin and sea-lagoon gradient was observed. The only exception in these two parameters were peaks of dissolved oxygen in a station of the central sub-basin and markedly higher values of concentration of water chlorophyll in March during the second sampling cycle.

The result of the Principal Component Analysis (PCA) on the beach seine net samplings data (fig. 15) showed that the first two components accounted for the 58.19% of the total variance of environmental data in the first sampling cycle and for the 59.85% in the second cycle. The temporal component is hard to observe both in the horizontal and the vertical axis, during both sampling cycles. Also, the PCA did not shows substantial differences between sub-basins except during the first sampling year (fig. 15A) when lagoon stations of the south sub-basin were characterized by high levels of organic content and chlorophyll in the sediment.

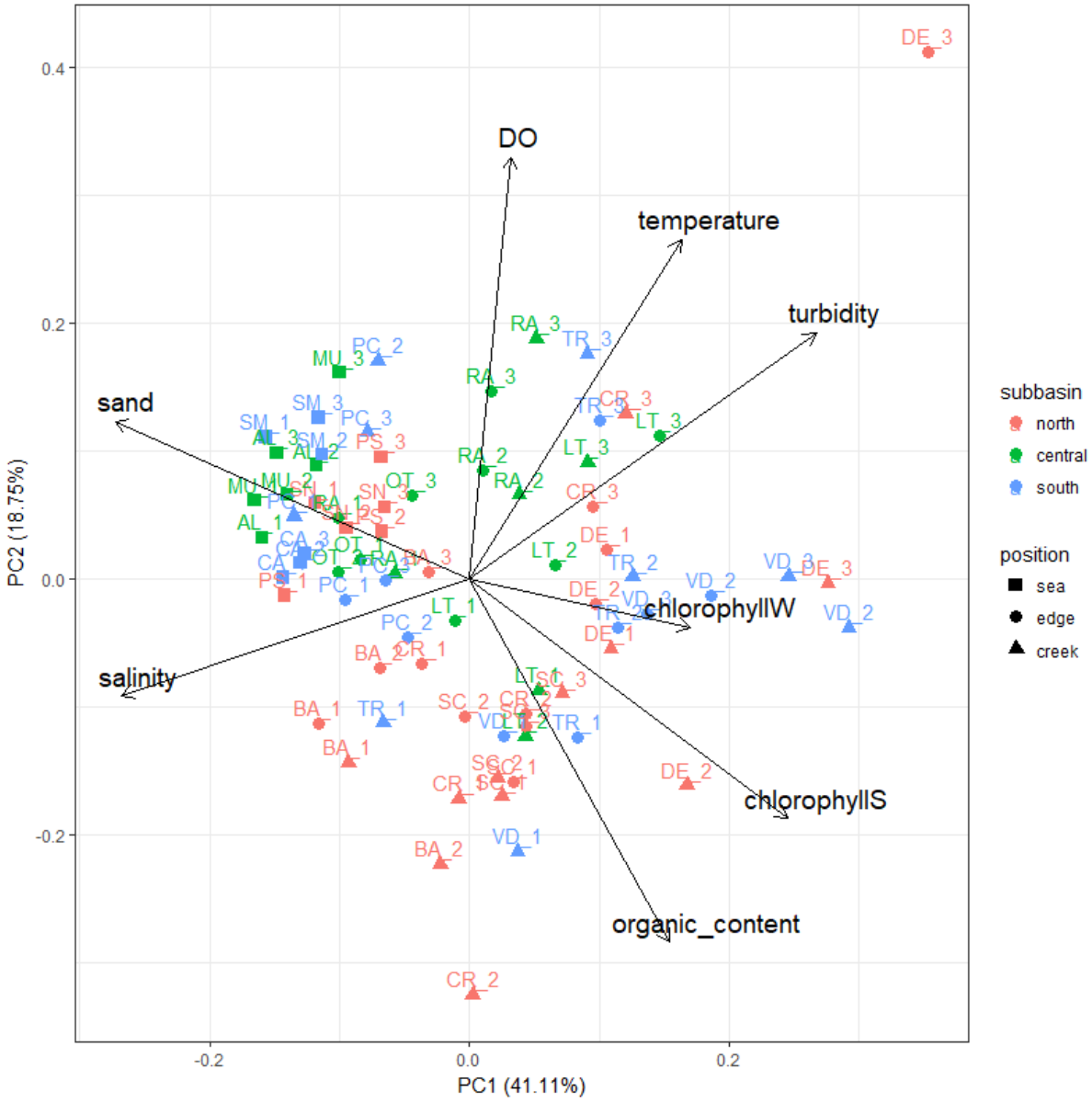
In both sampling cycles the horizontal axis (PC1, which explains 37.41% of the variance during the first sampling cycle and 41.11% during second cycle) is mainly correlated with salinity, % of sand and chlorophyll concentration in water, allowing to distinguish rather clearly marine and lagoon stations (fig. 15). The second axis (PC2, which explains 20.78% of the variance during the first sampling cycle and 18.75% during the second cycle) is associated with a second confining gradient influenced positively by organic content and chlorophyll concentration in sediment and negatively by turbidity, dissolved oxygen and temperature (fig. 15).

Overall, marine stations are characterized by high values of salinity and % of sand. Therefore, it is present a typical gradient proceeding from the sea towards the inner lagoon areas characterized by an increase in organic content and chlorophyll concentration in sediments. From the pictures (fig. 15) it is possible to observe a great variability of the lagoon samples both years, while marine stations appear more grouped together, especially during the second year (fig. 15B). Also, with the PCA is difficult to observe great differences between the tidal creek and the saltmarsh edge stations, both years (fig. 15).

From the analysis of the environmental parameters, it is possible to observe that the three sub-basins still show some distinctive characteristics (fig. 15). The sediments of the northern sub-basin contain a higher concentration of organic matter. The central sub-basin shows greater marine influence (%sand and salinity), even in the lagoon stations while the stations of the south sub-basin are mainly characterized by the influence of fresh water.


organic_content
chlorophyllS
chlorophyllW
turbidity
DO
temperature
sand
salinity
PC1 (37.41%)
PC2 (20.78%)
subbasin
north
central
south
position
sea
edge
creek

A)



B)

Figure 15 – PCA ordination on environmental data recorded during sampling with beach seine net, A = first sampling cycle, B = second sampling cycle. Labels = sample stations and sampling campaign. DO = dissolved oxygen. Data were square root transformed and then standardized (scale values to zero mean and unit variance) in R.

Post-larvae and juvenile composition

The analysis of ichthyofaunal samplings allowed the identification of 41 taxa of Teleosts (28 taxa in the first cycle of sampling, 38 in the second cycle) belonging to 20 Family; 25 taxa were found in both cycles of sampling (tab. 6). The taxonomic composition of the estuarine resident fish was quite stable between the two sampling years: the differences found can be attributed essentially to the guild of the marine stragglers: 5 taxa during the first year and 11 taxa during the second sampling year. The marine migrant community (13 taxa) shows lower variations between the two sampling years, such as the appearance of *Belone belone*. All

the marine migrant species were found at least once in all three sub-basins except *Belone belone* that were found in the central sub-basin and *Chelon labrosus* that were found only in the north and south sub-basins.

Table 6 - List of taxa caught at postlarva and juvenile stages during beach seine net sampling in both cycles. In bold are highlight marine migrant taxa. ER = estuarine resident, MM = marine migrant, MS = marine straggler. n = found in north sub-basin, c = found in central sub-basin, s = found in south sub-basin.

Family	Taxon	Guild	Code	I cycle	II cycle
Atherinidae	*Atherina boyeri*	r	ABO	X,ncs	X,ncs
Belonidae	***Belone belone***	**mm**	**BBE**		**X,c**
Blenniidae	*Salaria pavo*	r	SPA	X,ncs	X,ncs
	Aidablennius sphynx	ms	ASP		X,nc
Bothidae	*Arnoglossus kessleri*	ms	AKE		X,c
Clupeidae	***Sardina pilchardus***	**mm**	**SPI**	**X,nc**	**X,ns**
	Sprattus sprattus	**mm**	**SSP**	**X,ncs**	**X,ncs**
Cyprinodontidae	*Aphanius fasciatus*	r	APFA	X,ncs	X,ncs
Engraulidae	***Engraulis encrasicolus***	**mm**	**EEN**	**X,ns**	**X,n**
Gobiidae	*Gobius niger*	r	GNI		X,n
	Knipowitschia panizzae	r	KPA	X,ncs	X,ncs
	Pomatoschistus canestrinii	r	PCA	X,nc	X.ncs
	Pomatoschistus marmoratus	r	PMA	X,ncs	X,ncs
	Pomatoschistus minutus	**mm**	**PMI**	**X,n**	**X,ncs**
	Zebrus zebrus	r	ZZE		X,n
	Zosterisessor ophiocephalus	r	ZOP	X,n	X,n
Labridae	*Symphodus roissali*	ms	SRO		X,n
Moronidae	***Dicentrarchus labrax***	**mm**	**DLA**	**X,nc**	**X,ncs**
Mugilidae	***Chelon labrosus***	**mm**	**CLA**	**X,s**	**X,ns**
	Chelon auratus	**mm**	**CAU**	**X,ncs**	**X,ncs**
	Chelon ramada	**mm**	**CRA**	**X,ncs**	**X,ncs**
	Chelon saliens	**mm**	**CSA**	**X,ncs**	**X,ncs**
Pleuronectidae	***Platichthys flesus***	**mm**	**PFL**	**X,ns**	**X,ncs**
Sciaenidae	*Umbrina cirrosa*	ms	UCI		X,s
Scophthalmidae	*Scophthalmus rhombus*	ms	SRH	X,s	X,cs
	Scophthalmus maximus	ms	SMA		X,c
Scorpaenidae	*Scorpaena porcus*	ms	SPO		X,s
Soleidae	***Solea solea***	**mm**	**SSO**	**X,ncs**	**X,ncs**
	Microchirus sp.	ms	Micr.sp.		X,s
	Pegusa sp.	ms	Pegu.sp.		X,c
Sparidae	*Boops boops*	ms	BBO	X,c	
	Diplodus puntazzo	ms	DPU	X,c	
	Sarpa salpa	ms	SSA	X,c	
	Sparus aurata	**mm**	**SAU**	**X,ncs**	**X,ncs**
Syngnathidae	*Nerophis ophidion*	r	NOP	X,ncs	X,nc
	Syngnathus abaster	r	SAB	X,ncs	X,ncs
	Syngnathus taenionotus	r	STA	X,ns	X,cs
	Syngnathus typhle	r	STY	X,c	X,nc
	Hippocampus guttulatus	r	HGU		X,ns
Trachinidae	*Echiichthys vipera*	ms	EVI		X,c
Triglidae	*Chelidonichthys lucerna*	ms	CLU	X,n	X,n

Analyzing the percentage composition of juvenile catches (Appendix, fig. A5, A6), only in the northern sub-basin, both years, the lagoon fish population was dominated by marine migrant species. Among the migrant taxa, *C. ramada* appears to be the most abundant species, caught in all the three sub-basins. *C. auratus* also contributes to the lagoon fish population especially during the first campaign of both years in the central and in the south sub-basins. The presence of these two species (*C. ramada* and *C. auratus*) in the central and south sub-basin was mainly associated with marine stations, especially during the first cycle. The contribution of *C. saliens* was less important except during the first sampling campaign of the first sampling year in the central and south sub-basin, where they represent more than 50% of the catch. Differently from ichthyoplankton component, in case of post-larvae and juvenile, the contribution of clupeids is less important: *S. sprattus* were caught only during the third campaign in the north sub-basin both sampling yeas and *S. pilchardus* appears in the samples only the second year in the south sub-basin during the third campaign. Finally, among other marine migrant fish species, *D. labrax* were found with high relative abundance in the second sampling cycle in north sub-basin in marine station while S. aurata were found with relevant relative abundance in all three sub-basin and campaigns both years and along the whole sea-lagoon gradient.

Differences in juvenile density were tested only on marine migrant taxa, considering the following factors: sampling cycle (first or second), the sub-basin (north, central and south), the campaign sampling (I, II and III) and the position (sea and lagoon) (tab. 7).

Table 7 – Results of statistical test (ANOVA, GLM, Negative Binomial Family) on density of juvenile fishes calculated on total marine migrant and most abundant species (C. auratus, C. ramada, C. saliens and S. aurata). Here position = sea and lagoon

Factor	JUV TOT	CAU	CRA	CSA	SAU
Sub-basin	*	*	*	*	*
Campaign	n.s.	n.s.	n.s.	*	*
Position	*	*	*	*	0.05
Cycle	n.s.	n.s.	n.s.	*	n.s.
Sub-basin x Campaign	n.s.	*	n.s.	*	*
Sub-basin x Position	*	*	*	n.s.	*
Sub-basin x Cycle	n.s.	n.s.	n.s.	n.s.	*
Campaign x Position	n.s.	*	*	n.s.	n.s.
Campaign x Cycle	*	*	*	n.s.	*
Position x Cycle	n.s.	*	n.s.	*	n.s.
Sub-basin x Campaign x Position	*	*	*	n.s.	n.s
Sub-basin x Campaign x Cycle	*	n.s.	*	n.s.	n.s
Sub-basin x Position x Cycle	n.s.	*	*	n.s.	n.s
Campaign x Position x Cycle	*	n.s.	n.s.	n.s.	*
Sub-basin x Campaign x Position x Cycle	*	*	n.s.	n.s.	n.s

Considering the entire marine migrant component (tab. 7, fig. 16), the statistical tests on density of individuals show differences between sub-basins and positions and significant effect in the interaction of all considered factors. The interaction of different factors makes it difficult to interpret the results. However, it is possible to see some common patterns. The south sub-basin owns the highest density of fish collected in the sea during the first sampling year (fig. 16). In the north sub-basin, where conversely it is possible to observe the lowest overall densities regarding the other sub-basins in the first year, relevant densities of juvenile marine migrant fish, compared to the sea, were found within the lagoon in both years (fig. 16). Differences between positions (sea and lagoon) were found in central sub-basin, with the highest density in the sea stations (tab. 7, fig. 16). In the north and south sub-basin, no difference in position was found but even without statistical differences, in these two sub-basins relevant concentrations of juvenile marine fishes within the lagoon were found, except during the second campaign of the first sampling year in south sub-basin (fig. 16).

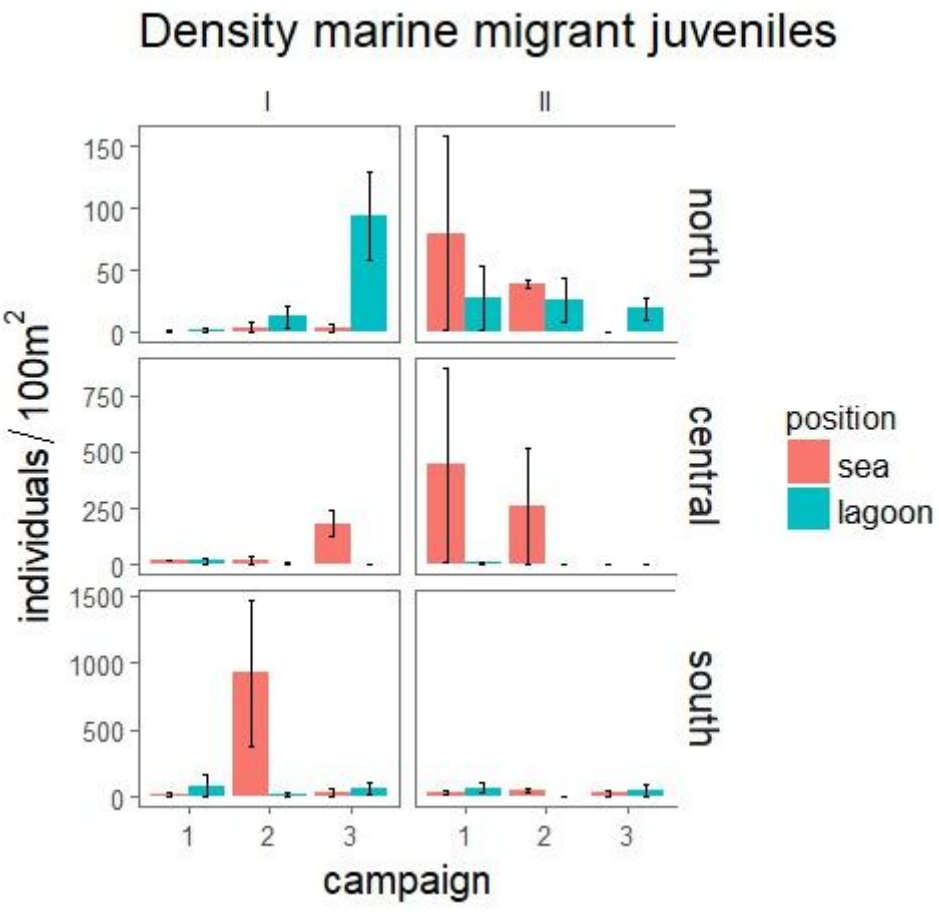


Figure 16 - Mean densities (± St. Err.) of the whole marine migrant juvenile community collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.

Statistical tests were performed also on the most abundant migrant species: *Chelon auratus*, *C. ramada*, *C. saliens* and *Sparus aurata* (tab. 7, fig. 17-20). *C. ramada* was the species found with the highest density, with a peak in the sea stations of the south sub-basin during the second campaign of the first year (fig. 17). Even

if each species seems to behave with a different spatial and temporal pattern, it is possible to notice some common features: for all the considered species, statistical differences between sub-basins and position were present (tab. 7).

The presence of *C. auratus* (fig. 18) and *C. ramada* (fig. 19), the most abundant species, changed temporarily in sampling years and in sampling campaigns, manifesting statistical differences in the interaction of factors (tab. 7). However similar patterns were observed for these species. Even if only in one occasion, the third campaign of the first sampling year, in the north sub-basin a higher density of *C. auratus* and *C. ramada* were found within the lagoon compared to the sea stations (fig. 17, 18). Significant differences in position were found only in the central sub-basin, where individuals were always caught exclusively in the marine stations. Instead, the general pattern appears quite stable in the other two sub-basins where, especially in north sub-basin, density of individuals belonging to these species were comparable between marine and lagoon stations (fig. 17, 18).

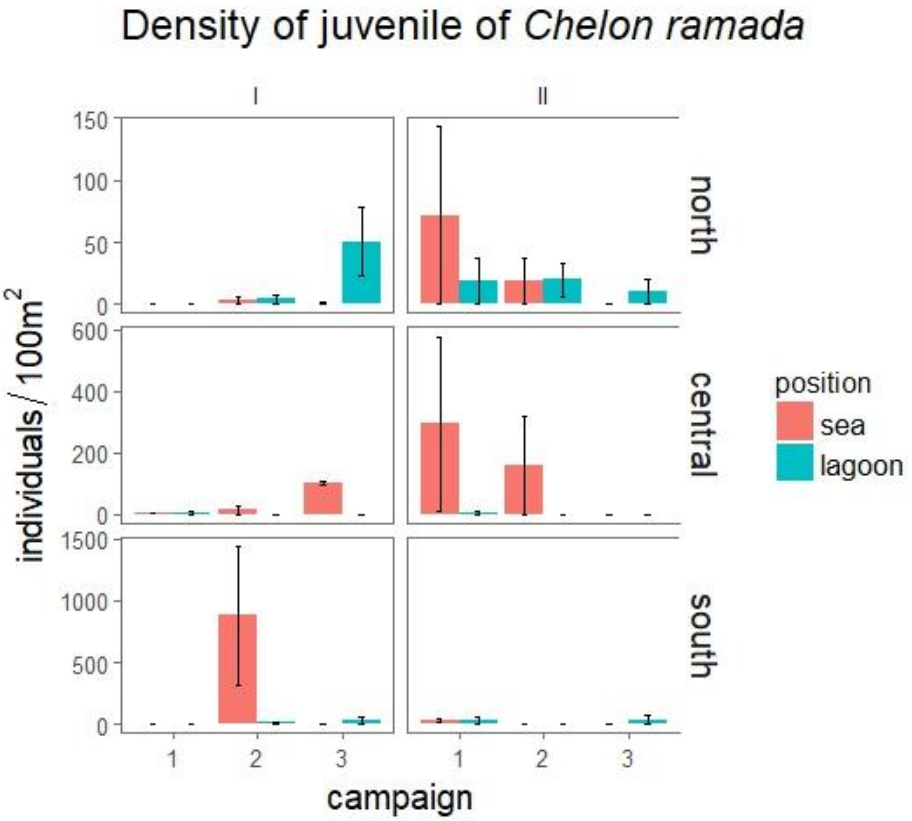


Figure 17 - Mean densities (± St. Err.) of Chelon ramada *juvenile collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.*

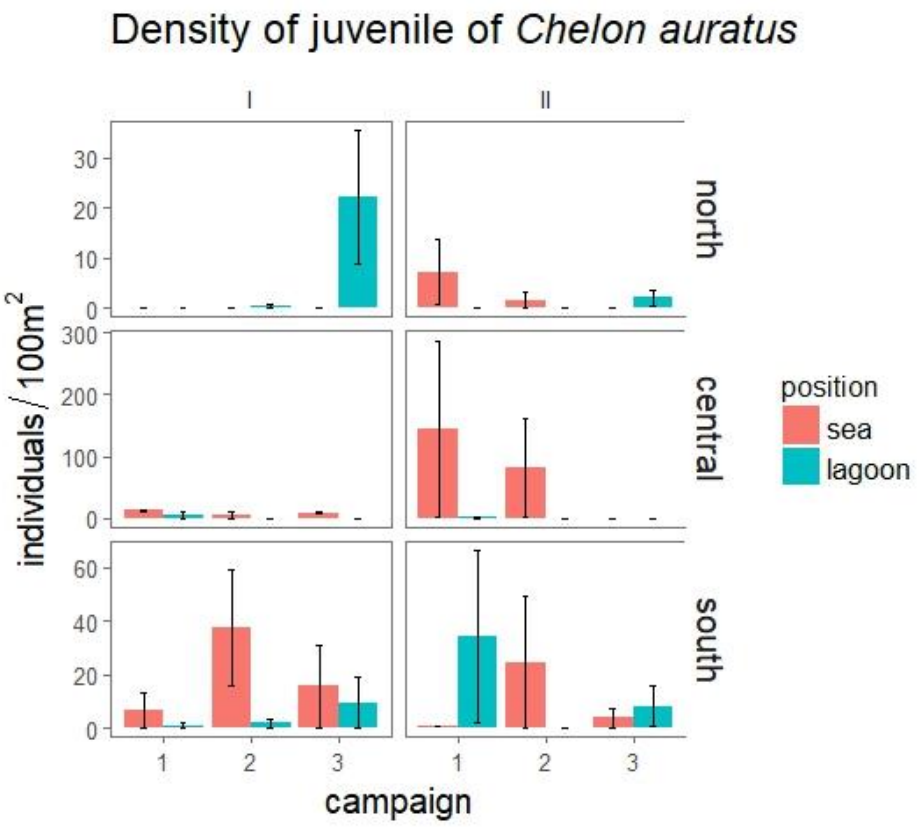


Figure 18 - Mean densities (± St. Err.) of Chelon auratus *juvenile collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.*

Compared to the other two species of the genus *Chelon*, *C. saliens* was the less abundant and the catch was limited to the first sampling year (fig. 19). Statistical differences for this species were found between sampling position, cycle, campaign and sub-basin (tab. 7). In general, in all the three sub-basins results show that even with different amounts, this species was found almost exclusively in the lagoon stations, except during the second sampling year in the south sub-basin, when density in marine stations was higher than in the lagoon stations (fig. 19).

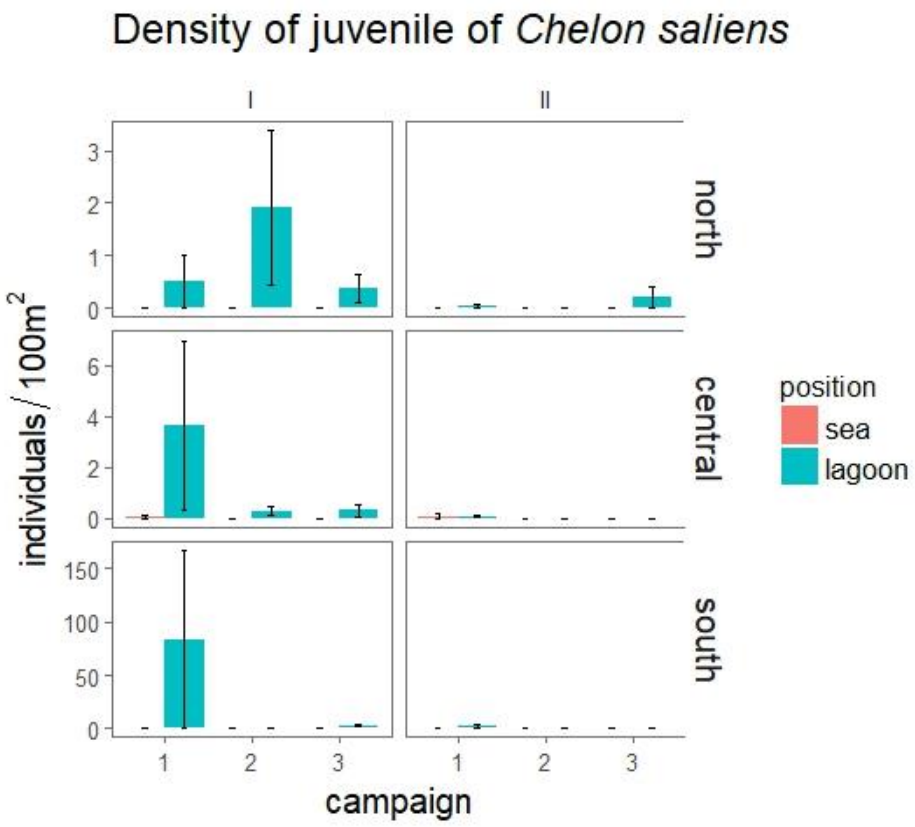


Figure 19 - Mean densities (± St. Err.) of Chelon saliens *juvenile collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.*

Regarding *Sparus aurata* (fig. 20), a peak of individuals was observed in marine stations of the central sub-basin during the third campaign of the first sampling year. Again, differences between sub-basins and positions were found (tab. 7): in the central sub-basin the density of individuals was always higher in marine stations than in lagoon stations (fig. 20). Opposite situations occur in the north sub-basin, where lagoon stations always have a higher density of S. aurata individuals compared to marine stations, while a more variable pattern can be observed in the south sub-basin (fig. 20).

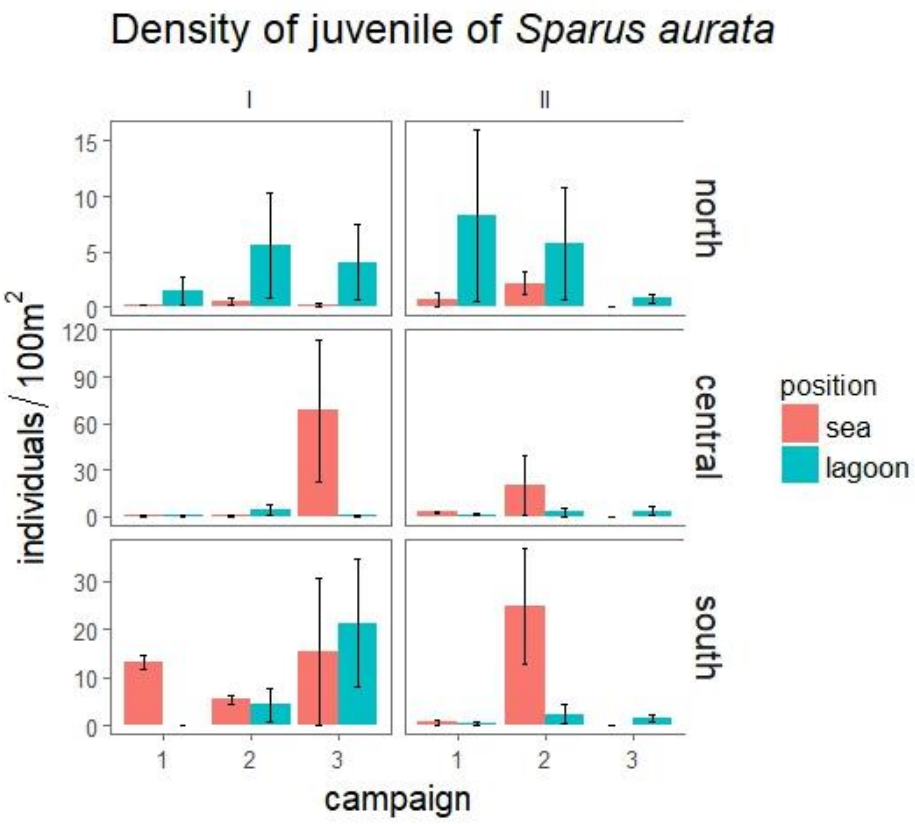


Figure 20 - Mean densities (± St. Err.) of Sparus aurata *juvenile collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (sea/lagoon). I = first sampling cycle, II = second sampling cycle.*

The previous results must be taken into account considering that two portions of saltmarsh were explored in the lagoon stations: a saltmarsh edge and a tidal creek (except for one station in the central sub-basin where these two habitats were not present). After having analyzed the different patterns between marine and lagoon stations in the three-different lagoon sub-basins, additional tests were performed to observe the contributions of tidal creeks in the lagoon stations (tab. 8). The results shown on Table 8 and Figure 21, and 22 display the differences in juvenile density between saltmarsh edge and tidal creek stations, considering the same previous factors: sampling cycle (first or second), the sub-basin (north, central and south), the campaign sampling (I, II and III) and the position (sea and lagoon) (tab. 8).

Table 8 - Results of statistical test (ANOVA, GLM, Negative Binomial Family) on density of juvenile fishes calculated on total marine migrant and most abundant species (C. auratus, C. ramada, C. saliens and S. aurata). Here position = saltmarsh edge and tidal creek.

Factor	JUV TOT	CAU	CRA	CSA	SAU
Sub-basin	*	*	*	n.s.	*
Campaign	*	*	n.s.	*	*
Position	*	*	n.s.	*	n.s.
Cycle	n.s.	n.s.	n.s.	*	n.s.
Sub-basin x Campaign	*	*	*	*	*
Sub-basin x Position	*	*	*	*	n.s.
Sub-basin x Cycle	n.s.	*	n.s.	n.s.	n.s.
Campaign x Position	n.s.	*	n.s.	n.s.	n.s.
Campaign x Cycle	n.s.	n.s.	*	n.s.	n.s.
Position x Cycle	n.s.	*	n.s.	n.s.	*
Sub-basin x Campaign x Position	*	n.s.	n.s.	n.s.	n.s.
Sub-basin x Campaign x Cycle	n.s.	n.s.	n.s.	n.s.	n.s.
Sub-basin x Position x Cycle	n.s.	n.s.	*	n.s.	n.s.
Campaign x Position x Cycle	n.s.	n.s.	n.s.	n.s.	n.s.
Sub-basin x Campaign x Position x Cycle	n.s.	n.s.	n.s.	n.s.	n.s.

Considering the entire marine migrant component (tab. 8, fig. 21), results of statistical tests highlighted differences both between sub-basins and positions (saltmarsh edge and tidal creek). Differently from sea-lagoon differences, where a high interannually variability was present, no difference between sampling years were found (tab. 8), indicating a stable situation regarding juvenile density distributions within the lagoon. As observed with the sea-lagoon differences, the south sub-basin is still the one that owns the highest density of juvenile fishes, collected with a peak during the first campaign of the first year in a tidal creek (fig. 21). Results also confirm the lowest density in the lagoon stations of the central sub-basins. Instead, differences between saltmarsh edge and tidal creek were present only in the north sub-basin (tab. 8), where inside the tidal creeks the density of marine migrant fishes were always higher than saltmarsh edges.

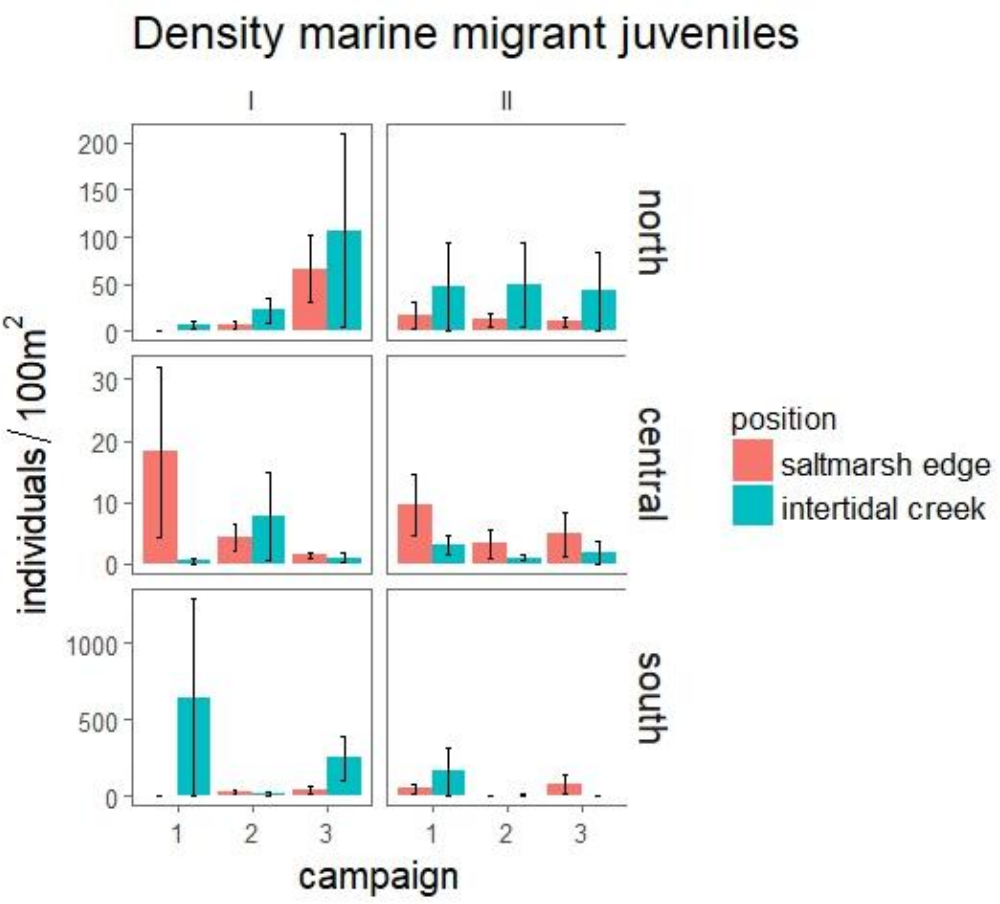


Figure 21 - Mean densities (± St. Err.) of the whole marine migrant juvenile community collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (saltmarsh edge/tidal creek). I = first sampling cycle, II = second sampling cycle.

Statistical tests were performed also on the most abundant species: *Chelon auratus*, *C. ramada*, *C. saliens* and *Sparus aurata* (tab. 8, fig. 22) and results show a different behavior from species to species. The presence of *C. auratus* within the lagoon seems to be the most variable, with a peak during the first campaign of the second sampling years inside the tidal creeks of the south sub-basin. In the other two sub-basins this species seems to concentrate in the saltmarsh edge. For *C. saliens*, whose presence is greatest especially during the first sampling year, it seems that the tidal creeks play an important role in influencing the distribution within the lagoon: in the north and south sub-basins this species concentrates significantly in tidal creeks. More stable pattern appears in *C. ramada* and *S. aurata* for which, in both years, even if without significant differences between position inside the saltmarsh habitats, they seem to concentrate inside the tidal creeks only in the north sub-basin.

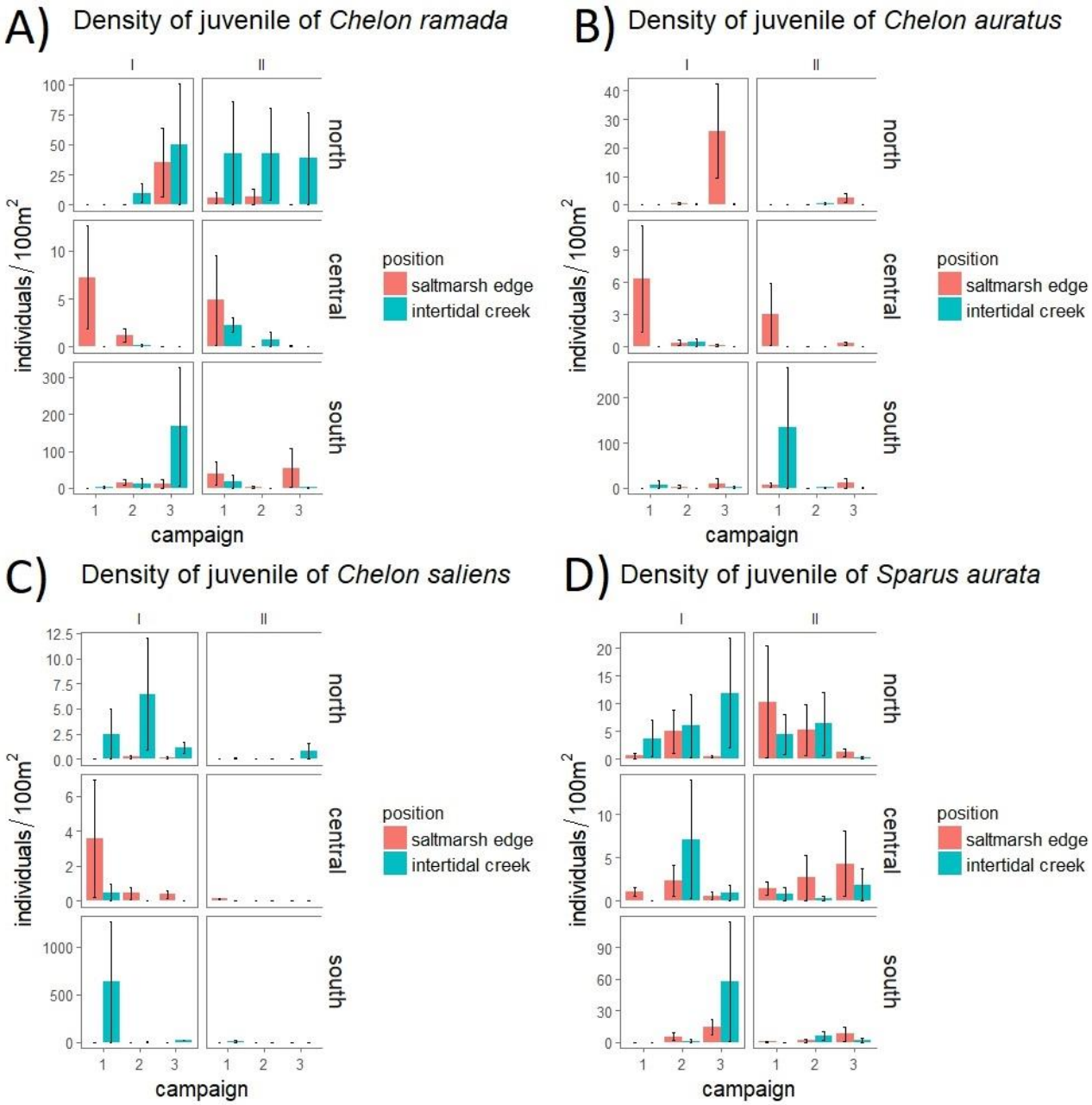


Figure 22 - Mean densities (± St. Err.) of C. auratus *(A),* C. ramada *(B),* C. saliens *(C),* S. aurata *(D) juvenile collected, divided for sampling cycle, sub-basin and sampling campaign. Colors indicate the different positions (saltmarsh edge/tidal creek). I = first sampling cycle, II = second sampling cycle.*

1.3.3 Colonization Index (I_C)

The densities of eggs, larvae and juveniles measured during samplings were used to calculate the colonization index (I_C) (tab. 9, 10). The index was calculated by cumulating for each sub-basin and each sampling cycle the densities of the sampling campaigns. The index has been calculated separately for eggs and larvae and juveniles of the entire marine migrant component. The index has been also calculated for eggs and larvae of *S. sprattus*, and the most abundant juveniles species (*C. auratus*, *C. ramada*, *C. saliens*, *S. aurata*).

Both for the total marine migrant eggs and for those of *S. sprattus*, the index has values ranging from 0.20 to 0.46, indicating a weak transport of eggs within the lagoon in both years. Considering the total larvae of marine migrant, the threshold of 0.5 were instead exceeded in all the three sub-basins, indicating an accumulation of larvae within the lagoon. For both eggs and larvae, the colonization index shows similar values during the two sampling cycles and the three sub-basins.

Table 9 – Colonization Index calculated separately for eggs and larvae of MM and S. sprattus

	Total MM eggs		**Eggs *S. sprattus***		**Total MM larvae**		**Larvae *S. sprattus***	
Sub-basin	I sampling year	II sampling year	I sampling year	II sampling year	I sampling year	II sampling year	I sampling year	II sampling year
North	0.25	0.46	0.43	0.20	0.52	0.52	0.38	0.48
Central	0.38	0.37	0.42	0.45	0.55	0.55	0.43	0.33
South	0.32	0.39	0.31	0.45	0.55	0.55	0.59	0.42

Considering both the entire marine migrant juvenile community and the single marine migrant most abundant species, the colonization index I_C (tab. 10) highlights a different pattern between the two sampling years and between sub-basins. Only the central sub-basin shows low values of the index both years, indicating an extremely low accumulation of individuals inside that sub-basin.

During the first sampling year, considering the juvenile component (tab. 10), the north sub-basin seems to be the only one having extremely high values of I_C (values near 1), indicating that individuals concentrate mostly inside this sub-basin. During the second sampling year the south sub-basin seems to be the ones with higher values, even if it does not reach, for any species, the values achieved by north sub-basin during the first year. In the north sub-basin *C. saliens* and *S. aurata*, during both sampling years, continue to have high I_C values (tab. 10), higher than the other sub-basins, and it seems to indicate that these species still concentrate preferably in this basin. The other two most abundant species, *C. auratus* and *C. ramada*, as observed with density distributions, show a similar pattern affected by high interannual variability. Results of the colonization index show that, even if with high interannual variability, north and south sub-basins are the only ones in which marine migrant fish concentrate within the lagoon stations (tab. 10). However, the north sub-basin is the one that reaches the highest value and where two species, *C. saliens* and *S. aurata*, concentrate both years.

Table 10 – Colonization Index calculated separately for entire marine migrant juvenile components and for the four most abundant specie (CAU = C. auratus*, CRA* = C. ramada*, CSA* = C. saliens*, SAU* = S. aurata*).*

	Total Juv MM		**CAU**		**CRA**		**CSA**		**SAU**	
Sub-basin	I sampling year	II sampling year	I sampling year	II sampling year	I sampling year	II sampling year	I sampling year	II sampling year	I sampling year	II sampling year
North	0.93	0.38	0.98	0.20	0.93	0.35	1.00	1	0.93	0.84
Central	0.10	0.02	0.19	0.01	0.06	0.01	0.98	0.46	0.07	0.25
South	0.15	0.50	0.18	0.59	0.05	0.68	1.00	0.87	0.43	0.16

1.3.4 Centre of Gravity Index (COG)

The densities of individuals measured during the sampling campaign in each sea-lagoon transect were used to calculate the center of gravity (COG), separated for eggs, larvae and juvenile for the whole marine migrant community and for the most abundant species (fig. 23, 24, 25).

Results of COG (fig. 23), considering the entire marine migrant sampled community, highlight a strong spatial, between sub-basins, and temporal, between years, variability. Except few occasions, eggs seem to be concentrated in marine stations, as seen in colonization index (I_C) (tab 9). The Centre of Gravity of the eggs has generally lower or similar to the ones of the sea inlets, indicating that densities of marine migrant eggs were caught mostly in the marine stations or in the stations near the sea inlets. Larvae, in each sub-basin, have COG values higher than eggs and it is located near the sea inlet stations, indicating a progressive shift of marine migrant concentration within the lagoon. Analyzing the COG of juvenile marine migrant is possible to observe that in the north sub-basin, both years, individuals are located in the inner part of the sea-lagoon transect from the first sampling campaign, indicating that they can move from the sea to the lagoon edge gradually since the first arrival in the lagoon. In central sub-basin, during the second year, and south sub-basin, both years, the Center of Gravity of juvenile marine migrant fish fauna was located near the lagoon edge only during the third sampling campaign. In the central and south sub-basins individuals seem to be present first in stations near the sea inlets or in marine stations and then move to inner stations suddenly during the last campaign. The presence of juvenile individuals in the inner station were observed especially during the second sampling year, in each sub-basin. In second sampling year the third campaign, due to meteorological conditions, was conducted at the beginning of May and not in April. These differences in the sampling dates, however, do not seem to influence the environmental parameters collected during the various campaigns of the two years.

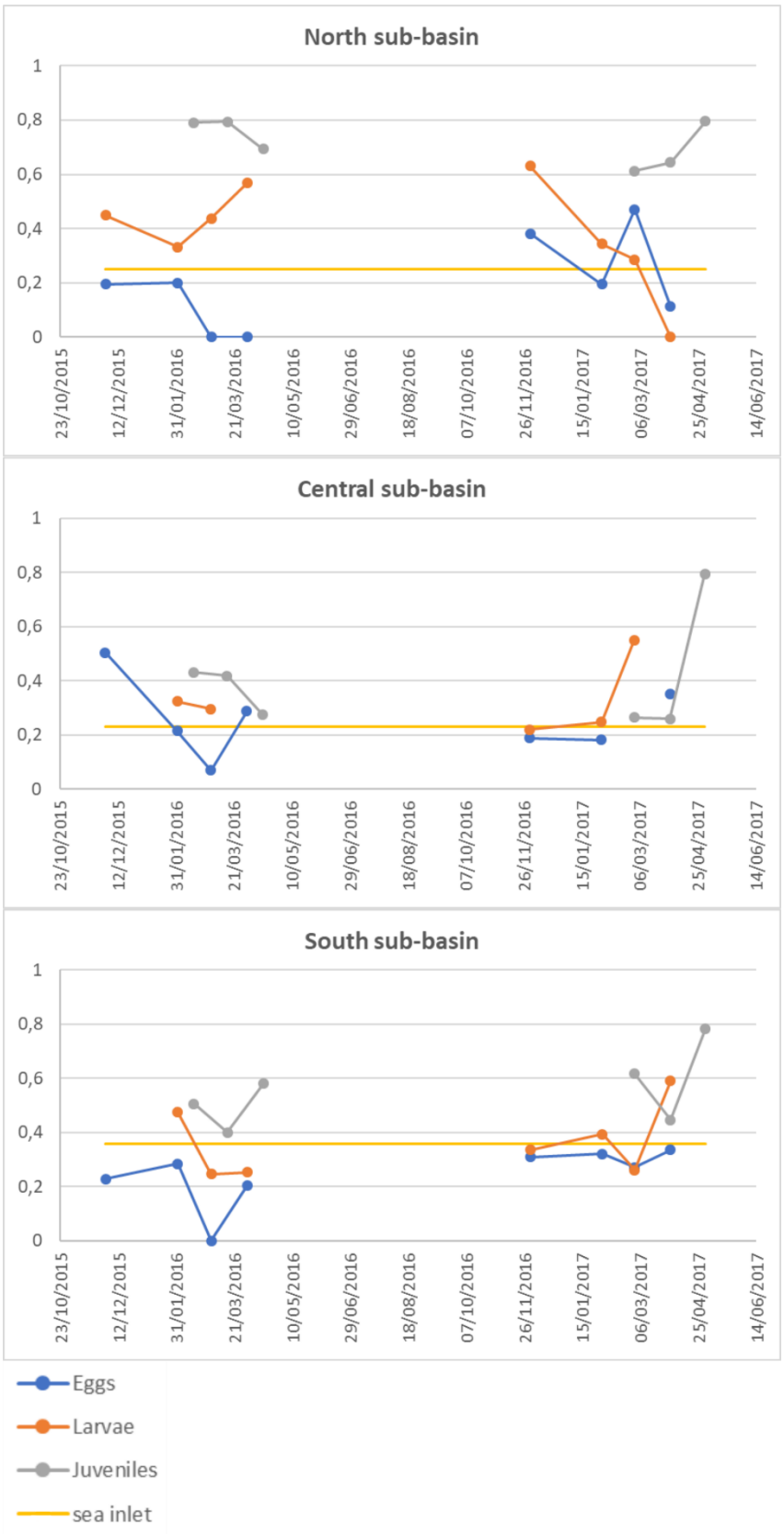


Figure 23 – COG calculated for eggs, larvae and juvenile of marine migrant community, separated for sub-basin. The yellow line corresponds to the sea inlet station. X axis = sampling dates; Y axis = COG value. Blu line = eggs; Orange line = larvae; grey line = juvenile. Missing point means that no individuals were caught during that campaign.

Analyzing the center of gravity (COG) calculated on the density of eggs and larvae of *Sprattus sprattus* (fig. 24), separated for sub-basin, results show many missing points, indicating that often individuals were not caught. Generally, as observed with the entire marine migrant ichthyoplanktonic component, eggs were located, both years, mostly outside the lagoon, while fish, especially during the first year and in north and south sub-basins, started entering the lagoon at the larval stage.

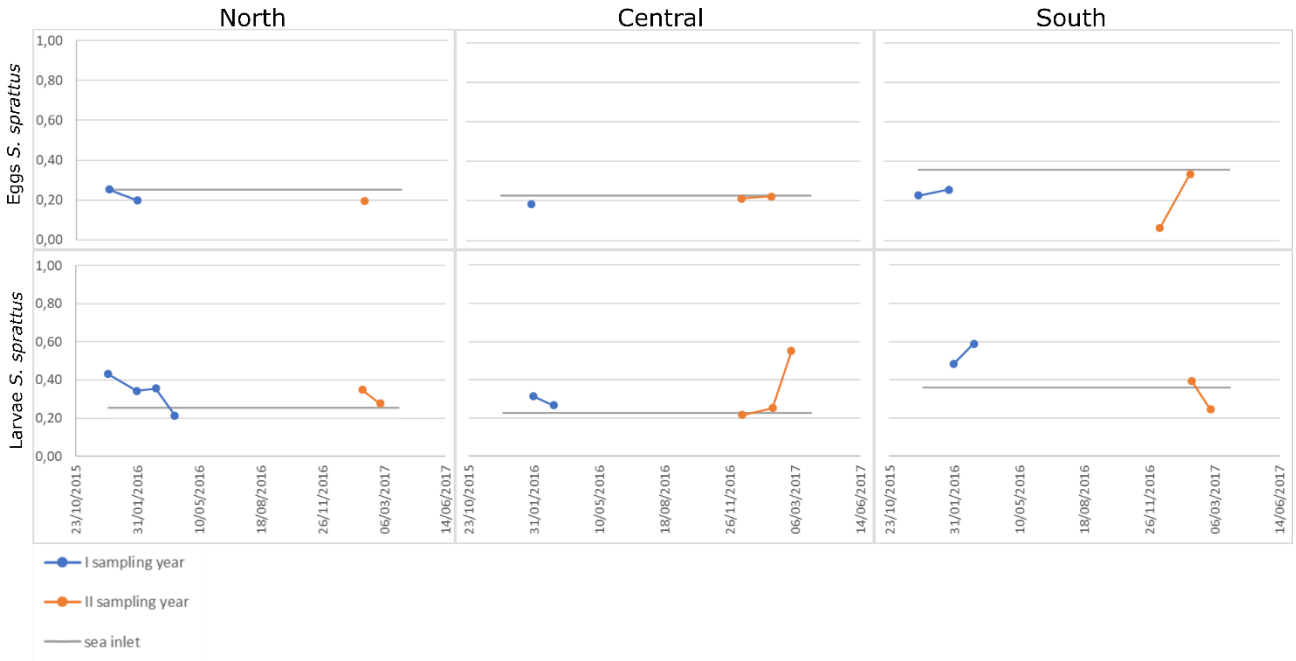


Figure 24 – COG calculated for eggs and larvae of Sprattus sprattus*, separated for sub-basin. The grey line corresponds to the sea inlet station. X axis = sampling dates; Y axis = COG value. Blu line = first sampling year; Orange line = second sampling year. Missing point means that no individuals were caught during that campaign.*

Observing the Center of Gravity (COG) calculated on density of juveniles of the most abundant species (*C. auratus*, *C. ramada*, *C. saliens* and *S. aurata*) (fig. 25), results show a similar pattern of total marine migrant community across sub-basins. Especially during the first year, for all the four species, the north sub-basin seems to have the higher values of COG, indicating that generally individuals are distributed within the lagoon up to inner stations. In the central sub-basin, the COG values highlight that individuals of *C. auratus*, *C. ramada* and *S. aurata* remain with higher density in sea stations, both years, except during the third campaign of the second sampling year. Results from central sub-basin show that individuals were first found in the sea stations and then gradually move inside the inner lagoon stations. The south sub-basin shows the presence of individuals within the lagoon only considering *C. saliens* and *C. ramada* during the first and second sampling year.

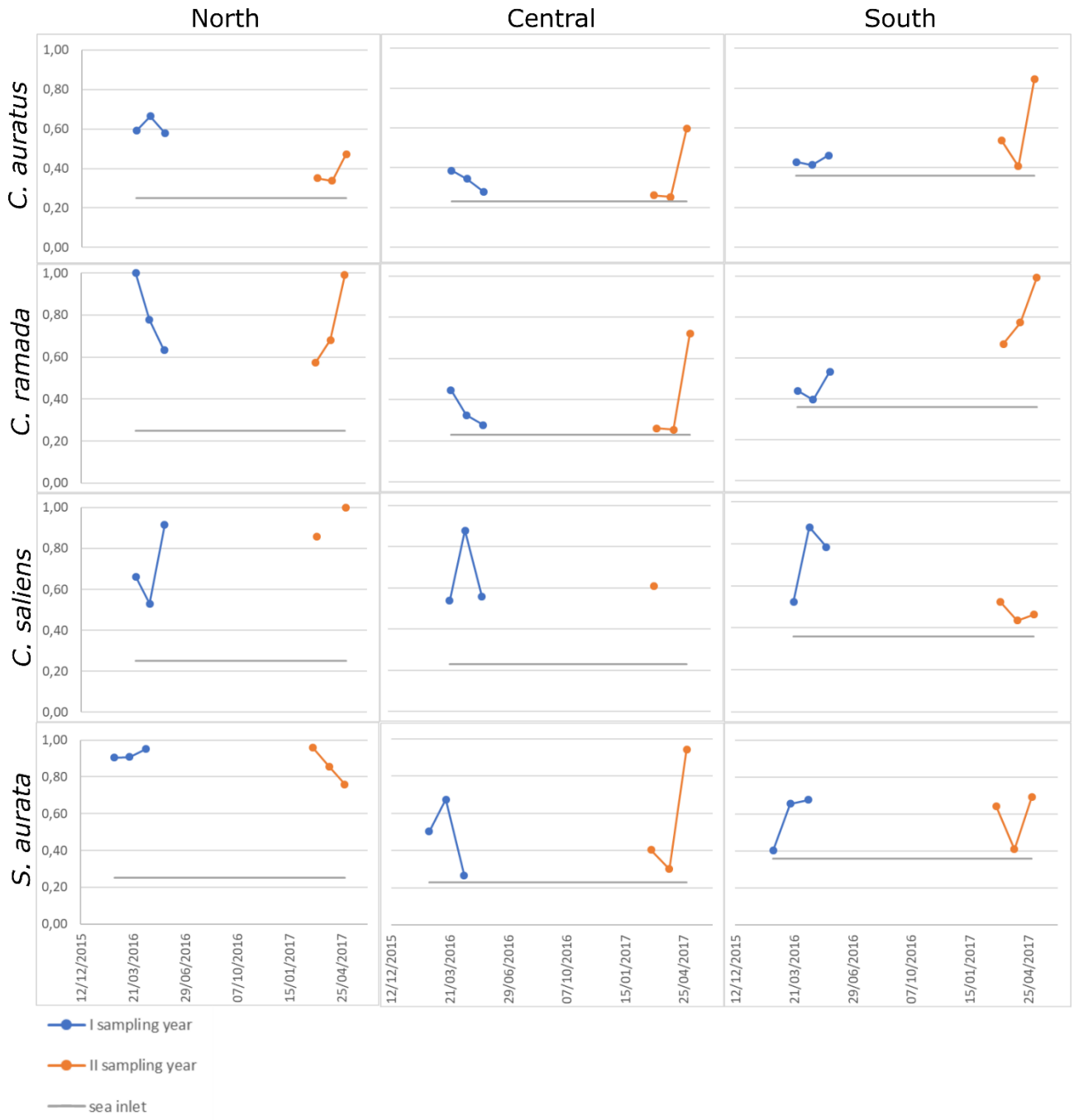


*Figure 25 – COG calculated for juvenile of the most abundant species (*C. auratus, C. ramada, C. saliens, S. aurata*), separated for sub-basin. The grey line corresponds to the sea inlet station. X axis = sampling dates; Y axis = COG value. Blu line = first sampling year; Orange line = second sampling year. Missing point means that no individuals were caught during that campaign.*

1.4 Discussion

This two-years study aimed to broaden the knowledge on biological connectivity of the whole Venice lagoon with the sea, quantifying and getting information about the presence and the composition of the marine migrant fish fauna at different ontogenetic stages (eggs, larvae and juveniles), both inside and outside the sea inlets, and also exploring the different role of the three sub-basins in attracting and concentrating marine migrant fish. From the literature on the subject, it is in fact known that the ichthyoplanktonic component

represents a good indicator of the hydrodynamic characteristics of a system (Bolle et al., 2009; Chiappa-Carrara et al., 2003; Perez-Ruzafa et al., 2004; Robins et al., 2012), useful to monitor the flows that pass through the sea inlets. The approach used in this study deepened the knowledge about the presence and entrance of marine taxa from the sea to the lagoon. The standardized sampling plan, which provided samples of ichthyoplankton (eggs and larvae) and juvenile fish, analyzed the entrance into the lagoon of the different fish taxa, highlighting different behaviors both in spatial and temporal terms.

From the observations of taxa composition, it seems to emerge that, except some occasions, for most of the species not all the developmental stages were found. Only a few marine migrant species, such as *Solea solea* and *Platichtys flesus*, have been found within the lagoon in all the three life stages considered (eggs, larvae and juveniles) and in this case the settlement in the areas of shallow water coincides with the metamorphosis from larva to juvenile. For some species, as clupeids, the presence of eggs and larvae were greater than those of juveniles. Contrarily, some species, as *Dicentrarchus labrax*, have been found in ichthyoplankton community only occasionally. Furthermore, it is extremely important to underline how some of the species found only in few occasions, and with no considerable densities in the ichthyoplankton communities are extremely abundant as juveniles, suggesting that these species enter the lagoon in a more advanced ontogenetic stage (e.g. Mugilidae sp. pl. and *S. aurata*). Therefore, at least in this case, the entrances into the lagoon do not seem to be attributable to a mere passive transport with tidal currents, since the juveniles are active swimmers. From the collected data it seems to emerge how the three analyzed ontogenetic stages (eggs, larvae and juveniles) show different behaviors during the migration phase in the lagoon, according to the different taxa found. While *S. pilchardus* and *S. sprattus* seem to enter the lagoon already as egg or larvae, other very common species, such as Mugilidae or *S. aurata* seem to complete the larval phase at sea and then enter the lagoon only at the juvenile stage (Perez-Ruzafa et al., 2004). Furthermore, in terms of abundance, it seems that the most significant access in the lagoon water occurs at a more advanced ontogenetic stage, according to what was observed by Perez-Ruzafa et al. (2004). The reason for these differences in the lagoon entrance can be attributed to various factors. Different species can spawn in different marine areas, even very far from the Venice lagoon. The passive transport of eggs and larvae of these species and their arrival near the coast could thus vary in time in relation to spawning area and distance from the lagoon, as well as in relation to the marine currents. As well there may be the preference towards different lagoon habitats. Eggs and larvae were collected in canal areas characterized by a great depth while juveniles in shallow water stations. Even if the selected stations have been carefully chosen, some species, as clupeids for example, during the juvenile phase, could prefer canal areas rather than shallow water areas, making it difficult to capture them.

In the case of the ichthyoplankton, the data obtained during the two years mostly agree with the results obtained for the southern sub-basin of the Venice lagoon by Varagnolo (1964) and Ziraldo (1996), for central

and north sub-basins by Spartà (1942) and more recently for the north sub-basin (2013-2015) by Cavraro et al. (2017a). For instance, *Sprattus sprattus*, the most abundant species found in the present bongo net sampling especially in December and January as eggs and in January and February as larvae, were found as eggs from November to January by Varagnolo (1964), from January to March by Ziraldo (1996) and from December to February by Cavraro et al. (2017a) while as larvae from December to April by Cavraro et al. (2017a). *Sardina pilchardus* in this work were found during all sampling periods, but especially in December and March as eggs, and from January to April as larvae; these periods of occurrence were the same observed in 2013-2015 by Cavraro et al. (2017a). Eggs of *Engraulis encrasicolus* were found from the first days of April, thus during the warm season, as observed by Ziraldo (1996), while the eggs of *Platichthys flesus* were found during the cold season, especially in February, as observed by Varagnolo (1964). Conversely the larvae of *P. flesus* were found especially in February as observed by Cavraro et al. (2017a). Finally, eggs of *Solea solea* were found during the first months of the year, from January, as observed by Cavraro et al. (2017a). The analysis of the ichthyoplanktonic populations found in these two years also agree with the taxonomic composition in other Mediterranean coastal lagoons (Perez-Ruzafa et al., 2004) and with the spawning periods of many marine species in the Mediterranean and Adriatic Sea. Furthermore, marine migrant species have more relevant densities inside the transitional water ecosystems during late winter and spring, according to the biological cycles of the marine species sampled (Rossi, 1986; Rossi et al., 1999). Considering ichthyoplankton composition, even if no significant differences in density were observed between sampling campaigns and years, it was possible to observe a strong interannual variability: in the second year, higher density values of both eggs and larvae of *S. pilchardus*, *P. flesus* but especially *S. sprattus* were observed in respect to the first year. Instead, the higher abundance of eggs, but especially larvae, of *S. sprattus* during the second campaign of both years correspond to the peak of the reproductive period of this species, which occur during winter months (Dulcic, 1998; Legovini, 2008; Teskeredzic, 1978; Ticina et al., 2000; Tsikliras, 2010) in a wider area of the upper Adriatic Sea, on the Rovinj-Po Delta profile. The second most abundant larvae belong to Gobiidae, presents especially in spring. Eggs of Gobiidae are not present in the samples since these taxa do not have pelagic eggs.

To interpret the interannual variability and the extremely high density of larvae of *S. sprattus* during the second year, superficial water temperature has been considered using Environmental Marine Information System (EMIS, 2017). Indeed, this parameter could influence the reproduction, the distribution and the presence of fish fauna and ichthyoplankton in marine water (Damirel, 2015; Genner et al., 2010; Marques et al., 2006). During the two-year study (2015-2016 and 2016-2017), the mean water temperature values did not change drastically (tab. 11), except in February, and no extreme high or low temperature events occurred, probably in part justifying the absence of statistical differences in density of fish eggs between sampling years or campaigns. The interannual variability could also be interpreted by considering the life-history of sprat. It is indeed well known that *S. sprattus* can be characterized by an extended reproductive period and many

spawning events per year (Alheit, 1988; Solberg, 2015; Ticina et al., 2000; Torstensen, 1992) and probably during the second sampling campaign of the second sampling cycle, a peak of these spawning events has been captured.

Table 11 – Mean temperature (°C) of sea surface water temperature by Environmental Marine Information System (EMIS, 2017) of the sampling period.

	2015-2016	2016-2017
November	14.1	14.8
December	10.6	9.9
January	8.0	8.1
February	9.0	7.3
March	10.2	11.2
April	14.9	15.0

Results of ichthyoplankton composition do not show significant differences between sub-basins or between positions (sea-lagoon). The absence of differences in the density of eggs and larvae between marine and lagoon stations suggests the existence of a mainly passive transport of eggs and larvae through all three sea inlets (Bolle et al., 2009; Chiappa-Carrara et al., 2003; Jenkins et al., 1999; Perez-Ruzafa et al., 2004). Moreover, eggs and larvae of *S. sprattus* were found with high abundance even in the inner station in both of the three sea-lagoon transects. This is very important to consider because differences in structure of the three sea inlets are present, as well as the direction and strength of forcing winds and currents, and thus in water exchange (Bellafiore et al., 2008; Gacic et al., 2002, 2004). In the Venice lagoon, the southern and the central inlets (Chioggia and Malamocco respectively) are about 500 m wide, whereas the northern inlet (Lido) is nearly 1000 m wide (Bellafiore et al., 2008); besides for Malamocco and Lido inlets the maximum depth is around 14 m and 8 m for Chioggia inlet (Ghezzo et al., 2010). The presence of different meteorological contribution can strongly affect the water exchange dynamics through sea inlets (Bellafiore et al., 2008; Gacic et al., 2002). Any sea/lagoon differences between sub-basins in water exchange could be influenced by the different orientation and conformation of the sea inlets in relation to the prevailing winds and currents. In the Venice lagoon a high frequency of the winds from the first quadrant (directions between N and E, bora wind) and second quadrant (direction between E and S, Scirocco wind) (Massalin and Canestrelli, 2004) is present. In particular, from October to February the wind from the first quadrant, such as bora wind, dominate the wind composition (Massalin and Canestrelli, 2004). Considering the prevailing winds and orientation of Venice lagoon, if all marine migrant fish species spawn in the North Adriatic Sea between Venice and Trieste, the northern sub-basin and Lido sea inlet should be the ones in which organisms arrive first. However, the meteorological characteristics and the structure of the sea inlets do not seem to create differences between sub-basins: the passive transport within the lagoon and the strong sea-lagoon connection seem to always remain stable in all three sub-basins.

A strong connection between sea and lagoon has been observed with the colonization index (I_C) and center of gravity (COG) calculated on eggs and larvae distribution both for the entire marine migrant component and *S. sprattus*, the most abundant species. The indices show similarities between years and sub-basins. Eggs of marine migrant fish species, which are spawned in the sea, can be found mainly in sea stations and the larvae then start to colonize and concentrates in lagoon stations. Even with marked differences in flows, wind and current between the sea inlets (Bellafiore et al., 2008; Gacic et al., 2002; Massalin and Canestrelli, 2004), the ichthyoplanktonic component, which is strongly related to hydrodynamic characteristics of a system (Bolle et al., 2009; Chiappa-Carrara et al., 2003; Perez-Ruzafa et al., 2004; Robins et al., 2012), seems to highlight the absence of differences between sub-basin and suggests the existence of a mainly passive transport through all three sea inlets. However, the construction of the mobile barrier of Mo.S.E., to protect Venice from high tide extreme events (Campostrini et al., 2017), could affects the current situation. From a model of Ghezzo et al. (2010), the mobile barrier construction does not affect water levels, but differences can be detected analyzing velocities and phase shift of fluxes. The variation will not affect the overall balance of the water within the lagoon as the relative flows through each inlet (Ghezzo et al., 2010). These variations in flows inside lagoon, in the future, could therefore modify the entrance and the retention of organisms within the lagoon habitats.

When considering the post-larval and juvenile component, the pattern resulting from the comparison of densities between the two sampling years was instead more complex, probably due to their different swimming behavior in the water masses and to their different arrival time in the lagoon waters. Significant interactions were highlighted between the considered factors, with different spatio-temporal dynamics in the three sub-basins.

Excluding resident taxa, which are represented mainly by *Atherina boyeri* and *Aphanius fasciatus*, marine migrant taxa were mostly represented by *Chelon* sp. pl. and *S. aurata*, found in all three sub-basins with high abundance in both sampling years. The major differences between the two sampling cycles however were observed for *C. saliens*: even if less frequent and abundant in samples respect congeneric species, in the second sampling year was captured only occasionally and with few individuals. Even if this could be a natural inter-annual fluctuation in the densities of a fish population, the decrease of a species with summer reproduction such as *C. saliens* (Rossi, 1986; Franzoi et al., 1989, 2005) could be attributed to the recent invasion of lagoon waters by *Mnemiopsis leidyi*, a predator ctenophore of zooplankton but also of eggs, larvae and juvenile fishes which appears especially in summer period (CIESM, 2015). Further studies are needed to evaluate the effect of this ctenophore on the distribution, abundance and disappearance of some fish species.

Analyzing spatial differences in juvenile abundance, it is possible to observe that the areas under the influence of the Malamocco and Chioggia inlets (central and south sub-basins) are characterized by the

highest abundance of marine migrant fish but these are concentrated in marine stations (e.g. *C. auratus* and *C. ramada*). Instead, in the north sub-basin, high density of marine migrants, especially *S. aurata*, can be found in the lagoon stations, indicating a quite stable access and entrance of individuals inside this portion of the lagoon. Lastly, *C. saliens* seems to concentrate within the lagoon stations in all the three sub-basins.

These observations can be confirmed also analyzing the colonization index (I_C) and the center of gravity index (COG). The central sub-basin is the one which has the lowest index values, in both years, indicating that the juveniles remain in the marine stations rather than accumulate within the lagoon. The south sub-basin appears to be the most variable: during the first year, values of I_C are extremely low while they increase during the second year. This pattern in the south sub-basin could be observed also for the COG index. The north sub-basin indeed, markedly during the first year, has the highest value of colonization index, both considering the total marine migrant community and the most abundant species. Observing the center of gravity index, again, in the north sub-basin, the same pattern can be observed, both years: individuals concentrate mostly in the lagoon station, up to the inner ones, still from the first sampling campaign. In particular, *C. saliens* and *S. aurata* were found almost exclusively within the lagoon stations in both years. The anomalous low value of I_C observed in the north sub-basin during the second year could be justified knowing that generally individuals arrive near the coast at different times, in relation to spawning events, which, for the most abundant species in Venice lagoon, could occur more than once during the breeding season (Ferrari and Chieregato, 1981; Franzoi et al., 1989, 2005; Rossi 1986).

These results could indicate that the juvenile marine migrant fish are mostly attracted by the north portion of the Venice lagoon and they concentrate inside this sub-basin before the others, but the reasons of high indices values could be different. Juvenile fish, partly as eggs and larvae, can indeed arrive near the coast of Lido inlet before the other sub-basins due to hydrodynamic and meteorological favorable condition and thus concentrate in this portion of the coast first. As no enough information about the hydrodynamic and meteorological conditions were obtained in this study, further studies with targeted samplings could unravel this point. A second reason that could explain the concentration of marine migrant fish in the north sub-basin of Venice lagoon, as for ichthyoplankton, can be attributed to the different morphology of the three sea inlets (Bellafiore et al., 2008; Gacic et al., 2004) and the coast nearby the inlets (e.g. breakwater), which can facilitate or slow down the entry of individuals from the coast. Lastly, the three sub-basins can be colonized in different ways since the three sub-basins show a different mosaic of habitat types (Franco et al., 2006a, 2009; Franzoi et al., 2010; Malavasi et al., 2004; Tagliapietra et al., 2009).

However, juveniles of marine migrant fishes, which can move actively towards more suitable habitats (Able et al., 2005; Beck at al., 2001; Elliot and Hemingway, 2002; Elliott et al., 2007), are probably influenced more by the different habitats present along the sea-lagoon transect, respect to the direct hydrodynamic characteristics of sub-basins. The presence of different interconnected types of habitat (seagrass beds, sand

flats, mud flats, saltmarshes and tidal creek), playing different functional roles, could influence the active entrance of juvenile marine migrants from the sea still from their arrival near the coast especially if located also near the sea inlet. In particular, as it will be seen in the second chapter of the thesis, among the different habitats, saltmarsh habitats and tidal creeks characterized by low salinity values seem to play an important function for the juveniles of marine migrant fish. Salinity is an important factor to understand the differences between sub-basins because freshwater, and the substance coming from the mainland through the rivers, are sought by juvenile through sensory organs during their sea-lagoon migration (Able et al., 2005; Bos and Thiel, 2006; Whitfield, 1999).

The three sub-basins own different morphological characteristics, with the presence and distribution of habitats changing along the sea-lagoon gradient. The north sub-basin is typically characterized by a high level of spatial heterogeneity, owing a variety of interconnected shallow water habitats. It is also the one with the lowest salinity due to the presence of the main freshwater tributaries in the lagoon (Zonta et al., 2005). Even if the distance from the sea inlet to the lagoon edge is the greatest, in this sub-basin the first natural habitats which fish and organisms can colonized are located evenly along the sea-lagoon gradient, starting a few kilometers from the sea inlet. Also, in the north sub-basin there is the biggest coverage of saltmarsh habitats and tidal creeks, likely facilitating the colonization of this portion of the lagoon by fishes. Results indeed show that, especially in the north sub-basin the tidal creeks own the highest abundance of individuals, in particular for *C. saliens* and *S. aurata*. The central sub-basin is characterized by a large channel (Malamocco-Marghera Channel) which connects the Malamocco sea inlet to the harbor of Marghera. Producing the highest water exchange with the sea (Cucco and Umgiesser, 2002) among the three sea inlets and allowing the passage of numerous ships, this channel determined significant changes in the morphological and hydrological conditions of the Venice lagoon (Molinaroli et al., 2009; Parnell et al., 2016; Zaggia et al., 2017). The constant hydrodynamic stress caused by the opening of the Channel produced a loss of more than 3 km^2 of saltmarshes (Molinaroli et al., 2009), leading to a general flattening of the morphology (Saretta et al., 2010). This situation (i.e. the absence of a high structured mosaic of habitats distributed along all the sea-lagoon edge gradient, the absence of saltmarsh habitats near the sea inlets, the absence of a linear salinity gradient) could have negatively influenced the distribution of marine migrant juvenile fishes, making this sub-basin the less suitable for the colonization of juvenile individuals. The south sub-basin, which hosts the town of Chioggia, is characterized by the lowest exchange of water with the sea and the lowest freshwater inputs (Cucco and Umgiesser, 2002; Franco et al., 2006a). Although simpler than the north sub-basin, the south sub-basin is characterized by a more complex mosaic of habitats respects to the central sub-basin. Moreover, in this sub-basin, even if the sea-lagoon edge gradient is the shortest, the distance between the sea inlet and the first natural saltmarsh is greater than the north sub-basin. These characteristics could justify the relatively low and variable colonization of the lagoon stations in this sub-basin. Instead, the great abundance of fish at sea

stations of the south sub-basin, at least for the first sampling campaign, could be analyzed through further future studies concerning wind and currents near the Chioggia sea inlet.

Overall, the differences between sub-basins that have been observed considering sea-lagoon connectivity should be related to different factors: different hydrodynamic and meteorological favorable conditions, which may have concentrated the fish outside a specific sea inlet; different morphological and structural complexity of the three sea inlets, which may have facilitated or slowed down the entry of individuals; different morphological and structural complexity of the three sub-basins and different freshwater supply, which may have influence the colonization of the lagoon. Therefore, the presence of a complex mosaic of interconnected habitats, distributed along a more linear gradient which begins near the sea inlet may explain the higher colonization of the north sub-basin, having influenced the distribution of fish.

Samples of eggs, larvae and juveniles have highlighted the presence of numerous species, also of commercial interest (sprat and sardine, sea bass, sea bream, flounder, mullets and sole), which migrate within the lagoon in the period from late autumn to early spring. This is also the period in which the "high water" phenomena and the Scirocco wind occur, causing the uplift of the MOSE mobile barriers and the consequent interruption of the incoming flows of eggs, larvae and juvenile fish. From this derives the importance of continue over time the study of this component of the ecological sea-lagoon connectivity to have a robust "zero state" (not influenced by oscillations due to occasional and punctiform phenomena arising, for example, from particular weather-climatic conditions), that will allow to assess the possible effects deriving from future interruptions in the sea-lagoon connectivity following the implementation of the MOSE.

In this chapter the sea-lagoon connectivity at scale of the entire lagoon basin has been analyzed and the results seems to highlight the importance of the north sub-basin relatively to colonization by marine migrant species, in particular juveniles. Considering what has been observed up to this point, the presence of a more complex mosaic of habitats could be one reason that explain why this sub-basin is massively colonized. Thus, in the second chapter of the thesis, considering only the north sub-basin, the distribution of juvenile fish in different habitats during the period of growth within the lagoon will be analyzed.

CHAPTER 2

MARINE MIGRANT JUVENILES DISTRIBUTION DYNAMICS IN THE NORTHERN VENICE LAGOON

2.1 Introduction

Estuaries and lagoons are commonly considered highly productive and valuable ecosystems, which provide numerous habitats for fish (Able, 2005; Costanza et al., 1997; Vasconcelos et al., 2010). These ecosystems, and the complex mosaic of interconnected habitats (e.g. seagrass meadows, marshes, tidal creeks, mangrove forests, for a detailed description Elliott and Hemingway, 2002) perform different functions for many fish species, which use them as feeding and spawning grounds, pathways in diadromous migrations and as nursery areas (Elliott et al., 2007; Franco et al., 2008; Sheaves et al., 2015). Among the other functions, estuaries and coastal lagoons support a great abundance and diversity of fish (Boesch and Turner, 1984; Gillanders, 2005; Nagelkerken et al., 2015). The occurrence of juvenile marine migrant fish within different habitats of coastal ecosystems can be related to several advantages (Beck et al., 2001; Cabral et al., 2007; Miller et al., 1985). For instance, within the lagoons and coastal ecosystems, marine migrant fish find more suitable conditions for a rapid growth compared to the marine environment, due to higher water temperature and a lower predation (Beck et al., 2001; Minello et al., 1985; Selleslagh et al., 2009; Vasconcelos et al., 2010; Whitfield and Pattrick, 2015). Juveniles of marine migrant species can exhibit a preference for different areas or habitats inside lagoons as a result of the response to multiple environmental characteristics (Vasconcelos et al., 2011) and as a tradeoff between food availability and predation risk (Cattrijsse and Hampel, 2006).

For marine aquatic species, a habitat is defined as “essential” when it is necessary for the completion of the biological cycle (Schmitten, 1999). For example, shelter, foraging or nursery habitats could represent “essential” habitats. Moreover, usually, nursery habitats are considered as “essential” habitats but only few marine migrant species are confined to a single nursery habitat (Nagelkerken, 2007; Nagelkerken et al., 2015; Ribeiro et al., 2012; Whitfield and Pattrick, 2015) and the movement of organisms between habitats is vital for the populations’ persistence and productivity (Olds et al., 2012). The complex composition of different type of habitats, that often can be found in close proximity one to each other, allows many species to make multiple ontogenetic habitat shifts (e.g. Bostrom et al., 2011; Adams et al., 2006; Elliott and Hemingway, 2002; Nagelkerken et al., 2015). Determining the movement of fish, site fidelity and ontogenetic habitat use

pattern among estuarine habitats is an important tool to understand which juvenile habitats are functioning as nursery and which environmental characteristics juveniles prefer during growth and ontogenetic shifts. Temperate lagoons and estuaries are characterized by rapid and intense fluctuations in physicochemical factors, which translates into strong spatial and temporal heterogeneity and variability (Elliott and Hemingway, 2002; McLusky and Elliott, 2004; Vasconcelos et al., 2011). These fluctuations, mainly influenced by meteorological, seasonal, hydro-morphological and biotic variations inside the lagoon, can occur at different temporal and spatial scales. The characteristics of each habitat thus change rapidly even on a small spatial and temporal scales. Consequently, the specific function performed by each habitat could change rapidly and therefore fish moves to found suitable habitat for their life function. The nursery function of a whole lagoon must be evaluated considering the relationships between each habitat and their biotic and abiotic characteristics and ecological roles for the juveniles (e.g. feeding grounds, shelter), that can change rapidly in time and space (McLusky and Elliott, 2004; Pasquaud et al., 2008; Sheaves et al., 2015). As a result, during their permanence inside lagoons juveniles of many species can show a complex dynamic in their preference toward certain habitats or environmental conditions (Adams et al., 2006; Beker and Sheaves, 2005; Brown et al., 2006; Minello et al., 2003). Spatial and temporal patterns of occupancy of habitats differ as a function of age and life history strategy, development stage or habitat availability (Able and Fahay, 1998; Elliott and Hemingway, 2002), because each species during the growth can change its preferences as physiological response to abiotic and biotic factors. Moreover, fish can change their preferences towards habitat or environmental conditions during growth within the lagoons in relations with changes in body morphology, swimming ability, development of digestive tract, teeth-age adaption and die preferences (Georgalas et al., 2007; Russo et al., 2007; Tancioni et al., 2003).

It is commonly reported that identifying and protecting the most valuable Essential Fish Habitats and nursery habitats, especially those particularly vulnerable to degradation or loss, is very important to create sustainable fisheries management and to safeguard and conserve the estuarine ecological integrity (Adams et al., 2006; Avigliano et al., 2017; Beck et al., 2001; Brown et al., 2006; Reis-Santos et al., 2015). The individuation and characterization of juvenile and larval fish habitats is a critical step for the management of the nursery function of transitional water ecosystems. Nevertheless, given the different environmental and habitat preferences among species and size classes, this task cannot be fulfilled without considering the dynamics of the use and the movements of individuals among habitats throughout ontogeny (Green et al., 2012; Nagelkerken et al., 2015).

In the upper Adriatic Sea, many marine species that represent important stocks exploited for fishing, at the juvenile stages concentrate in shallow water habitats of coastal and transitional water environments, like the Venice lagoon (Rossi et al., 1986; Franzoi et al., 1999; Franzoi e Pellizzato, 2001; Provincia di Venezia, 2009). Among these species, gilthead sea bream *Sparus aurata*, sea bass *Dicentrarchus labrax*, European flounder

Platichthys flesus, common sole *Solea solea*, mullets *Chelon ramada*, *C. auratus*, *C. saliens*, *C. labrosus* and *Mugil cephalus*, anchovy *Engraulis encrasicolus* and clupeids *Sardina pilchardus* and *Sprattus sprattus* are particularly frequent and abundant. In the Venice lagoon, studies on fish fauna have been conducted, highlighting the importance of lagoon shallow bottom habitats as potential nursery areas for marine migrant fish species (Cavraro et al., 2017a; Franco et al., 2010; Franzoi et al., 2005; Zucchetta et al., 2009, 2010) and stressing, in particular, the role of the northern part of the lagoon (Franzoi e Pellizzato, 2002; Franco et al., 2006a; 2010; Zucchetta et al., 2010). However, although a complex mosaic of habitat types is present in Venice lagoon (Franco et al., 2006a, 2009, 2010; Franzoi et al., 2010), no studies were conducted on environmental preferences of juveniles of marine migrant fish at the habitat scale. Hence, the specific nursery role of different habitats and the changes in habitat's preference during fish permanence in the transitional waters are still not well known (Zucchetta et al., 2010; Zucchetta, 2010).

Generally, an effective family of tools to identify Essential Fish Habitats is represented by Species Distribution Models (Elith et al., 2008; Phillips et al., 2017; Young and Carr, 2015; Zucchetta, 2010). These models are based on the quantification of the relationships existing between species distribution and environmental parameters (or any biotic or abiotic factor that influences the distributions). In this study, the influence of environmental characteristics on the distribution of juveniles of marine migrant fish in the northern sub-basin of the Venice lagoon were analyzed using Species Distribution Models. We focused on this part of the basin, because it showed an important role for marine migrant species, as it has been reported in the first Chapter of this thesis and in past studies (Cavraro et al., 2017a; Franco et al., 2006a; Zucchetta et al., 2009, 2010). Habitats as saltmarsh, marsh creeks, mud and sand flats or seagrass beds, with different biotic and abiotic characteristics and located in different position of the sub-basin, were considered to explore the environmental preferences of fish. To represent different ontogenetic stages, data were organized considering different size classes according to the available literature. The general aim of the study was to characterize the habitat and environmental characteristics preferred by marine migrant fish, investigating their potential changes during ontogeny in the short time period of lagoon occupancy, in order to evaluate the relative importance of different habitats as nursery grounds. The hypothesis of this chapter is that each habitat within the lagoon, differenting from the other for abiotic (e.g. freshwater input, hydronynamic) and biotic conditions (e.g. predation and trophic resources), is used by each marine migrant species for a different function. Moreover, a second hypothesis is that individuals belonging to the same species use different habitats in relation to their life stage.

The specific objectives of this part of the study are:

- Identifying - on the basis of available knowledge, mainly regarding diet and morphology - the main ontogenetic stages of the marine migrant species present in the lagoon;

- Modeling the habitat and environmental relationships for the most important species of juvenile migrants;
- Testing if there are significant changes in the environmental requirements of the juvenile marine migrant species that use the lagoon waters, by contrasting a single class model (no differences among size classes) with a multi-class approach (considering a class-specific model for each ontogenetic stage);
- Identifying the habitats within the lagoon where the densities of juvenile marine migrant fish are higher.

2.2 Materials and Methods

2.2.1 Data collection

Samplings were conducted in 2016 in the Northern sub-basin of the Venice lagoon from late winter to early summer, when inward fish migration within the lagoon has its maximum intensity (Franzoi et al., 2010; Rossi, 1986). Data were collected in 16 shallow water stations located at different position of the sea inlet-lagoon edge gradient (fig. 26) (for a detailed descriptions of Venice lagoon see the chapter "Study Site"). Sampling stations were distributed to encompass the environmental variability of the sub-basin and different habitat typologies. Sampling took place during daylight hours monthly in February, March and June, and fortnightly in April and May in each sampling occasion. A beach-seine net (2 mm knot disatance) was trawled on shallow waters over an average area of 480 m^2. According to the site-specific environmental conditions, the width of the net aperture and the length of the haul varied respectively from 8 to 20 m and from 20 to 80 m. The area swept by the net during each sampling was estimated on the basis of the length and width, in to order standardize catches as number of specimens per 100m^2. All fish collected were sacrificed with an excess of 2-phenoxyethanol, preserved refrigerated until the arrival in laboratory and then frozen at -20°C.

In each station, together with fish samples, the main abiotic characteristics were collected during each sampling occasion. Water temperature (±0.1 °C), salinity (±0.01 PSU), dissolved oxygen (±0.1 % saturation) and turbidity (±0.1 FNU) were recorded for the water column with a multi parameter probe (Hanna Instrument 9829). Simultaneously, 200 mL of water were filtered on Whatman GF/F 47 mm diameter filters and three cores of sediment (diameter 2 cm) were collected, to determine the total chlorophyll concentration in the water column (±0.01 µg/L) and in the upper 2 cm sediment (±0.01 µg/g). In April, a core of sediment (diameter 3 cm; height 10cm) was collected in each sampling station to determine grain size composition. During each sampling occasion, in each site, the presence/absence of macroalgae and the seagrass coverage (% coverage) were also recorded. In addition to the environmental data collected simultaneously to fish fauna

samplings, water residence times were also attributed to each station on the basis of its geographical position (days; Ghezzo et al., 2010) and used to quantify the degree of confinement.

Habitats were defined firstly according to the main morphological characteristics, namely if the sampling area was included within or very close (<10 m) to saltmarshes or if it was located in a sand or mud flat, and then considering the presence of submerged vegetation. Each sampling station was characterized according to the main vegetation cover observed in the field, i.e. in terms of presence/absence of macroalgae and seagrass coverage. To better understand the role of saltmarshes, marsh sampling sites were divided into two sampling position located very close to each other: Saltmarsh Edges (i.e. sites in which the net was trawled on one open edge of the saltmarsh) and Marsh Creeks (i.e. sites in which the net was trawled within the margins of creeks located inside the saltmarsh). This classification allowed to distinguish bare bottom sites and seagrass sites, with or without macroalgal cover.

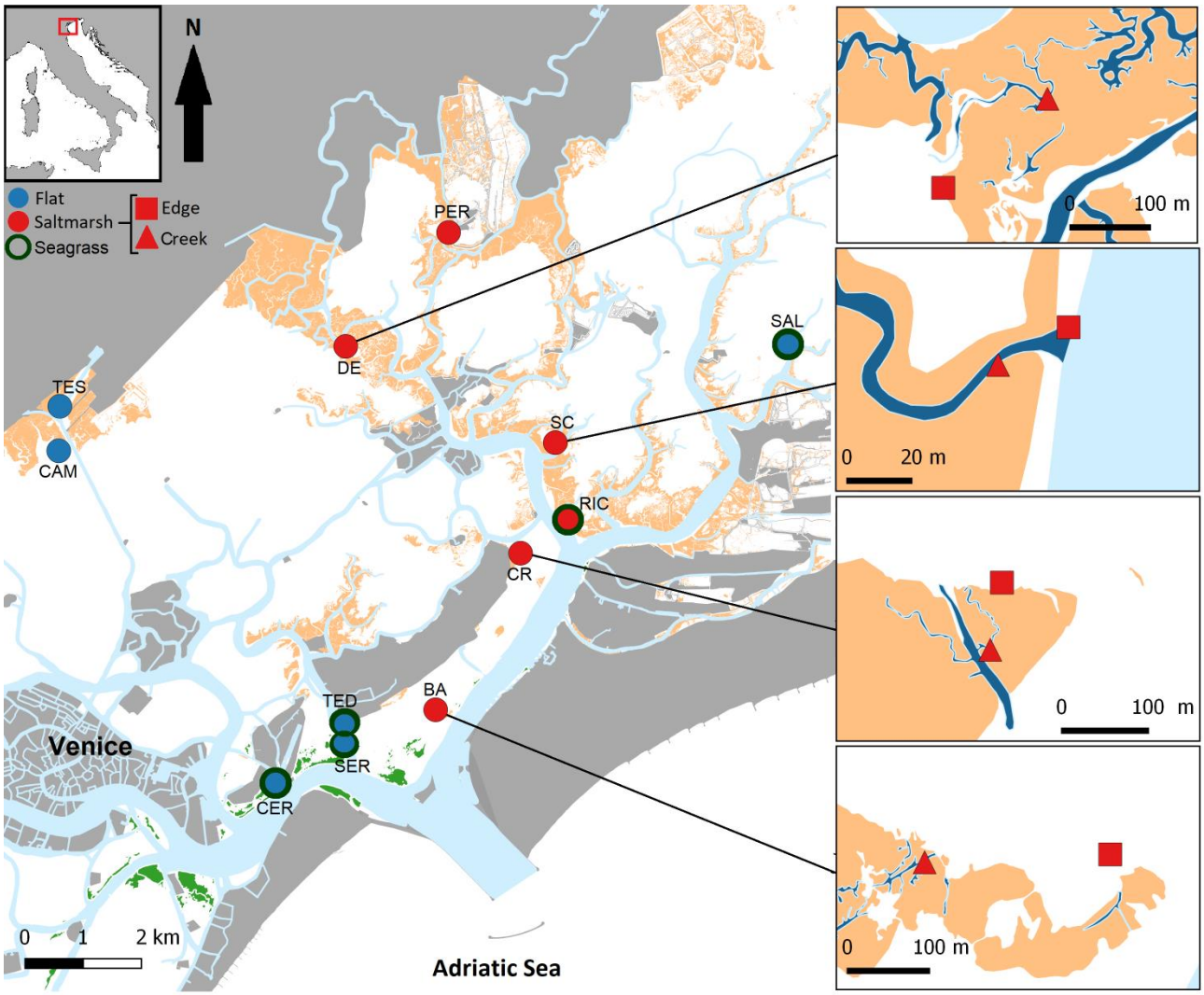


Figure 26 - Sampling sites in northern sub-basin of Venice lagoon. In grey the land, in light blue the channels, in dark blue the marsh creeks, in green the seagrass beds and in light orange the saltmarshes.

2.2.2 Laboratory activities

In laboratory, fish were thawed at 6 °C for 24 hours. All individuals were identified at the species level, counted, measured (Standard Length, SL, ±0.1 mm) and weighed (Total Weight, TW, ±0.01 g). In case of abundant samples, random subsamples of at least 100 individuals were measured. Fish were identified following the scientific literature: Arias and Drake (1990), D'Ancona and Lo Bianco, (1932-1933), Fisher et al. (1987), Gandolfi et al. (1991), Ré and Meneses (2009), Tortonese (1970, 1975) and Whitehead et al. (1984-1986, 1988). In case of postlarvae and juveniles of Mugilidae, the classification was confirmed after the observations of pattern of distributions of chromatophore (Cambrony, 1983; Franzoi et al., 1989; Serventi et al., 1996). The identification of individuals belongings to this Family was validated after leaving them three weeks in 8% buffered formaldehyde. Species were classified as marine migrants following Franzoi et al. (2010).

Chlorophyll concentration in water and sediment was estimated following Lorenzen (1966), with Trylogy Laboratory Fluorometer. Sediment cores were processed to determine the percentage of sand in the upper 10 cm following the methodology reported in Sfriso et al. (2003) and the content of organic matter through loss of ignition method (Heiri et al., 2001) at 550°C.

2.2.3 Data analysis

Preliminary analysis

To analyze the effects of habitat type and environmental variables on juvenile distributions, and to test for preference changes during the growth inside the lagoon, data of marine migrant species found in more than 25 observations were organized considering different size classes. For the definition of size classes, together with the research group's experience, literature concerning mostly diet, morphometry and organ development was considered.

The environmental data, after being standardized scaling values with zero as mean and unit as variance, were analyzed using Principal Component Analysis (PCA). Principal component analysis was employed to summarize patterns in environmental parameters and to identify the main spatial and temporal gradients. The principal components were thus considered as predictors of environmental variability in statistical models.

Models calibration

Species density was used as response variable. Only species collected at least in 10 occasions were included in the analysis. Total and size class species densities were independently modeled with generalized linear mixed models (GLMM; Bolker et al., 2009) to analyze the habitat and environmental preferences to take into

account the potential lack of independence among observations. Stations were included as random factor in the GLMMs. Indeed, stations were replicated over time and furthermore, in some stations sampling points were very close to each other (e.g. saltmarsh edge and tidal creek). After an exploratory analysis of the datasets, the Poisson distribution was used to model response variables. As regarding predictors variables, three predictor variables categories were considered in the models (tab. 12), namely time, habitat and environmental factors.

Table 12 – Candidate predictor variables considered in the models.

Predictor variables	**Codes and description**
Time factor	Month (from February to June)
Habitat factors	Marshes (Yes/No) Creeks (Yes/No) Seagrass cover (%) Macroalgae (Yes/No)
Environmental factors (combined in spatial and temporal gradient according to the PCA axes)	Temperature (temp)
	Salinity (sal)
	Residence time of the water (tres)
	Oxygen saturation (od_sat)
	Turbidity (torb)
	Chlorophyll concentration in water column (chlH2O)
	Chlorophyll concentration in upper sediment (chlSED)
	Granulometry (% sand) of the upper 10 cm sediment (sand)
	Content of organic matter of the upper 10 cm sediment (sost.org)

Four categories of models were considered (tab. 13) for development following a hierarchical approach by progressively adding new predictors variables to represent an increasing level of complexity. It was thus possible to explore different hypotheses: 0) response variable (density of the given species/size class) is not affected by any of the variables considered in this study; 1) response variable is affected by temporal factor (category m1), indicating that the seasonality alone explains the distribution of a given species within the lagoon; 2) response variable is affected by habitat characteristics, including habitat type and vegetation coverage, in addition to the temporal factor (category m2); 3) response variable is affected by environmental parameters in addition to the previous variables (category m3). Category m1 included only one model, while more than one GLMM formulation were made for the other categories, resulting in a series of candidate

alternative models for each category (tab. 13). Category m2 was built by adding to category m1 either habitat type (marshes or flat), the morphological structure (marsh creek or not), the seagrass coverage or the presence of macroalgae. Category m3 was built by adding to category m2 either the principal component(s) representing the spatial gradient, or the one(s) representing the temporal gradient in environmental variables. Category m2 was built regardless of the influence of temporal factor on response variable. Similarly, category m3 was built regardless of the influence of temporal and habitat factors on response variable.

Table 13. Structures of models used to link density distribution to temporal, environmental and habitat factors. Best mod. = previously selected best model.

Model category	Label	Model structure	General hypothesis
0. No factors	m0	$Y_i \sim constant + \varepsilon_i$	Response variable is not affected by any of the considered predictors
1. Temporal factor	m1	m0 + months	Response variable is affected by time only
2. Habitat factors	m2.0	Best mod. + marshes	In addition to model selected in the previous steps, response variable is affected by Marshes (Yes/No)
	m2.1	Best mod. + creeks	In addition to the model selected in the previous steps, response variable is affected by Creeks (Yes/No)
	m2.2	Best mod. + seagrass	In addition to the model selected in the previous steps, response variable is affected by Seagrass cover
	m2.3	Best mod. + macroalgae	In addition to the model selected in the previous steps, response variable is affected by Macroalgae presence (Yes/No)
3. Environmental factors	m3.0	Best mod. + spatial gradient	In addition to the model selected in the previous steps, response variable is affected by PCA axes representing spatial gradient
	m3.1	Best mod. + temporal gradient	In addition to the model selected in the previous steps, response variable is affected by PCA axes representing temporal gradient

To better interpret the responses of the different species to the environmental factors, values of variables included in the PCA were back transformed to their original scale, reporting the effects of the single environmental parameters rather than the PCA axes.

Selection of best models for each size class of each species

For each response variable (densities for each size class), starting from category m0 the best candidate model between category or within each category was selected by using the Akaike Information Criterion approach (AIC; Burnham and Anderson, 2004), choosing the model with the lower AIC value. In the case of model comparison with an inadequate support for the identification of the best model (AIC difference lower than

2) the most parsimonious formulation was selected. Then, each subsequent category was built adding predictors to the best model selected from the previous one.

Model evaluation (comparison between the single class and multi-class model)

For each species, the best models were used to explore the different preferences during ontogeny. To understand if the stage-specific approach performed better than the total one (no size classes), the prediction of total density obtained using all the stage/size-specific models of a given species was compared to the density estimated with the total model. For each species, to estimate the abundances, a *joint model* was created cumulating all the single size-class models. For each species, the correlation between observations and predictions of the *joint model* was the correlation given by cumulating all the single size-class models (e.g. the sum of the predicted abundance of all the size classes for each sampling station). Spearman correlations between observed and predicted densities were used to determine the goodness of fit: if the correlation of the best model created considering all the individuals together (no size classes) was lower than the correlation of the one estimated considering separately the size classes (*joint model*), the single stage-specific estimation (with multiple models) was chosen to represent the environmental and habitat preferences of that species.

2.3 Results

2.3.1 Size classes definition

In total, 85 observations were included in the dataset. The observations were well distributed among habitat typologies, with tidal flats accounting for 35% of the observations, saltmarsh edges accounting for 27% and saltmarsh creeks for 38%. Stations belonging to one of these habitats but also covered by seagrass beds during all sampling periods represented 29% of the records while macroalgae were found in 34% of records.

During the seven sampling campaigns in the 16 stations 9489 marine migrant fish were collected. They belonged to nine families and 13 species: *Belone belone*, *Chelon auratus, C. labrosus*, *C. ramada*, *C. saliens*, *Dicentrarchus labrax*, *Engraulis encrasicolus*, *Platichthys flesus*, *Pomatoschistus minutus*, *Sardina pilchardus*, *Solea solea*, *Sparus aurata* and *Sprattus sprattus*. Among the collected fish species, *B. belone*, *C. labrosus*, *D. labrax*, *E. encrasicolus, P. minutus and S. solea*, being present with very few observations, (number of observations, n = 2, n = 5, n = 6, n = 8, n = 5, n = 8 respectively) were excluded from further analyses.

As previously stated, during their growth fish change diet and can use more than one type of habitat to exploit the best resources. Each species changes their dietary and environmental needs at different ages or sizes. *S. pilchardus* and *S. sprattus* specimens smaller than 30 mm (Total Length) and *E. encrasicolus* smaller than 40 mm (Total Length) were considered postlarvae (Baldo and Drake, 2002), feeding predominantly on

Copepoda (Baldo and Drake, 2002); at larger sizes preferences change towards Cladocera for *S. pilchardus* and *S. sprattus* and towards Mysidacea and fish for *E. encrasicolus* (Baldo and Drake, 2002). For *P. flesus*, the main ontogenetic shift in the diet composition appears at 45 mm of total length (Aarnio et al.,1996), when it shifts from a diet insisting on meiofauna, focused mainly on Harpacticoida and Copepoda, to a diet dominated by macrofauna, in particular by Oligochaeta, Amphipoda and Chironomidae (Aarnio, 2000). For Mugilidae like *C. auratus*, *C. ramada* and *C. saliens* diet shifts were observed by Ferrari and Chieregato (1981). Individuals with Standard Length (SL) below 30 mm feed mainly on zooplankton, including Polychaeta larvae, Rotatoria, nauplii of Copepoda, Cirrepedia, Calanoida and Cyclopoida. The importance of these preys decreases, and diet completely changes for individuals with average SL above 53 mm, feeding mainly on meiozoobenthos, Bivalvia larvae, Polychaeta, Diatoms, Nematoda and Harpacticoida. In the studies by Ferrari and Chieregato (1981) and Salvarina et al. (2016), microalgae, silt and sand are the preferential for fish with an average SL above 50 mm, as these items represent the only food source found in the analyzed samples. Detailed studies were conducted in the past decades on ontogenetical changes occurring during growth in *S. aurata*, often providing detailed information on changes of body shape during development (e.g. Russo et al., 2007), on the development of organs (e.g. digestive tract; Elbal et al., 2004) and on adaptation of teeth in relations to the growth (Cataldi et al., 1987). All these authors agree that, as described for other species, changes during ontogeny in *S. aurata* are strongly linked to changes in feeding habits and swimming ability (Russo et al., 2007). The diet of *S. aurata* shifts from zooplankton, during larval stage, to meio- and macrozoobenthos once reached the juvenile and adult stages (Andolina, 2017; Ferrari and Chieregato, 1981; Cataldi et al., 1987; Russo et al., 2007; Elgendy et al., 2016). According to Elbal et al. (2004) and Cataldi et al. (1987), in *S. aurata* post-larvae up to 20 mm SL, canine teeth, gastric channel and stomach musculature are barely developed, allowing the ingestion of small planktonic preys only. Later, from 20-25 mm up to 35 mm in SL, according to Ferrari e Chieregato (1981) and Cataldi et al. (1987), changes in diet towards microbentivore prey is associated to the presence of three concentric rows of canine teeth and some "transitional teeth" that later become molars. Once fully developed, when individuals reach a standard length above 35 mm, molar teeth allow them to eat hard prey as bivalves, Decapoda and in general benthic animals (Elgendy et al., 2016; Ferrari and Chieregato, 1981; Zucchetta, 2010).

Following these indications on diet and morphological changes, 2 to 4 size classes were defined for the different species included in the analysis (tab. 14).

Table 14 – Size range (in standard length), number of observations and references for each species and size class. In brackets the number of observations in which that species/size class was found.

		Selected size class range (Total or Standard Length, mm)				Bibliography used to select size class
Taxa	**Total**	**Cl 1**	**Cl 2**	**Cl 3**	**Cl 4**	
Sparus aurata	(31)	< 20 (20)	20 – 34.99 (14)	35 – 49.99 (7)	≥ 50	Ferrari and Chieregato, 1981 Elbal et al., 2004 Cataldi et al., 1987 Russo et al., 2007
Chelon auratus	(30)	< 30 (16)	30 – 49 (18)	≥ 50 (8)		Ferrari and Chieregato, 1981 Baldo and Drake, 2002 Salvarina et al., 2016
Chelon ramada	(30)	< 30 (29)	30 – 49 (7)	≥ 50 (4)		
Chelon saliens	(28)	< 30 (15)	30 – 49 (16)	≥ 50 (17)		
Engraulis encrasicolus	(8)	<40	≥ 40			Baldo and Drake, 2002
Clupeidae: ***Sardina pilchardus*** ***Sprattus sprattus***	 (13) (14)	< 30	≥ 30			
Platichthys flesus	(10)	< 45	≥ 45			Aarnio et al., 1996

For marine migrant species frequently observed in the dataset, *Sparus aurata*, *C. auratus*, *C. ramada* and *C. saliens*, collected in at least 25 occasions, data were organized considering different size classes as reported in Table 14. For the other cases such an approach was not possible, because the limited numbers of occurrence would have resulted in some size classes represented by only few specimens. These species (*P. flesus*, *S. pilchardus*, *S. sprattus*) were considered without being divided in size classes.

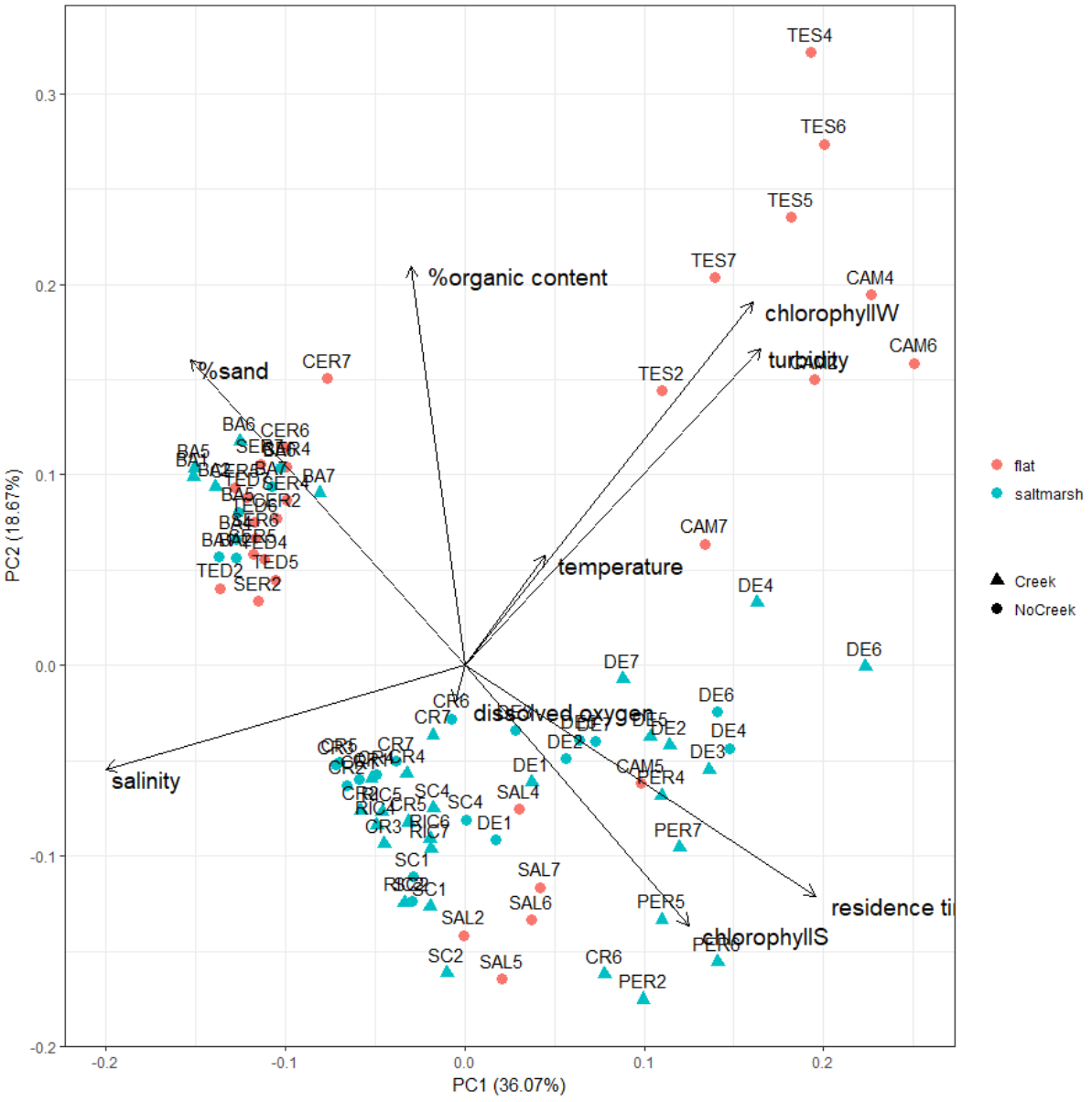
2.3.2 Environmental factors

The first three axes, which accounted for the 71.47% of the total variance, were chosen to represent the environmental data in the model analysis.

The first axis of the PCA (fig. 27A) (PC1, which explains 36.1% of the variance), is related with a spatial sea-lagoon edge gradient dominated by salinity (negatively), residence time, turbidity, chlorophyll concentration in water and percentage of sand in the upper layer of sediment. Stations located near the sea inlet are characterized by high values of salinity and percentage of sand, while inner stations are represented by high

values of water residence time and chlorophyll concentration in sediment. The second axis of the PCA (fig. 27) (PC2, 18.7% of the variance) is related with a second spatial pattern dominated by organic content, turbidity and chlorophyll concentration in water. Finally, PC3 (16.73% of the variance) (fig. 27B) is influenced mainly by the concentration of dissolved oxygen and water temperature, indicating that this axis, being influenced by variables showing strong seasonal /temporal fluctuations, can be considered as representative of the temporal pattern.

Considering PC1 and PC2 (fig. 27A) temporal replicates of the same station are generally grouped together, with the exception of some stations (TES and CAM), characterized by high average values and large temporal variability of chlorophyll and turbidity. PC2 distinguishes between two types of confined stations: more stable sites characterized by low values of turbidity and more variable stations in terms of turbidity and chlorophyll concentration. All the stations located at the sea inlet, both saltmarshes and flats, with or without vegetation cover, are grouped together with high values of percentage of sand. Seagrass beds stations are not grouped together, and the effect of vegetation does not influence the PCA graph (fig. 27A). Gradually, stations located near the lagoon edge show high values of chlorophyll concentration in waters. Station SAL, which is a seagrass bed station, is the only one flat located inside the lagoon with low turbidity values, suggesting that PC2 does not characterize only a sea-lagoon edge distance. Some stations, located right at the edge of the lagoon (TES and CAM), are characterized by flat environments extremely turbid that can largely vary over time. No remarkable differences (e.g. clear point separation) between tidal creeks and saltmarsh edges sites can be observed from the PCA analysis. For some confined stations (e.g. DE) the temporal gradient (fig. 27B) did not show a linear trend as the driving variables (e.g. the water temperature) did not increase with the sampling campaign, as conversely observed in general in BA station, located near the sea inlet (fig. 27B).


TES4
TES6
TES5
TES7
CAM4
CAM6
%organic content
chlorophyllW
turbidity
%sand
CER7
TES2
CAM7
temperature
DE4
DE6
DE7
dissolved oxygen
salinity
DE1
CAM5
PER4
SAL4
SC4
PER7
SC1
SAL7
SAL6
PER5
residence ti
SAL2
chlorophyllS
SC2
SAL5
CR6
PER2
PC2 (18.67%)
PC1 (36.07%)
flat
saltmarsh
Creek
NoCreek

A)

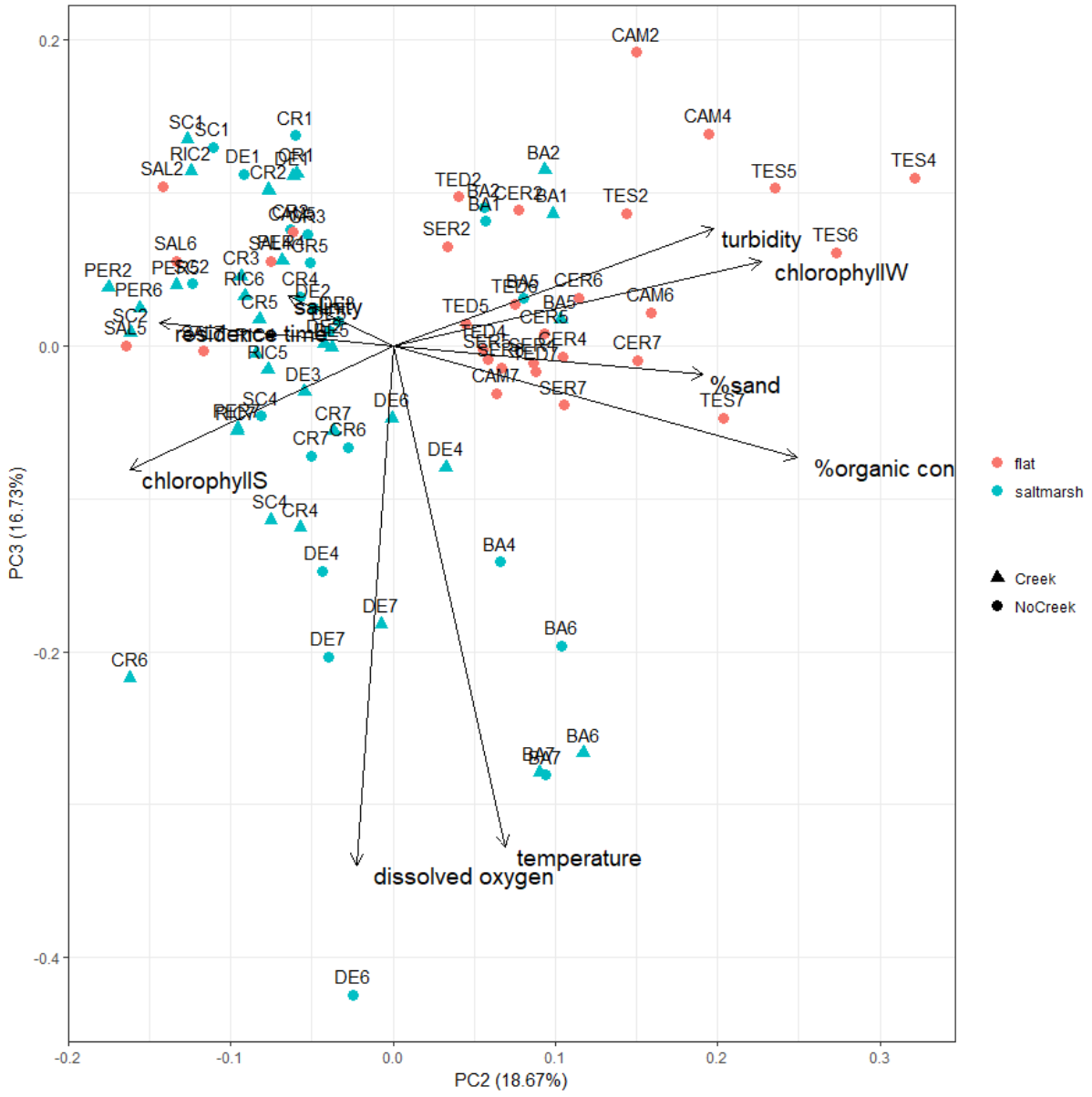


Figure 27 - PCA ordination on environmental data recorded during sampling with beach seine net. A = PC1 horizontal axis and PC2 vertical axis; B = PC2 horizontal axis and PC3 vertical axis. Light blue = saltmarsh stations, orange = flat stations, circle = haul performed in open stations, triangle = haul performed in tidal creek stations. Labels represent station code and number of sampling campaign.

2.3.3 Habitat model calibration

Model selection

Observing the best model selected for each species and size classes (tab. 15), is possible to see that models of the family m3, which consider the environmental parameters, together with temporal and habitat factors, are the most frequently selected. Models of the family m3 were always selected as best models to explain distribution of densities when for each species all individuals were considered together (i.e. no size classes). Models m3.1 were the most frequent best model considering the size classes separately, generally preferred

over models m3.0, indicating an influence of the temporal variability, namely due to temperature and dissolved oxygen, in addition with the contribution of the sampling month, (model m1). In most cases, the habitat factor was included in the m3.x models. It is possible to observe that when different size classes of a species were analyzed separately, the best models selected were, in general, different from the model chosen cumulating all individuals (the single stage models do not belong to the same family of the overall models). Among the individual size classes, also simpler models (e.g. m2 or m1, hence considering the temporal aspects alone or the temporal aspects and the habitat factors) were selected as best models in some cases, but the family of models more frequently selected is still the one that includes also the environmental variables in addition to the other variables (m3.1; tab. 15). It is worth noting that for no species the same types of model were selected for the three size classes.

Models that consider only temporal factor related with months (m1) were selected only for larger *S. aurata* (size class 3), whose distribution resulted to be influenced mostly by the seasonal dynamic. Models that consider habitat factors explained the density distribution of small individuals (size class 1) belonging to *C. auratus* and *S. aurata* and size class 2 individuals of *C. saliens*. Finally, for all the other species and size classes (*C. auratus* size class 2 and 3, *C. ramada* size class 1, 2 and 3, *C. saliens* size class 1 and 3 and *S. aurata* size class 2), models that consider also the environmental factors were selected as best models.

Model evaluation (comparison between single class and multi-class model)

The explained deviance of single size class model was higher than the ones calculated cumulating all individuals together (no size class). Considering, *P. flesus*, *S. pilchardus* and *S. sprattus*, for which was not possible to divide individuals in size classes, the explained deviance of the best models was always over 0.83.

The correlations between observations and model predictions ranged from 0.31 (*S. aurata* classes 3) to 0.96 *C. aurata* size class 3), with an average value of 0.77. In general, all best models created for the different species and size classes, except those for *C. ramada* and *S. aurata* size class 3, show a correlation value between observations and predictions higher than 0.57, indicating a good agreement. Moreover, the best models of the different size classes of *C. auratus* and *C. saliens* show always a high correlation between predicted densities and observed densities. Considering species not divided in size classes, the lower correlation between model prediction and observation was observed for *S. pilchardus* (0.64) while the correlation of *P. flesus* and *S. sprattus* was respectively 0.77 and 0.96. For species divided in size classes (*C. auratus*, *C. ramada*, *C. saliens*, *S. aurata*), to evaluate if it was better to consider the single size class models or the model created cumulating all individuals, the correlation of predictions against observations was analyzed, showing that for all four species, and especially for *C. auratus* and *S. aurata*, the correlations of the single size class models (*joint models*) were always higher than the correlations obtained cumulating all

individuals (*tot*, no size class models). These results highlighted that, even if for some single size class models the correlation was low (e.g. *C. ramada* size class 2 and 3 or *S. aurata* size class 3), creating different single size class models has increased the agreement level between predictions and observations.

Table 15 – Types of the selected models (In bold the best models chosen for each species (sp) and each size classes (cl) and basic statistics: deviance explained, correlation of predictions against observations for the different models. CAU = C. auratus, *CRA* = C. ramada, *CSA* = C. saliens, *PFL* = P. flesus, *SAU* = S. aurata, *SPI* = S. pilchardus, *SSP* = S. sprattus.

Sp and cl	**Selected model**				**Deviance Explained by each size classes models**				**Correlation of predictions against observations for each size classes models**				**Correlation of predictions of *joint model* against observations**
	tot	**1**	**2**	**3**	**tot**	**1**	**2**	**3**	***tot***	**1**	**2**	**3**	
CAU	3.1	2.2	3.1	3.1	0.07	0.39	0.14	0.80	0.73	0.80	0.79	0.96	0.82
CRA	3.1	3.1	3.0	3.0	0.39	0.42	0.97	0.54	0.91	0.94	0.57	0.32	0.94
CSA	3.1	3.1	2.3	3.0	0.22	0.15	0.25	0.83	0.96	1.00	0.89	0.89	0.99
PFL	3.0				0.92				0.77				
SAU	3.1	2.1	3.1	1	0.31	0.96	0.87	0.97	0.57	0.95	0.67	0.31	0.75
SPI	3.1				0.92				0.64				
SSP	3.1				0.83				0.96				

Relationship with environmental factors of selected best models

The analysis of the different selected models (fig. 28) highlights that the different species and the different size classes behave in a different way in relation to temporal and habitat factors and environmental parameters, showing a high variability in the response to the different environmental factors. Indeed, also within each species, the individuals belonging to different size classes behave differently, exhibiting preferences for specific habitat and environmental characteristics. Due to this high variability in the responses of the best models of each species and size classes, the different species were analyzed separately.

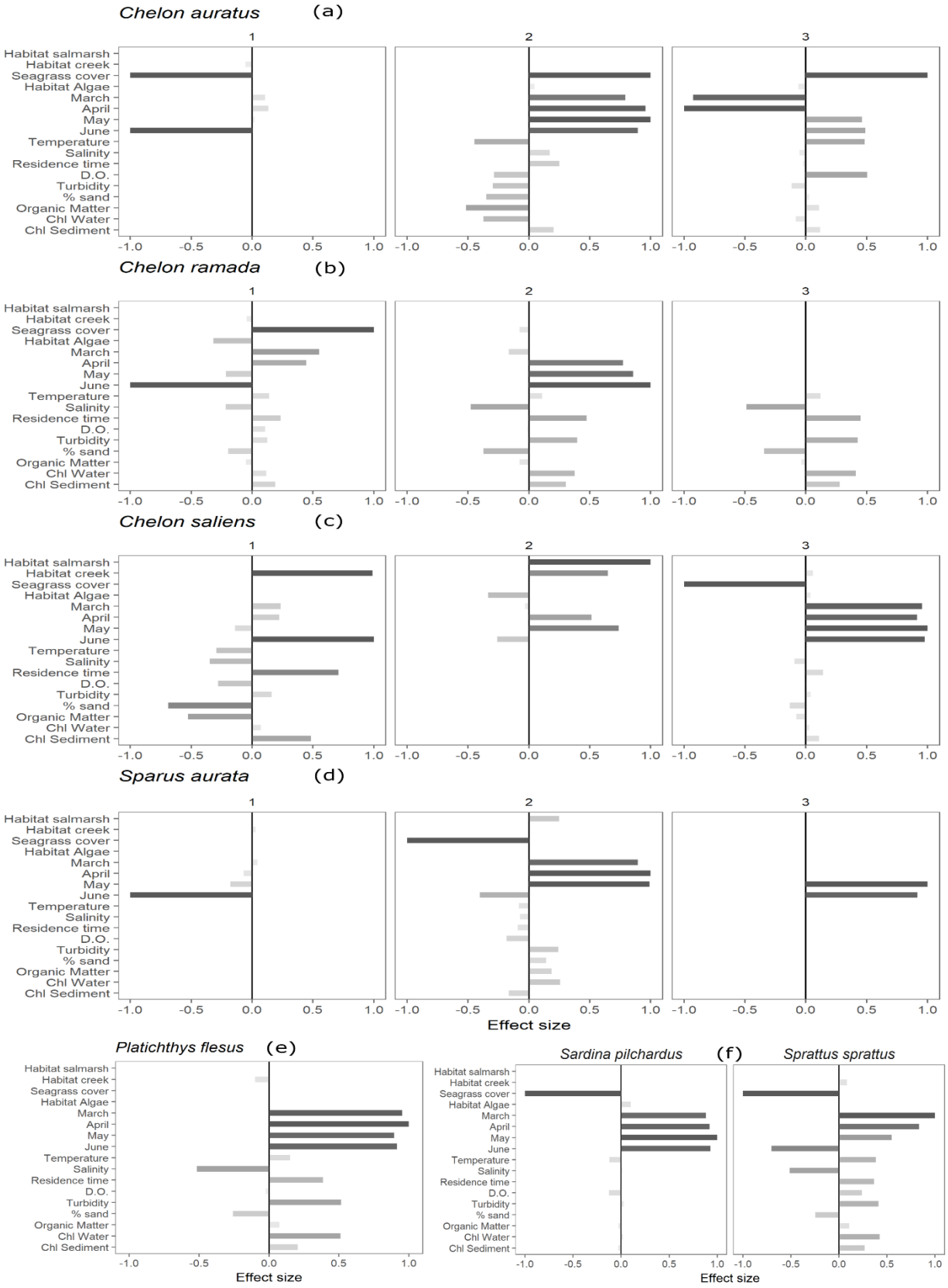


Figure 28 – Effects of the predictor variables of the best models on distribution of C. auratus *(a),* C. ramada *(b),* C saliens *(c),* S. aurata *(d), separated for size class, and* P. flesus *(e),* S. pilchardus *and* S. sprattus *(f). Horizontal axis = magnitude of the effect, from light grey to dark grey increase the magnitude of the effect; Vertical axis = predictor variable considered. The absence of effect means that the factor did not show a substantial improvement of the model.*

For *C. auratus* (fig. 28a) belonging to the first size class the model suggested that March and April, during which the higher abundances were recorded, were the most suitable months to find the smaller individuals of these species inside the lagoon, while the effect of time changes quickly in May and June, when the predicted abundances were very low. In terms of habitat use, small *C. auratus* were more abundant in unvegetated areas outside the marsh creeks. Densities of second size class individuals increased from March until the peak of May, and then decrease in June. Unlike what was observed for size class 1, class 2 of *C. auratus* size strongly preferred areas with a high vegetation cover: a strong positive effect was observed especially with higher seagrass coverage, rather than with macroalgae. These individuals were not influenced by habitat typology as marshes, flats or tidal creeks. However, considering environmental factors, density of *C. auratus* size class 2 was influenced by clear waters with quite high level of salinity. The preferred stations indeed were characterized by low percentage of sand, low organic matter content but high chlorophyll concentration in sediment. Regarding the largest sampled *C. auratus* (size class 3), the fitted model showed a strong positive relation with warmer months: May and June. Again, as for size class 2, seagrass coverage had a stronger positive effect than habitat typology (marshes, flats or tidal creeks). Among the environmental variables, a positive effect was given by dissolved oxygen and temperature while all the other parameters showed low and variable effect, indicating that *C. auratus* belonging to size class 3 preferred warmer temperature rather than a specific position along a spatial gradient.

As in the case of *C. auratus*, March and April had a positive effect on density of small *C. ramada* individuals (fig. 28b). *C. ramada* belonging to first size class preferred seagrass bed habitats outside marsh creeks. The second size class *C. ramada*, for which May and June were the months with the higher abundance, show a weak preference for high seagrass coverage and did not show any effect of habitat type. The third size class *C. ramada* did not show a preference for any habitats. Considering environmental factors, changes in preferences were observed from the first size class to larger individuals (size class 2 and 3) in particular concerning the importance of the variables. However, salinity and water residence time were always the most important variables while dissolved oxygen and organic content the less import. *C. ramada* individuals, generally, were concentrated in confided stations characterized by low salinity, high residence time and avoid stations with larger sediment grain size. Analyzing in detail the differences, small *C. ramada* individuals were concentrated in less sandy stations while the bigger individuals in stations with higher turbidity.

C. saliens individuals (fig. 28c) belonging to first size class, for which the model attributed the highest density in June, preferred strongly the creeks and, even if it was present also in other habitats, they did not contribute in explaining the abundance distribution within the model. Small individuals of *C. saliens* preferred the stations characterized by high residence time of the water, low salinity and low percentage of sand of the bottom sediment, indicating a preference towards the portion of the lagoon near the lagoon edge and in

particular for the stations characterized by high turbidity. Considering the second size class of *C. saliens*, for which the model did not include environmental factors, the marsh creeks showed the stronger effect on abundances. The size class 2 individuals of *C. saliens* avoided the stations with macroalgae. The third size class *C. saliens* had the same environmental preferences of the size class 1 individuals. Regarding habitat factors, size class 3 *C. saliens* continued prefer creeks, albeit less strongly than the first and second size classes individuals. Size class 3 *C. saliens* strongly avoided seagrass beds and preferred stations characterized by macroalgae.

Sparus aurata (fig. 28d) showed, more than the other species, large changes in habitat preferences during the study period and the abundance pattern explained by the models were largely influenced by temporal dynamics. The *S. aurata* individuals belonging to first size class - the one entering the lagoon in February and March - were found in higher abundances in marsh creeks, while spatial or temporal gradients did not influence the density distribution of these individuals. The second size class *S. aurata*, which is more present during April and May, were influenced by the environmental gradient, positively influenced by turbidity, chlorophyll concentration in water and organic content in sediment and negatively by water residence time and dissolved oxygen, indicating a preference towards more confined stations. A strong effect of habitat typology was also present for the second size class: individuals were positively influenced by saltmarsh habitat, while the effect of seagrass beds habitats was negative, suggesting that these size class can be found in high abundances in marsh-related unvegetated areas. Finally, the *S. aurata* individuals belonging to third size class, according to the models, were not influenced by habitat and environmental factors and were distributed following only the temporal factor: they were abundant in May and June.

The best model of *P. flesus* (fig. 28e) showed that this species, which had a peak of abundance in April, had the same probability of showing high abundances in the whole period March-June. Higher abundances of flounder juveniles can be found in relatively low salinity, turbid waters with high chlorophyll concentration in confined areas characterized also by fine grain size sediments.

Results of the models selected for Clupeidae (fig. 28f) show that *S. pilchardus* individuals were present in high densities with the same probability during the period March-June, with slightly higher values in May, while *S. sprattus* juveniles were abundant during cooler months, especially in March. Considering habitat typology factors, models showed that both species avoided areas characterized by seagrass beds. *S. sprattus* individuals, however, showed a specific preference towards creeks and *S. pilchardus* towards flats characterized by the presence of macroalgae. Regarding environmental parameters, the model selected for *S. pilchardus* showed that this species was influenced by the variables with a marked seasonality, being affected negatively especially by temperature and dissolved oxygen. *S. sprattus* instead responded both to spatial and temporal gradient, being linked with confined habitats with high turbidity, with low salinity values and muddy bottoms.

2.4 Discussion

In this work the habitat and environmental preferences of seven marine migrant species during their early phases of permanence inside transitional water ecosystems have been studied. For the most abundant species, a particular attention was also paid to the changes in preference during such period. Generally, our results confirm that species abundance was not stable during the considered period, with some peak of presence. Moreover, the different species and, within each species, the different size classes, use different habitats inside the transitional ecosystem. The preferences towards environmental or habitat factors, indeed can change even on the short period considered, although the relevance of some habitats emerges clearly (e.g. the importance of marshes). The preferences towards a specific habitat or set of environmental conditions for the studied species were analyzed with generalized linear mixed models (GLMMs), calibrated for different species considering as response variable both the total and the stage specific abundance, when possible. The sampling scheme was designed to focus on differences among habitats and environmental conditions. In general, the results of the models suggest that it is better to consider the different size classes separately, because stage-specific models showed different association with habitat and environmental conditions and offered a better habitat-species association description. Indeed, only few species are confined to a single nursery habitat in coastal and estuarine ecosystems (Nagelkerken et al., 2007, 2015; Ribeiro et al., 2012; Whitfield and Pattrick, 2015). The presence of many different types of shallow water habitats in the Venice lagoon (Franco et al., 2006a, 2010; Franzoi and Pellizzato, 2002; Franzoi et al., 2005) often found in proximity to each other - especially in the Northern sub-basin - allows species to occupy multiple habitats during their development, due to ontogenetic habitat shifts (e.g. Adams et al., 2006; Bostrom et al., 2011; Elliott and Hemingway, 2002). Accordingly, it is well known that, inside transitional water ecosystems, various factors such as environmental characteristics, spatial availability (Able and Fahay, 1998; Elliott and Hemingway, 2002; Herzka, 2005), changes in body morphology, swimming ability and development of digestive tract (Cataldi et al., 1987; Pita et al., 2002; Russo et al., 2007; Tancioni et al., 2003) can lead to changes in preference towards a certain habitat or environmental conditions (Adams et al., 2006; Beker and Sheaves, 2005; Minello et al., 2003). These changes can occur also during the first months of permanence of these species within the Venice lagoon, and in this work it has been showed that such changes can be effectively detected by considering two to three different size classes. However, the requirements of the stage-specific approach are quite data-demanding, and it was not possible to calibrate separate models accounting for the different stage classes for all the species. Indeed, considering the marine migrant species caught within this study (n = 13 species, compared to the total 16 marine migrant species caught from 2001 to 2017 in the Venice lagoon, Scapin et al., in press), dividing the species in size classes was relatively easy for some species (e.g. *S. aurata*, *Chelon* sp. pl. and *P. flesus*), for which many works are present in the literature (Aarnio et al., 1996; Ferrari and Chieregato, 1981; Russo et al., 2007), while for other (e.g. *S. pilchardus*, *S. sprattus*, *E. encrasicolus*) information were scarcer. Detailed studies were conducted in the past

decades on ontogenetic changes in *S. aurata*, often providing detailed information on changes in body shape during the development (e.g. Russo et al., 2007), on the development of organs (e.g. digestive tract; Elbal et al., 2004) and on the adaptation of teeth in relations to the growth (Cataldi et al., 1987). The choice of a certain habitat depends on morphological changes of body shape, the development of the organs, the adaptation of theeth and especially the diet changes because these are factors that affects the preferences of a species (Pita et al., 2002; Tancioni et al., 2003). Therefore, the three species belonging to Genus *Chelon* and *Sparus aurata*, the most abundant and frequent marine migrant species in the north sub-basin of Venice lagoon during the sampling period, were divided in different size classes following the literature about diet habits (Baldo and Drake, 2002; Cataldi et al., 1987; Ferrari and Chieregato, 1981; Russo et al., 2007). For other species such as *S. pilchardus*, *S. sprattus* and *P. flesus*, although their change in diet preferences during growth was known from literature, it was not possible to divide individuals in size classes due to the lack of sufficient number of observations. Robinson et al. (2011) proposed stage specific models for explaining species/habitat relationship of species with complex life cycles, changing environmental requirements and habitat occupancy over time, referring, in particular to marine organisms. Some authors proposed this approach also for studying the use of lagoon habitats by juvenile marine fish species (Leone et al., 2016; Zucchetta et al., 2009; Zucchetta, 2010). These examples, however, focus on the presence/absence only of the different species, while it was showed in this work that this approach can be effective also when considering abundance. The abundance of individuals is indeed an important aspect when considering the nursery role of a habitat (Beck et al., 2001; Dahlgren et al., 2006), even if the nursery values connot be defined considering abundance alone (Sheaves et al., 2015).

The entire sampling period corresponded to the one during which the majority of marine migrant species enter the Venice lagoon to exploit the more suitable condition (Franzoi et al., 1989; Rossi, 1986). However, all the species and size classes showed different peaks of abundance inside the lagoon and different temporal dynamics of entrance. According to their spawning season, *C. auratus*, *C. ramada* and *S. aurata* start to enter the Venice lagoon during the colder months, and younger individuals (first size class) have a peak of abundance in March and April, while older individuals (second and third size classes) concentrate their presence in warmer months (May and June), according with what observed by Schreiber et al. (1979). These results indicate that generally the three size classes of *C. auratus*, *C. ramada* and especially *S. aurata* have a stable alternation in presence and abundance inside the habitats of the Venice lagoon. In contrast, different patterns of distribution occurs for *C. saliens*: their entrance inside the lagoon happens especially in June (fist size class), but results show that larger individuals (size class 2 and 3) have a peak of abundance also in May, indicating both the presence of more than one period of entrance inside the lagoon (Gandolfi et al., 1991) and a constant presence inside the lagoon (Franzoi et al., 2010). Also, the presence and the abundance of *S. pilchardus* and *P. flesus* seem to be constant during all the sampling period while *S. sprattus*, as previously pointed out by Solberg et al. (2015) and Dulcic (1998), prefer colder month as March.

Considering habitat typology, except for *C. saliens*, which prefers marsh creeks and concentrates in saltmarsh habitats located near the lagoon edge, results show that the other species changes their preferences with ontogeny, according also to Nagelkerken (2007), Whitfield and Pattrick (2015) and Ribeiro et al. (2012). Overall, among the different habitats present in the northern sub-basin of Venice lagoon, seagrass beds do not seem to be preferred by many marine migrant species, as opposed to what is generally reported by other studies (e.g. Ford et al., 2010; Heck et al., 2003; Whitfield, 2016). Except for larger *C. auratus* and smaller *C. ramada*, which strongly prefer vegetated stations, seagrass beds are generally avoided by *S. pilchardus*, *S. sprattus* and by *S. aurata*, *C. auratus*, *C. ramada* and *C. saliens*. As a result, being colonized for a short time and by a few marine migrant species, this habitat does not seem to support massively the growth of the marine migrant individuals and thus it does not play any strong nursery role in the Venice lagoon. However, the seagrass meadows are considered to have a fundamental role for fish fauna and in maintaining populations of commercially and recreationally exploited fisheries (Jackson et al., 2011; Vizzini et al., 2002). Seagrass meadows generally perform important functions as feeding, shelter and nursery areas (Heck et al., 1997, 2003; Jackson et al., 2001; Nagelkerken et al., 2008, 2015; Whitfield, 2016) and are a major primary producer, supporting detritus-based trophic webs (Nordlund et al., 2016; Scapin et al., 2018b). The nursery function of seagrass beds is usually attributed to lower predation pressure, linked to high structural complexity (Franco et al., 2006a; Jackston et al., 2001; Rooker et al., 1998). Moreover, the biological characteristics of seagrasses can influence the structure of fish population by affecting also differential survival and growth rates (Ford et al., 2010; Heck et al., 2003). Among the multiple human pressures that coastal lagoons are subject (Elliott and Quintino, 2007), the degradation and loss of seagrass meadows is the one of the most significant for the fish fauna (Franco et al., 2009; Vasconcelos et al., 2007; Zucchetta et al., 2016) and evidence suggest that habitat fragmentation might impact on seagrass fish fauna (Bell et al., 2002). Generally, habitat architecture and then habitat degradation is a major environmental factor affecting fish distribution and fish assemblages in estuarine and coastal ecosystems (Scapin et al., 2018b). Moreover, the fragmentation of seagrass meadows, when coupled with a reduction in the habitat extent, could cause the decline of species that benefit from greater seagrass cover (Perez-Ruzafa et al., 2006). The possible degradation and fragmentation of seagrass meadows in the Venice lagoon caused by human impacts, could have influence the colonization of these habitat by fish fauna. However, in Venice lagoon, Scapin et al. (2018b) observe that a reduction of seagrass habitat coverage should be regarded for conservation of associated fish in coastal lagoons (Scapin et al., 2018b). According to species-specific habitat preferences, the magnitude and the effects of habitat fragmentation on fish can vary around a treshold level of fragmentation, below which the fauna does not apper to be negatively affected (Bell et al., 2002; Macreadie et al., 2009; Scapin et al., 2018b). Indeed, as observed in this study, Franco et al. (2006a) observe that, considering the whole Venice lagoon, the seagrass bed habitats own the lower densities of juvenile marine migrant fish, suggesting a minor nursery role. Considering Franco et al. (2006a), even seagrass beds habitats less

degradated, as the one located in the south sub-basin (Curiel et al., 2014; Sfriso and Facca, 2007), perform a minor nursery role. An explanation of these results could be the higher abundance of potential predators of juvenile stages, as *Z. ophiocephalus* and *S. typhle*, found in these habitats (Campolmi et al., 1996; Franco et al., 2006a; Malavasi et al., 2004). However, in this study, the spatial configuration of the patch mosaic of seagrass beds and their degradation was not evaluated. Further studies should then be conducted to evaluate in detail why marine migrant fish tend to avoid seagrass meadows of the norther sub-basin of the Venice lagoon and if this is related with the degradation and fragmentation of these habitats.

Conversely, saltmarsh habitats being colonized by more marine migrant species during at least one period of their life-cycle (e.g. *C. saliens*, *S. aurata* and *S. sprattus*), as observed also by other works both in the Venice lagoon (Franco et al., 2006a) and in other areas (Cattrijsse and Hampel, 2006; Deegan et al., 2000; Rebeiro et al., 2012; Whitfield, 2016), seems to act an important role for juvenile marine migrant fish. In detail, marsh creeks seem to affect positively the abundance of *S. aurata*, *C. saliens* and *S. sprattus*, as observed by other works for other species in different areas (Cattrjisse and Hampel, 2006; Jin et al., 2007; Patterson and Whitfield, 2000).

Among environmental parameters, salinity, turbidity and confinement affect the distribution of many species. Inner stations of the lagoon, characterized by low salinity values, low percentage of sand, long water residence time and high turbidity seem preferred by many marine migrant species (e.g. *S. pilchardus*, *S. sprattus*, *P. flesus*, *C. ramada*, *C. saliens*), as pointed out also by other authors (Bodinier et al., 2010; Harrison and Whitfield, 2006). This is probably due to their higher tolerance to salinity variations and to the higher abundance of trophic resources that characterize confined lagoon areas (Islam et al., 2006; Marshall and Elliott, 1998). Among the different considered habitats, the preferences of individuals of the different species toward a specific habitat (e.g. marshes) could be related to the lower predation risk. Indeed, predations can be an important process structuring post-settlement assemblages of fish (Bostrom et al., 2006; Choat, 1982; Heck, 2007; Hindell et al., 2000) and small fish are generally negatively associated with the abundance of piscivorous fish (Hixon, 1991; Hindell et al., 2000). Moreover, saltmarshes provide a good food-rich place to forage (Boesh and Turner, 1984; Irlandi and Crawford, 1997) as well as protection from predation (Boesh and Turner, 1984; Irlandi and Crawford, 1997; Minello and Zimmerman, 1983). However, the preferences toward a specific habitat could be probably also related to the trophic and feeding role of these habitats. Indeed, the diet seems to be the factor that strongly affect the first developmental stages (Pita et al., 2002; Russo et al., 2007; Tancioni et al., 2003). The potential preys distribute differently inside the transitional water ecosystems (De Biasi et al., 2003; Kneib, 1984; De Souza et al., 2013). *S. aurata* individuals, for example, once enter inside the lagoon, concentrates in marsh creeks independently from their location in the sea-lagoon edge gradient, probably for trophic or shelter reasons. After the entrance phase, *S. aurata* individuals start growing and they prefer saltmarshes located in the inner part of the lagoon, indicating a progressive entrance and colonization

of the lagoon habitats. Probably, for some marine migrant species (e.g. *C. saliens* and *S. aurata*), the presence of suitable habitats (e.g. marsh creeks) located near the sea inlets could facilitate the colonization of individuals from the sea and toward the inner part of the lagoon.

In transitional water ecosystems, ecological needs and human demands can conflict sharply (Borde et al., 2003; Chittaro et al., 2009) and some habitats used by juvenile fish (e.g. saltmarsh) are extremely vulnerable to degradation or loss (Brown, 2006) (Tagliapietra et al., 2011). The increasing difficulties associated to protecting entire ecosystems, due to limited time and funds (Mohan et al., 2015), led to the need of identification of conservation priorities. This work highlights that not the whole lagoon functions as nursery for any species. Moreover, individuals of the same species can use different lagoon habitats, often located in different portion of the sea-lagoon edge gradient, in relation with ontogenetic stage or can use the same habitat, as saltmarsh for example, as a stepping stone for the colonization of the inner part of the lagoon. Generally, these species perform movements also on a short spatial and temporal scale, to search and exploit the more suitable conditions. Consequently, it appears extremely important to identify and protect the habitats supporting more species and the most recruits to adult populations, even if they change with growth of individuals (Mohan et al., 2015; Sheaves et al., 2015). The identification of nursery areas is a very important tool to generate strategies for the maintenance of fishery resources (Avigliano et al., 2017; Beck et al., 2001). In the light of the results of this chapter, the study of the nursery role of different habitats should be tackled also considering the complex and interconnected habitats that surround it (Bostrom et al., 2011; Nagelkerken et al., 2015; Sheaves et al., 2015). In addition, a particular attention should be paid to the trophic and feeding relationships that could explain why individuals concentrate in some particular areas and within some particular position of transitional water ecosystems, such as the inner saltmarsh habitats in the case of the Northern sub-basin of the Venice lagoon.

CHAPTER 3

FEEDING ECOLOGY AND SECONDARY PRODUCTION OF GILTHEAD SEABREAM'S JUVENILES IN A SALTMARSH HABITAT OF VENICE LAGOON

3.1 Introduction

It is well known that within estuaries and lagoons some habitats (e.g. saltmarshes and tidal creeks), due to their high productivity, contain high nutrient loads and attract a large number of organisms that act as primary prey for fish (Boesh and Turner, 1984; Cattrijsse and Hampel, 2006). These habitats are dominated by the juveniles of marine migrant species (Weinstein, 1979; Weinstein and Walter, 1981; Mathieson et al., 2000; Martinho et al., 2008; Patterson and Whitfield, 2000, 2003; Ribeiro et al., 2012; Whitfield and Pattrick, 2015), and play a key role as a nursery and foraging ground (Minello et al., 2003; Allen et al., 2007; Beck et al., 2001; Boesh and Turner, 1984; Deegan et al., 2000; Green et al., 2012; Jin et al., 2007; Kneib, 1997).

Because of the increasing level of saltmarsh loss through erosion, the identification of nursery habitats is particularly important when some of the nearshore habitats used by juvenile fish are vulnerable to degradation or loss (Brown, 2006). A better understanding of food-web structure and functioning of marshes is crucial for protecting these vulnerable habitats which play a relevant role as nursery grounds (Ribeiro et al., 2012).

Ontogenetic changes in the diet of juvenile fish, related mainly with changes in the body morphology and growth of the digestive tract (Pita et al., 2002; Tancioni et al., 2003), together with the prey availability in different habitats, may strongly affect the distributions of different ontogenetic stages of juveniles of marine migrant species between habitats in transitional water ecosystems (Castillo-Riviera et al., 2000; Mendes et al., 2014). Understand the trophic role and the food habits of individuals during ontogeny is a fundamental tool to determine the actual nursery role and the value of a lagoon (Sheaves et al., 2015).

The stomach-content analysis, based on ingested prey, represents the diet of individuals on short term (few hours) while the simultaneous measurement of carbon and nitrogen stable isotopes in animal tissue reflects the diet over the previous week or month (Hobson, 1999), providing information on trophic relationships (Cocheret de la Moriniere et al., 2003; Herzka, 2005; Owens, 1987;). The dual isotope approach can provide important trophic information: $\delta^{13}C$ ratios show significant differences between different sources of primary

producers, whilst the $\delta^{15}N$ ratio increases with trophic level from prey to predator through accumulation (Green et al., 2012; Peterson and Fry, 1987).

The combination of fish gut content analysis and fish tissue stable isotope analysis are generally used as a powerful tool in many coastal food-web studies (Cocheret de la Moriniere et al., 2003; Escalais et al., 2015). Stomach content and dual stable isotope analyses can thus provide information about diets of fish at different life stages and in different habitats. Respectively short- and long- time scales, allow to observe short- and long- term dietary changes during ontogeny (Carassou et al., 2016; Cocheret de la Moriniere et al., 2003). Simultaneous studies of gut contents and multiple stable isotope are usually mostly limited to large piscivores (>100 mm) (Backer and Sheaves, 2005) and few are the informations that consider the early life stages of fishes in nursery habitats during ontogeny (Cocheret de la Moriniere et al., 2003). Fortunately, in recent years studies on spatial and ontogenetic variations of juvenile fish diet, using multiple approaches, are increasing (Carassou et al., 2016; Escalas et al., 2015; Pasquaud et al., 2008; Renones et al., 2002). Lastly, the study of the head morphology can help interpret changes in the diet. Some studies have indeed demonstrated how developmental modifications may be closely linked to ontogenetic changes in habitats (Loy et al., 1998; Ventura et al., 2017) and/or food use of resources (Linde et al., 2004; Hernandez and Motta, 1997; Russo et al., 2007). Changes in morphology can shape the diet through the modification of a fish's feeding capability (Wainright and Richard, 1995). Finally, the secondary production was used to assess the potential nursery value of a saltmarsh habitat, using the growth and production of 0-group fish as proxies (Franco et al., 2010).

The target species for these analysis was the gilthead seabream, *Sparus aurata*, a euryhaline and eurythermal species, who enter in coastal lagoons and estuaries in early spring (Arias, 1980; Gandolfi et al., 1991; Lasserre, 1974; Rossi et al., 1999; Mercier et al., 2012) and represent an important and high commercial value resource both in transitional lagoon fisheries and in "valliculture" in the Mediterranean region (Lasserre, 1974; Tancioni et al., 2003) and in particular in the brackish lagoons along the North Adriatic coast (Franzoi e Pellizzato, 2002; Franzoi et al., 1989, 1999; Rossi, 1986). This species was found to be particularly abundant during the sampling carried out for this thesis in the whole basin of the Venice lagoon but especially in the north sub-basin (see chapter 1). It has also been observed that *S. aurata*, during its early stages of life inside the coastal lagoon, despite changing its preferences in relation to environmental parameters, it prefers saltmarsh habitats and tidal channels when its length is minor than 20 mm (see chapter 2).

Juvenile stages of *S. aurata* are characterized by changes in habitat and the use of resources linked to ontogenetic progressive changes in anatomy, physiology and behavior, (Andolina, 2017; Bodinier et al., 2010; Cataldi et al., 1987; Russo et al., 2007; Tancioni et al., 2003). In the past decades, many detailed studies were conducted on morphological changes occurring during ontogeny in *S. aurata*, providing detailed information about the body shape changes (Russo et al., 2007), the development of the digestive tract (Elbal et al., 2004)

and the teeth-age adaption (Cataldi et al., 1987). All these authors agree that, as well as for other species, in *S. aurata*, changes during ontogeny is strongly linked to new behaviors related to feeding habits and swimming ability (Russo et al., 2007). The diet of *S. aurata* shifts from zooplankton, during larval stage, to meio-, and macrozoobenthos once reached the juvenile and adult stages (Elgendy et al., 2016; Ferrari and Chieregato, 1981; Francescon et al., 1987; Pita et al., 2002; Tancioni et al., 1998, 2003). According to Cataldi et al. (1987) and Elbal et al. (2004), in *S. aurata* post-larvae, up to 20 mm SL, canine teeth, the gastric channel and the stomach musculature are barely developed, allowing the only ingestion of small planktonic preys. From 25 to 35 mm in standard length, according to Ferrari e Chieregato (1981) and Cataldi et al. (1987), changes in diet towards meiobenthic prey is associated to the presence of three concentric rows of canine teeth and some "transitional teeth" that will develop in molars. When fully developed, the molar teeth allow to prey on hard-shelled or exoskeleton bearing organims as bivalves, Decapoda and in general benthic animals (Elgendy et al., 2016; Ferrari and Chieregato, 1981). As it has been done in the previous chapter (chapter 2), to represent different ontogenetic stages, according to the available literature, data was organized considering different size classes.

The aim of this study was to combine different approaches to understand how juveniles of *S. aurata,* during ontogenetic growth, use the saltmarsh habitats and which are the trophic relationships that increase the nursery value of a lagoon. The hypothesis of this chapter is that inside the saltmarsh habitats, some portions perform a better trophic function, by providing a greater amount of resources and food. Results should show that *S. aurata* and therefore in general the marine migrant fish, use these habitat as feeding grounds, even if the shelter function cannot be excluded. In particular, in this chapter, the stomach content, the stable isotope and the head's shape morphology analyses were used with purpose to a) investigate the differences among the saltmarsh edge and the tidal creek inside a saltmarsh habitat and b) describe ontogenetic diet changes during growth. Once analyzed the importance of the saltmarsh habitats (saltmarsh edge and tidal creek) for *S. aurata*, secondary production was calculated to evaluate the potential nursery role of these habitat.

3.2 Materials and Methods

3.2.1 Field and sampling activities

The sampling took place in two stations: Dese (DE) and Baccan (BA), placed in areas characterized by saltmarsh habitats but located in different positions along the sea-lagoon gradient, in the North sub-basin of the Venice lagoon (fig. 29). One station (BA), located close to Lido's lagoon inlet, had marine characteristics with a high level of water salinity and sand sediment. The other station (DE), located close to the lagoon edge was more confined and characterized by a lower salinity and a high content of organic matter. For each

station two positions were investigated: a tidal creek and a saltmarsh edge (fig. 30). The tidal creek of Dese station, contrarly to the one of BA station, did not empty completely during the low tide.

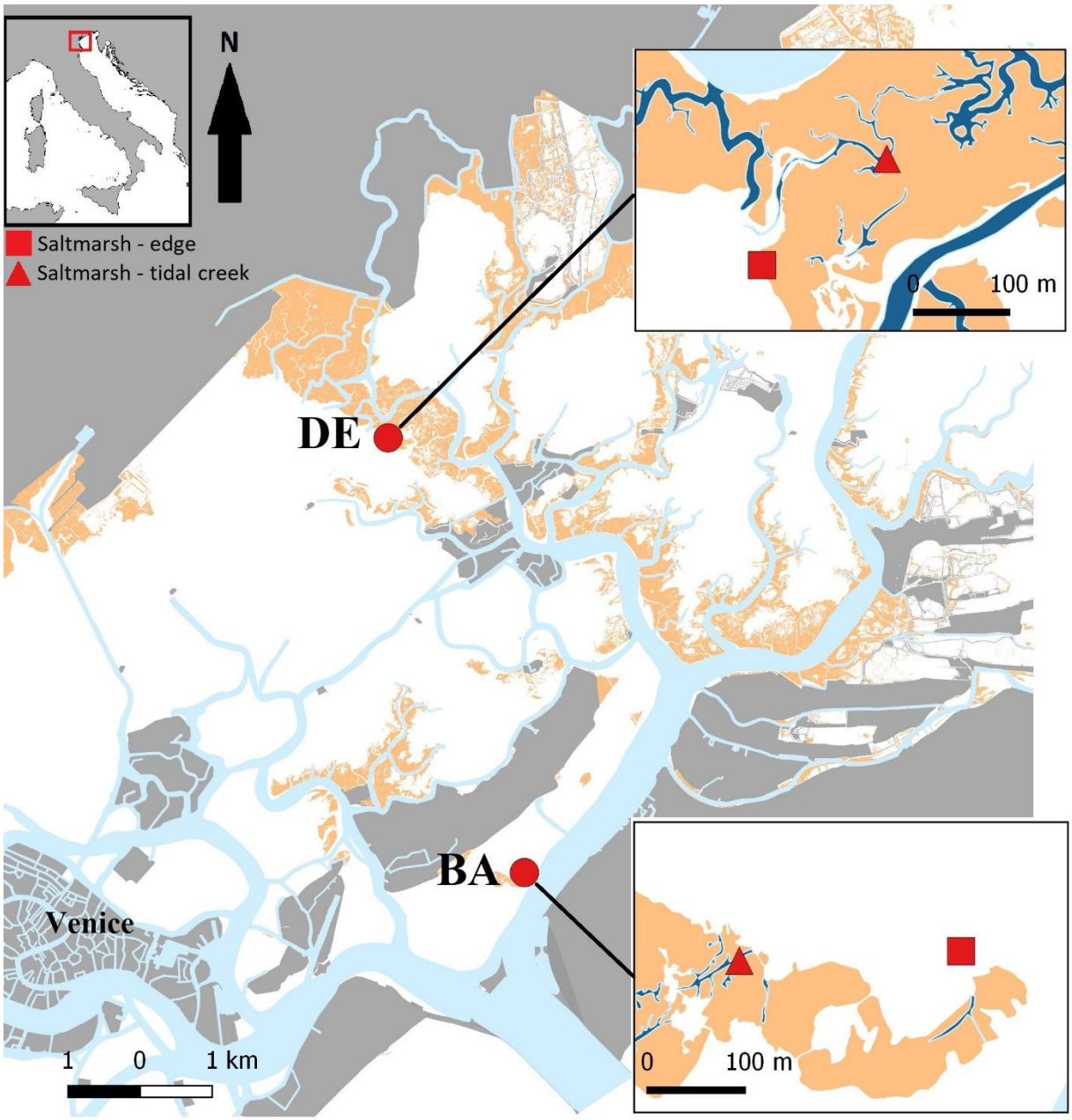


Figure 29 - Sampling stations (DE = Dese, BA = Baccan) in north sub-basin of Venice lagoon.

Figure 30 - Example of saltmarsh edge (right, orange) and tidal creek (center, light blue) in a saltmarsh area of Venice lagoon. Photos show the sampling of fish juveniles.

The juveniles of *S. aurata* were collected from the period of their migration into the lagoon (February – April) till late spring (June) (Rossi, 1986; Franzoi e Pellizzato, 2002). Sampling took place monthly in February, March and June and fortnightly in April and May 2016. In both stations and in each position three different sampling activities were performed: 1) the sampling of postlarvae and juvenile *S. aurata* for the stomach content and stable isotope analysis; 2) the integrated sampling of organisms associated to the most superficial sediment layer and the water column to it directly overlying (zoo-plankton, zoo-iperbenthos and zoo-benthos) for the analysis of prey presence and distributions; 3) the sampling of environmental sources of organic matter for stable isotope analysis; 4) the measurement of the main abiotic characteristics.

Juvenile *S. aurata* were collected using different beach-seine nets (2 mm inter-knot), trawled on shallow water. According to site-specific environmental conditions, the length of the net and the distance covered by the fishing action might vary. In saltmarsh edges the net was 12 m long and was trawled an average of 50 m, covering an average area of about 400 m^2 per haul. In tidal creeks, a morphologically more complex environment generally not larger than 5 meters, the net was 8 m long and was trawled for about 50-60 m, covering an average area of about 250-300 m^2 per haul. The bottom surface explored by the net during each sampling was calculated (trawled length x opening width of the net during sampling) in order to standardize the catch.

After the catch, all *S. aurata* specimens collected were sacrificed with an excess of 2-phenoxyethanol and were divided into two subsamples. One sub-sample was placed immediately in 8% neutralized formalin solution to stop digestive processes and transferred to the laboratory for later diet analysis, one was kept cool until the arrival in the laboratory and stored at -20°C for stable isotope analysis. For stomach content and stable isotope analysis a maximum number of respectively 50 and 25 individuals were collected, trying to get a representative number of individuals for each size class.

In two occasions, 21 March and 4 May 2016, two replicates of possible prey samples (zoo-plankton, zoo-iperbenthos and zoo-benthos) were collected immediately after the fish trawling in DE station. A modified square plankton-benthos net (fig. 31) (160 µm mesh-size) was towed horizontally above the substrate for 4 meters. This modified sampling tool, having a square structure (fig. 31) (35 cm side), was towed to the bottom to sample both iper-benthic organisms, located in the water near the sediment, and the benthic organisms placed approximately in the first 4 cm substrata. Furthermore, as the depth of the water almost never exceeded 40-50 cm, the sampling tool was able to catch also most of the planktonic organisms. The depth of the water to which the net was towed, remained constant during all the haul performed in the various positions in order to standardize the sampling procedure. On board, the collected plankton-benthos samples were then stored in buffered 5% formaldehyde.

Figure 31 – Modified net for integrating sampling S. aurata *possible preys (zoo-plankton, zoo-iperbenthos and zoo-benthos).*

Environmental sources of organic matter for stable isotope analysis were collected in March and in May 2016. When present, seagrasses, macroalgae and halophytes were collected randomly by hand in triplicate. Regarding the halophytes and macroalgae, only the most abundant species were analyzed in the laboratory and considered for the trophic assessment. Soil and particulate organic matter (respectively SOM and POM) were obtained by sampling three replicate of superficial sediment cores (3 cm diameter) and 2 L of superficial water respectively. Plankton was sampled by horizontal haul with a small plankton net with a mesh size of 160 µm. All the samples collected were maintained refrigerated in thermic boxes until arriving to the laboratory and stored at -20°C prior to the processing.

In each station and position, the main abiotic characteristics were also recorded. Water temperature (±0.1 °C), salinity (±0.01 PSU), dissolved oxygen (±0.1 % saturation) turbidity (±0.1 FNU) were recorded for the mid-water column with multiparameter probe Hanna Instrument 9829 during each sampling. Simultaneously 200 ml of water were filtered on Whatman GF/F 47 mm diameter filters and three cores of sediment (diameter 2 cm) were collected to determine, in laboratory, the total chlorophyll concentration in water column (µg/L) and in upper 2 cm sediment (µg/g) following Lorenzen (1966) methods, with Trylogy Laboratory Fluorometer.

In each station and position, in April, a core of sediment (diameter 3 cm) was collected to determine the granulometry (% sand) of the upper 10 cm following the methodology reported in Sfriso et al. (2003) and the content of organic matter through loss of ignition method (Heiri et al., 2001) at 550°C.

During each sampling campaign, in each station and position, the presence/absence and the coverage (% coverage) of macroalgae, seagrass and halophyte were recorded.

3.2.2 Laboratory activities

In the laboratory, all *S. aurata* juvenile individuals sampled for diet analysis were rinsed to remove any formalin residue and then were measured (Standard Length, SL, ±0.1 mm), weighed (Total Weight, TW, ±0.01 g) and divided in size classes (see later for details).

S. aurata specimens were firstly photographed on its left side with a stereo-microscope (Nikon SMZ1270, 6.3x-80x magnification) for a head shape morphology analysis; then the *S. aurata* collected for the stomach content analysis (n. 228) were dissected, eviscerated and weighted again (±0.01 g Eviscerated Total Weight ETW) to obtain the weight of digestive tract (fig. 32).

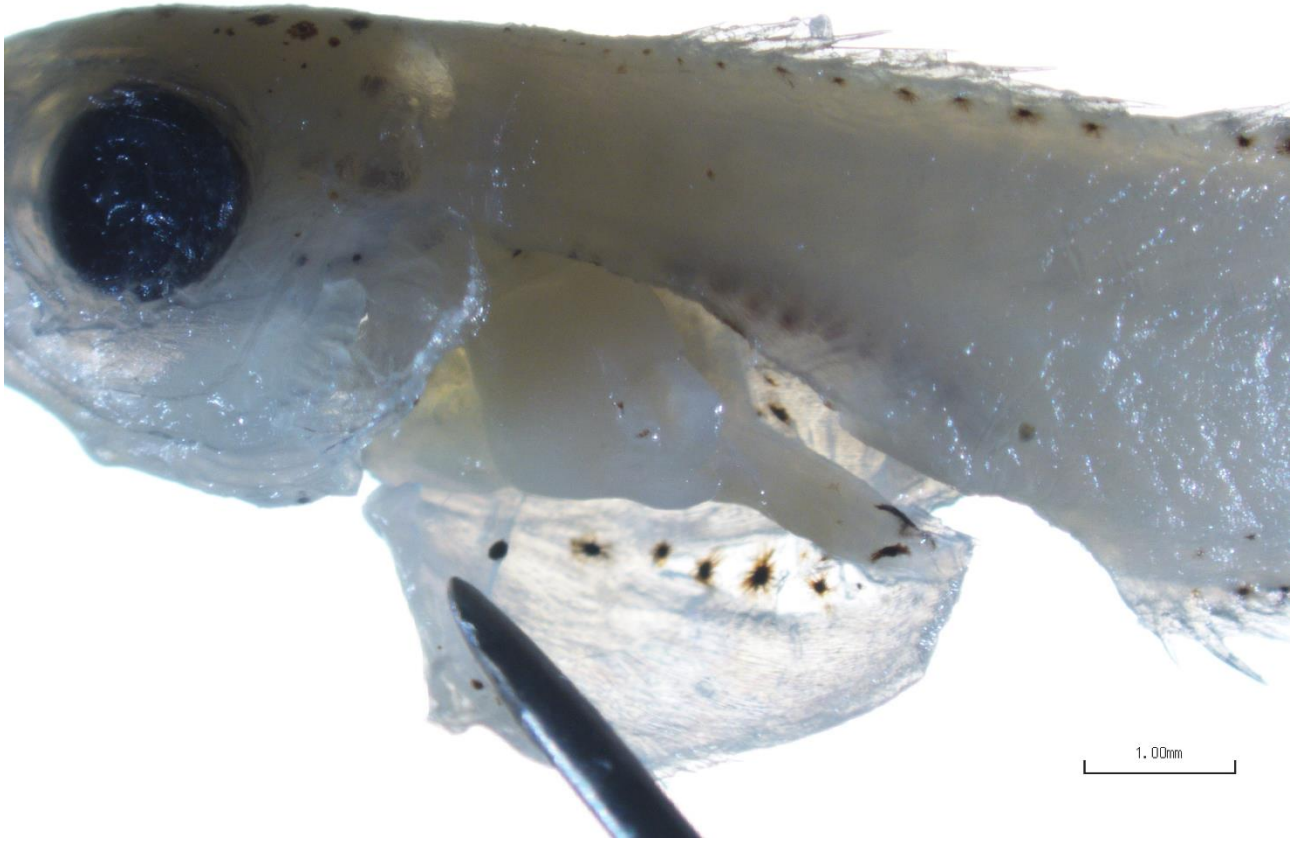


Figure 32 – Detail of stomach removal. Scale = 1 mm.

The dissection took place under a stereo-microscope (Nikon SMZ1270, 6.3x-80x magnification) and the entire stomach content of each fish was examined individually. For the diet analysis, only the stomach content was considered, defined as the material contained in the pyloric and cardiac stomachs. The content of the intestine was discarded to reduce bias caused by different rates of digestion and gut passage times (Baker et

al., 2014; Berg 1979; Buckland et al., 2017; Hyslop, 1980). Stomach fullness was estimated visually on a four-step scale (1 as empty, 2 as less than 50% full, 3 as more than 50% full and 4 as full stomach) (Garrido et al., 2008).

Prey items were identified up to Class, or Order in case of Copepod, according to the remains and their ability to provide enough information for a positive identification, counted, photographed using a high-performance camera attachment (Nikon DS-Fi2) coupled with a digital controller (Nikon DS-L3) (fig. 33), and then stored in ethanol 80%. Photos of prey items were used for the determination of individual prey length and width measurement, in order to calculate the bio-volume later.

Figure 33 – Stereo-microscope (Nikon SMZ1270) and high-performance camera (Nikon DS-Fi2) coupled with a digital controller (Nikon DS-L3).

In the laboratory, zoo-plankton, zoo-iperbenthos and zoo-benthos organisms, sampled with modified plankton-benthos net were filtered, rinsed to remove any formaldehyde residues and stained with Bengal Rose for 24 hours to ensure good contrast and facilitate sorting. Each sample was observed in full by the stereomicroscope (6.3x-80x magnification) to identify and separate any possible prey from the rest of the sample (sand, organic matter etc). As for prey items, organisms present in the zooplankton-benthos samples were identified up to Class, or Order in case of Copepod, counted, photographed and stored in buffered 5%

formaldehyde. In this case too, photos were used for the determination of individual length and width measurement, in order to calculate the bio-volume of different zooplankton and zoo-benthos taxa.

All *S. aurata* preserved for stable isotope analysis (n. 154) were then dissected using scalpel and tweezers to extract the dorsal muscle (fig. 34).

Figure 34 – Dorsal muscle removal from a juvenile S. aurata *(Standard length < 30 mm) for isotope stable analysis.*

Primary producers (seagrasses, macroalgae and halophytes) were identified at species level and gently scraped to remove epiphytes. Water samples collected for POM were filtered (200 mL) on GF/F Whatman filters (pore size 0.7 µm) previously combusted at 450°C for 4 hours. Sediment cores sampled for SOM were sliced to the top 1.5 cm and homogenized. Plankton samples were concentrated in Eppendorf tubes after accurate cleaning to remove any detrital material under binocular microscopy. After processing, all fish and source samples designed for isotopic analysis were oven dried (60°C for 48 hours) to obtain a constant weight and ground with a micro mill or a mortar and pestle to obtain a fine powder. An aliquot of each replicate of primary producers, SOM and POM was acidified with HCl 1 M in order to dissolve carbonates potentially affecting the carbon isotopic signature. Each sample, encapsulated in tin cups, was analyzed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Thermo-Electron Delta Plus XP) coupled to an elemental analyzer (Thermo-Electron Flash EA1112).

3.2.3 Data analysis

3.2.3.1 Stomach content analysis

Estimation of prey's biovolume

Many problems could emerge using only the number of prey in the gut analysis. When in the gut content there are prey of very different sizes, the numerical method tends to overestimate the importance of smaller

prey items (Hyslop, 1980). The volumetric method seems to be more representative of the contribution, in terms of energy, provided by the different prey to the diet of a given species. But also, the volumetric method presents some problems, e.g. is difficult to evaluate correctly the total ingested volume of organisms that present a different degree of digestion inside the fish gut (Hyslop, 1980). This problem has been partly overcome considering that juvenile *S. aurata* have a fast swallowing rate and they eat continuously and their stomachs are rarely found empty or with all their prey digested. Moreover, to avoid the errors related to the different degree of digestion of the prey, for the analysis of the diet only the contents of the stomach were considered, and not intestinal ones, where usually the prey showed a state of advanced digestion. Finally, the problems deriving from the different prey digestion rate have been considered and solved by calculating the biovolumes of individual prey reconstructing them by length and width measurements through specific formulas. These reconstructed biovolumes were calculated in the same way for each ingested and available prey item.

Each prey items found in the stomach or collected with modified plankton-benthos net were measured at their maximum length and width using the program ImageJ (fig. 35). Except Copepods, for each organism, one measure of length and one measure of width were recorded (fig. 35, left). Because Copepods are formed by two parts, a thorax and an abdomen, generally different in size, to get a more appropriate size measure, maximum length and width were obtained both for thorax and abdominal tracts (fig. 35, right).

Figure 35 – Examples of measures with ImageJ. For Copepod (right) length and width were collected separately for abdomen and thorax.

Due to Amphipoda's peculiar morphology, thickness was determinated instead of width and width was then estimated using two formulas: for *Corophium* sp., which has a more compact shape, Width = (1.0214 * Thickness) + 0.0748; for *Gammarus* sp. and other Amphipoda, Width = (0.823 * Thickness) + 0.0122. These coefficients are slope and intercept of the linear regression between width and thickness, calculated using a subsample of individuals for which had been measured both length, width and thickness.

Biovolumes were then estimated using Warwick and Price (1979) equations: $V(nL) = L * W^2 * C$, where L is length (mm), W is width (mm) and C is a conversion factor. For Amphipoda the Warwick and Price (1979)

equation were modified as: V(nL) = L * T * W', where L is length (mm), T is thickness and W' is width calculated with the coefficient previously estimated.

The conversion factors C, different for each morphological form and derived from a volumetric displacement of plasticine scale models (McIntyre and Warwick, 1984; Warwick and Gee, 1984), are reported in Table 16.

Table 16 - Conversion factor used for the determination of biovolumes

Taxa	Conversion factor
Calanoida, Cyclopoida, Mysidacea, Decapoda and Amphipoda	490
Polychaeta	530
Harpacticoida	400 or 485 or 490 or 560 in relation to their morphology form (see Warwick and Gee, 1984 for details)

Quantitative study of diet

According to Hyslop (1980), methods used to quantitatively describe the diet and the food items ingested were: percentage frequency of occurrence (%Fi), numeric percentage (%Ni) and volumetric percentage (%Vi) of a prey category i. The percentage frequency of occurrence (%F) of each prey is calculated according to the equations: %Fi = (Fi/Ft) x 100, where Fi is the number of stomachs containing the food item i and Ft is the total number of stomachs examined. The percentage of prey abundance in number (%N) is calculated according to the equations: %Ni = (Ni/Nt) x 100, where Ni is the number of food item i and Nt is the total number of food item in the stomach examined. The percentage of prey abundance in biovolume (%V) is calculated according to the equations: %V = (Vi/Vt) x 100, where Vi is the biovolume of food item i and Vt is the total biovolume of food item in the stomachs examined. To assess the contribution of each prey category to fish diet, the Index of Relative Importance (IRI, Hyslop, 1980), expressed in percentage of each prey (%IRI), was calculated. %IRI combines the three previous indices and it is calculated according to the equations proposed by Pinkas et al. (1971) and Prince (1975): %IRI = (IRI / $\sum^{n}_{a=1}$IRI) x 100, where IRI = %F x (%N + %V) and n is the number of different prey categories.

Graphical approach for feeding strategy

Amundsen's et al. (1996) graphical method was used to describe prey importance and feeding strategy of different *S. aurata* size classes, both for prey abundance and biovolume. Feeding strategy was given by a two-dimensional graphic representation where the vertical axis represents the feeding strategy in terms of specialization or generalization (Amundsen et al., 1996). In graphs (fig. 36), frequency of occurrence, expressed in fraction rather than in percentage, is plotted against prey-specific abundance of prey i (Pi).

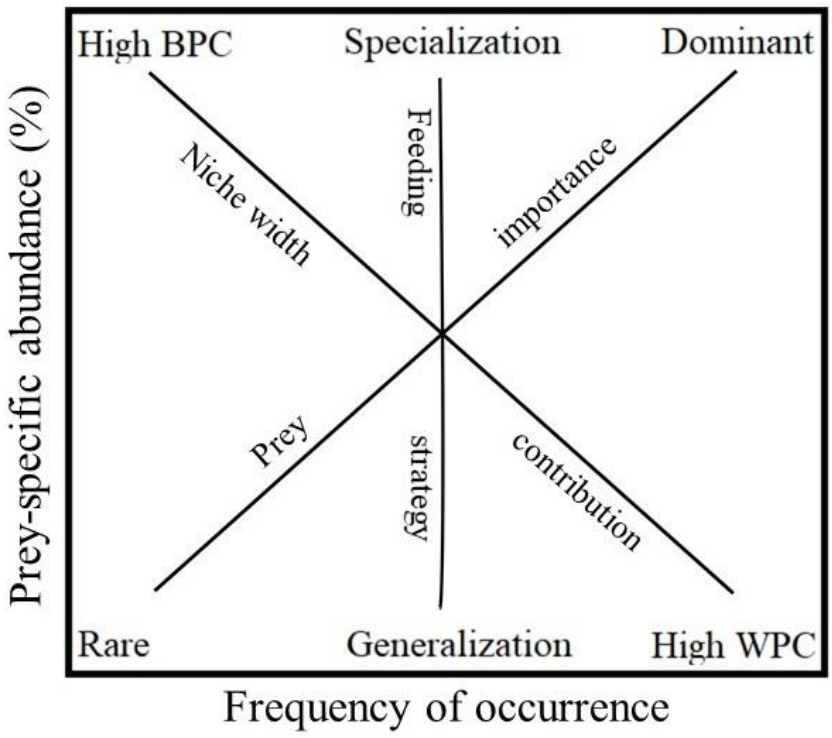


Figure 36 – Diagram for interpretation of feeding strategy, niche width contribution and prey importance from method proposed by Amundsen et al. (1996). BPC = between-phenotype component. WPC = within-phenotype component.

For each *S. aurata* stomach and each food item i, the percentage of prey abundance (in number %Ni and volume %Vi) were calculated. Prey-specific abundance (Pi) of prey i is defined as the mean of prey abundance (%Ni or %Vi) of those predators with prey i in their stomach.

The vertical axis represents the feeding strategy (specialization or generalization) of the predator: prey positioned in the lower part of the graph have been eaten more occasionally (generalization) while predators have specialized on prey types positioned in the upper part (fig. 36) (Amundsen et al., 1996). Prey points located at the lower left of the diagram would be indicative of prey rare in the diet of analyzed population while prey points located at the upper right of the diagram would be indicative of dominant prey in the diet of the whole analyzed population. A population with high between-phenotype component is composed by individuals specialized on different resource types, whereas a population with high within-phenotype component is composed by individuals who utilize many resource types simultaneously (fig. 36) (Amundsen et al., 1996). Prey points located at the upper left of the diagram would be indicative of specialization at the level of individuals: few individuals are specialized towards a specific prey type. Instead the points located at the top right would be indicative of specialization at the level of all predator population: all individuals are specialized towards the same prey type.

Selectivity and preferences of food items

Not all organisms which are present in the environment are equally chosen by *S. aurata*, some prey are actively selected with respect to the others. The selection process, is the one in which an animal chooses a

resource among alternative food that is available. To determine which food items are selected more often than others, in order to evaluate the relationship between ingested prey and organisms present in the environment, two selectivity indices were used.

The measure of prey selection was assessed by calculating the Strauss' linear selection index (L) (Strauss, 1978) and the Vanderploeg and Scavia's relativized selectivity index (E*, second selectivity index) (Vanderploeg and Scavia, 1979a, b). Selectivity indices, L and E* were calculated for each prey category separately for sampling position and size classes. Indices were applied for the dates during which possible preys were collected simultaneously to *S. aurata* individuals: 21 March and 4 May 2016. To avoid the problems arising from use of number of prey (Hyslop, 1980), biovolumes were used.

The Strauss' linear selection index (L) was calculated using the formula:

L = ri – pi

where ri is the proportion of the *i* prey category in the digestive tract content and pi is the proportion of the *i* prey category in the environment.

The Vanderploeg and Scavia's relativized selectivity index (E*), derived from Chesson index (Vanderploeg and Scavia, 1979) was calculated using the formula:

$$E_i^* = \frac{W_i - n^{-1}}{W_i + n^{-1}}$$

where $W_i = \frac{(r_i/p_i)}{\sum_1^n (r_i/p_i)}$, ri and pi defined as in Strauss' linear selection index (L) and n is the number of prey items.

These indices range from -1 (avoidance or inaccessibility) to +1 (maximal positive selection or preference) and the expected value of the indices for random feeding is zero. Values of the indices close to zero suggest that fish are opportunistic, whereas specialist fish exhibit values close to |1| (Selleshlag and Amara, 2015). Extreme values occur when prey item is rare but consumed almost exclusively or is highly abundant but rarely consumed. However, in practice, the value of +1 can be attained only under unrealistic condition with one food item in the gut which does not occur in the environment and the number of food types is infinite. Strauss's index is not amenable to comparison of electivity for an item sampled at sites with differing abundances of items in the environment or diet and this essentially precludes any field comparison of electivity (Lechowicz, 1982). Furthermore, both indices are vulnerable to sampling error for rare and moderately common food items (in the environment or in the diet) (Manko, 2016). Vanderploeg and Scavia's index, however, provides a better estimate of the effort exerted by the predator in selecting a given prey. Advantages of the E* are that it includes a measure of the feeder's perception of a food's value as a function of both its abundance and the abundance of other items of food in the environment. Also Confer and Moore

(1987) found this index as appropriate in field studies with high variability of the number of food items in diet and the relative abundance of food resources. These properties were the reasons why Lechowicz (1982) accepts this index as the best and most useful.

3.2.3.2 Stable isotope analysis

Carbon and nitrogen stable isotope ratios were expressed in δ unit notation, as parts per mil deviation from the international standards (respectively Pee Dee Belemnite and atmospheric N2 for carbon and nitrogen) and determined as follows: δX=[Rsample/Rstandard)-1]x103, where X is ^{13}C or ^{15}N and R is the relative $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The analytical precision of the measurement was 0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively.

In order to observe differences between sampling site, position and size class in the isotopic niche width and relative metrics, standard ellipse areas (SEAc, corrected for small sample size) were estimated by Bayesian statistics, using the R package SIBER v2.0.2 (Stable Isotope Bayesian Ellipses in R). Community-wide metrics were calculated using the R package SIAR 4.2.2 (Parnell et al., 2010). Metrics were estimated independently for different size classes (1, 2 and 3), different stations (BA and DE) and different sampling position (saltmarsh edge and tidal creek), cumulating the sampling campaigns. Among the community-wide metrics, proposed by Layman et al. (2007), the followings were calculated for the purpose:

i) $\delta^{15}N$ Range (NR), difference between the most enriched and most depleted $\delta^{15}N$ values, estimates the trophic length;
ii) $\delta^{13}C$ Range (CR), difference between the most enriched and the most depleted $\delta^{13}C$ values, is a measure of the diversity of basal resources used;
iii) mean Distance to Centroid (CD), average Euclidean distance of each species to the centroid $\delta^{13}C$ -$\delta^{15}N$, quantify the trophic diversity and species spacing within the isotopic space;
iv) mean Nearest Neighbor Distance (NND), Euclidean distance of each individual to the nearest neighbor, provide information on individuals density and packing within the population (trophic redundancy);
v) Standard Deviation of the Nearest Neighbor Distance (SDNND) provides information on the evenness of individuals packing.

Environmental sources were analyzed to detect the difference of $\delta^{13}C$ and $\delta^{15}N$ among different stations and sampling positions. Additionally, Bayesian mixing models were then applied for DE station for each sampling position (tidal creek and saltmarsh edge) and for three size classes detected (class 1 and 2), with the purpose to trace the trophic pathway supporting the species at different development stages, to investigate the role of different habitat and to estimate the contribution of each source. As basal sources we considered all the

organic matter sources available in the specific habitat, that included sedimentary and particulate organic matter (SOM and POM), seagrasses when present, algae, pooled halophytes and plankton.

3.2.3.3 Secondary production of *S. aurata*

The potential nursery value of saltmarsh station in Venice lagoon was also analyzed by using growth and production of *S. aurata* juveniles collected in DE station, both in the saltmarsh edge and in the intertidal creek. This station was chosen for the constant and abundant presence of juvenile of *S. aurata* 0-group during all the sampling periods, from February to May.

Firslty, absolute growth rates (AGR, mm/day) were determined according to Martinho et al. (2008): $AGR = \frac{L_t - L_{t-1}}{\Delta t}$ where L_t and L_{t-1} are the mean total length at consecutive sampling dates, and Δt is this time interval. *Sparus aurata* production was then calculated for each sampling date (t) as the net increment in mean wet weight of the individuals per unit area, according to: $P_{cn}(t) = (w_t - w_{t-1}) \cdot (\frac{N_{t-1}+N_t}{2})$ where N is the density (ind/m^2), w is the mean individual weight (g w.w.), and t-1 and t consecutive sampling dates. Value was then standardized over a 30 days temporal interval to obtain the monthly production (g w.w./m^2/month).

3.2.3.4 Statistical analysis

Diet analysis: differences between sampling positions

The differences in stomach fullness, estimated on a four-step scale (1 as empty, 2 as less than 50% full, 3 as more than 50% full and 4 as full stomach) (Garrido et al., 2008), between tidal creek and saltmarsh edge were statistically tested with a chi-squared test on a Generalized Linear Model with binomial family.

To observe the differences between sampling positions (saltmarsh edge and tidal creek), following the available literature (Cataldi et al., 1987; Elbal et al., 2004; Russo et al., 2007) three size classes were defined as follows:

1) Post-larvae, SL<20 mm
2) Juveniles I, 20≤SL<35 mm
3) Juveniles II, 35≤SL<50 mm

A non-multimeric multidimensional scaling plot (nMDS) was performed on the distance matrix to visualize diet similarities between sampling position and size classes. Euclidean distances were calculated using the single linkage rule. To observe similarities in diet, the %IRI was considered. To test the null hypothesis showing spatial differences in fish diet, ANOSIM was applied. ANOSIM was also used to test differences in fish diet between size classes.

Differences in length, weight and diet composition of *S. aurata* between tidal creek and saltmarsh edge were statistical tested with Generalized Linear Models using the most suitable distribution family for each case (generally negative binomial). The most suitable post-hoc comparisons were then chosen to observe the differences in detail. Shapiro-Wilk test was used to verify if the distribution of the variables tested with the GLMs was normal while Bartlett test was used to test the homogeneity of the variance. Although the first part of the study focused mostly on the spatial differences (among tidal creek and saltmarsh edge), the temporal factor (sampling campaign) was included in the analysis. Tests were performed comparing means of standard length (SL, mm), total weight (TW, g) and eviscerated total weight (ETW, g) without dividing individuals in size classes. Then, to consider the changes in stomach size and standardize the results, the means of total bulk content, both total number and biovolume (nanoliters, nL), were tested dividing individuals in size classes.

Diet analysis: differences in ontogenetic shift of diet

To analyze in detail the ontogenetic shift of diet, rather than differences between sampling positions, *S. aurata* individuals were later also divided in smaller size classes (range of 5 mm). New size classes were defined as:

A) $SL < 20$ mm;
B) $20 \text{ mm} \leq SL < 25$ mm
C) $25 \text{ mm} \leq SL < 30$ mm
D) $30 \text{ mm} \leq SL < 35$ mm
E) $SL > 35$ mm

To analyze the ontogenetic shift of diet (dividing individuals in new size classes having 5 mm range), all individuals were considered together and no distinction between sampling campaign or sampling station were made. Again, as for difference between sampling position, difference between size classes were statistically tested with Generalized Linear Models using the most suitable distribution family for each case. The most suitable post-hoc comparisons were then chosen to observe the differences in detail and Shapiro-Wilk test and Bartlett test were used to verify if the distribution of the variables and the homogeneity of the variance of considered data. Tests were performed on total biovolume and total number of ingested prey and on mean size of an ingested prey. Tests were also performed on the main prey taxa found in the stomach content.

The ontogenetic shift was also observed with an analysis of head shape morphology. Photos of the 99 *S. aurata* analyzed for the stomach contents were digitalized in ImageJ 1.51k to obtain the body shape data for Elliptic Fourier analysis (EFA). Because of the different position of the mouth during death, it was not possible to use all the individuals photographed, and a sub-sample of individuals was made. Again, individuals were

separated in different size classes (range of 5 mm). Using a polygon selection tool, 8-bit.jpeg black and white images of head region was created manually for each selected individual (fig. 37). The interpolate and fit spline tools of ImageJ were also used to minimize the irregularities of drawing. Three landmarks on head outlines were defined (fig. 37, red circles) in order to align the shapes. Each shape, which consist of series of x and y coordinates for each pixel, were then converted into harmonically related equations using Elliptic Fourier analysis (for a detailed description of the method refer to Ventura et al., 2017). The differences between the head mean-shape of the different size classes were observed through Thin-Plate Spline (TPS) visualization in the form of "arrows maps". Differences among size classes were tested by appropriate Generalized Linear Model and specific post-hoc. These analyses were performed in R environment using the Momocs package (Bonhomme et al., 2014).

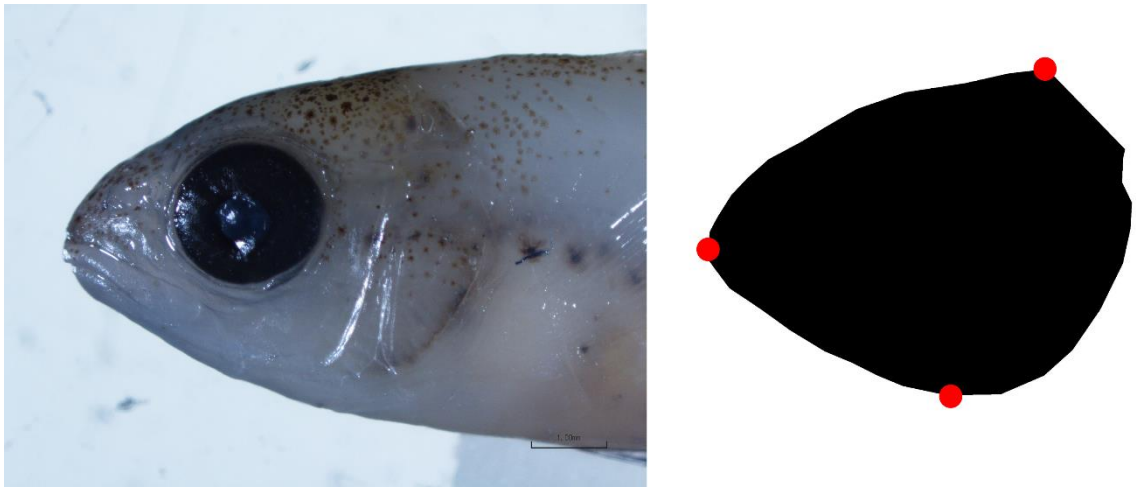


Figure 37 – Example of head shape morphology. Left = S. aurata *individual, Right = black and white images of head region created with ImageJ and in red the chosen three landmark.*

Stable isotope analysis

Carbon and nitrogen isotopic data were then analyzed separately to observe differences between size classes (class 1, 2, 3) and position (saltmarsh edge and tidal creek). Linear regression models were applied to observe the relationship between body length and isotopic signature for each size class in different sampling sites and position; t-test of the slopes was later used to explore differences.

Due to non-normality of data, to assess differences in multiple comparison among sites, sampling position and size classes, three-way permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) were used, using R (R Core Team, 2017), testing $\delta^{13}C$ and $\delta^{15}N$. PERMANOVA was also used to assess differences of basal sources among sites and sampling position, testing $\delta^{13}C$ and $\delta^{15}N$. Significant differences, considering the level of $p<0.05$, were further investigated using Pair-Wise t-tests where p values were calculated for permutations. For DE stations, where the sources were collected both in March and May, the mean isotopic values between sampling campaign were used while for BA stations only the values of the sources collected in May were used.

To compare the isotopic niche widths and the community metrics of the different size class, position and sampling station, additional bootstrapping of data (n = 10000 replicates of bootstrapping interaction to perform, indicated with '$_B$') based on the minimum sample size of data (n = different for each sampling station, position and size class) was performed allowing comparison among conditions due to the different sample effort (Jackson et al., 2011, 2012). All metrics were determined using Stable Isotope Analysis in R (SIAR) package (Parnell et al., 2010) and Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al., 2011), all statistical analyses were performed using R with significance for statistical tests set to $\alpha = 0.05$.

To investigate the role of different habitats and to estimate the contribution of each source, Bayesian mixing models were applied for DE station, both for tidal creek and saltmarsh edge. It was not possible to apply the Bayesian mixing models to the samples collected in BA station because the results did not satisfy the assumptions of the model: individuals did not fall inside the mixing polygon created by the food sources (Phillips, 2012). Taking into consideration fish as second level consumers, trophic enrichment factors (TEFs) were used. In detail TEFs used for the mixing model were 4.52‰±0.75 for $\delta^{15}N$ (Vander Zanden and Rasmussen, 2001) and 2.52‰±0.67 for $\delta^{13}C$ (Post, 2002).

3.3 Results

A total of 606 *S. aurata* were collected during the whole sampling period, from February to June, particularly in DE station (61,4%) (tab. 29). Standard length of *S. aurata* varied from 12.9 mm to 49.8 mm while total weight ranged from 0.0259 g to 3.0492 g. Respectively, the 33.7%, 62.7% and 3,6% of the collected *S. aurata* specimens belongings to size class 1, 2 and 3. The size class 1 *S. aurata* individuals were mostly found in February and March (91,7%) while size class 2 individuals in April and May (98.7%). When possible, *S. aurata* collected were divided in subsamples for diet analysis (36.3% of individuals) and stable isotope analysis (16.8% of individuals) (tab. 17).

Table 17 – Subdivision of the S. aurata *collected in different station during different sampling campaign for the different analyzes. CL1 = size class 1, CL2 = size class 2, CL3 = size class 3.*

data	station	position	total *S. aurata*				*S. aurata* analized for diet				*S. aurata* analyzed for stable isotope			
			total	CL 1	CL 2	CL 3	total	CL 1	CL 2	CL 3	total	CL 1	CL 2	CL 3
19/02/2016	BA	saltmarsh edge	2	2	0	0	0	0	0	0	0	0	0	0
19/02/2016	BA	tidal creek	1	1	0	0	0	0	0	0	0	0	0	0
19/02/2016	DE	saltmarsh edge	8	8	0	0	8	8	0	0	0	0	0	0
19/02/2016	DE	tidal creek	22	22	0	0	22	22	0	0	0	0	0	0
21/03/2016	BA	saltmarsh edge	19	19	0	0	0	0	0	0	11	11	0	0
21/03/2016	DE	saltmarsh edge	64	60	4	0	33	30	3	0	11	10	1	0
21/03/2016	DE	tidal creek	76	75	1	0	32	32	0	0	21	20	1	0
07/04/2018	DE	saltmarsh edge	22	11	11	0	10	4	6	0	11	6	5	0
07/04/2018	DE	tidal creek	6	3	3	0	6	3	3	0	0	0	0	0
15/04/2018	BA	saltmarsh edge	3	2	1	0	0	0	0	0	3	2	1	0
15/04/2018	DE	saltmarsh edge	4	0	4	0	4	0	4	0	0	0	0	0
15/04/2018	DE	tidal creek	104	0	104	0	46	0	46	0	10	0	10	0
04/05/2018	BA	saltmarsh edge	209	1	191	17	30	0	24	6	17	0	10	7
04/05/2018	DE	saltmarsh edge	8	0	8	0	0	0	0	0	8	0	8	0
04/05/2018	DE	tidal creek	55	0	53	2	26	0	24	2	10	0	10	0
26/05/2018	DE	saltmarsh edge	2	0	0	2	2	0	0	2	0	0	0	0
21/06/2016	DE	tidal creek	1	0	0	0	1	0	0	1	0	0	0	0

3.3.1 Stomach content and diet analysis

3.3.1.1 Differences between sampling positions

Analyzing stomach content, from a total of 220 juvenile *S. aurata* only 6% had empty stomachs. In this analysis, if a stomach was full for more than 50%, it was considered full. Stomach fullness analysis shows that 79,09% of size class 2 individuals were caught with full stomachs, while only 53.54% size class 1 had full stomachs. Extremely important is the position inside the saltmarsh stations where individuals with full stomachs were caught. Considering size class 1, 33.33% of all *S. aurata* collected in saltmarsh edge had full stomachs, while 68.48% of all *S. aurata* collected in tidal creek had full stomachs. Considering size class 2, 59.46% of all *S. aurata* collected in saltmarsh edge had full stomachs, while 89.04% of all *S. aurata* collected in tidal creek had full stomachs. Differences in stomach fullness between the sampling positions, separated by size class, were also tested with Chi-square test and the results were statistically significant ($p<0.001$).

Statistical tests (GLM) were performed on standard length, total weight and eviscerated total weight to observe differences between tidal creek and saltmarsh edge. These tests were conducted only for individuals collected in DE station because only in this station enough individuals were collected both in saltmarsh edge and in tidal creek during all the sampling campaign. Differences ($p<0.05$) were observed only considering

sampling campaign for standard length and total weight (fig. 38, 39). No differences between sampling station and sampling campaign were observed for eviscerated total weight (38, right).

Considering temporal factor, standard length increased during all sampling campaigns, in both saltmarsh edge and tidal creek; significant differences ($p<0.05$) were observed between the second and the third campaign, the third and the fourth and between the fourth and the fifth sampling campaign (fig. 39). Mean individual total weight, even if increased over time as standard length, showed significant difference ($p<0.05$) only between fourth and fifth sampling campaign (fig. 38).

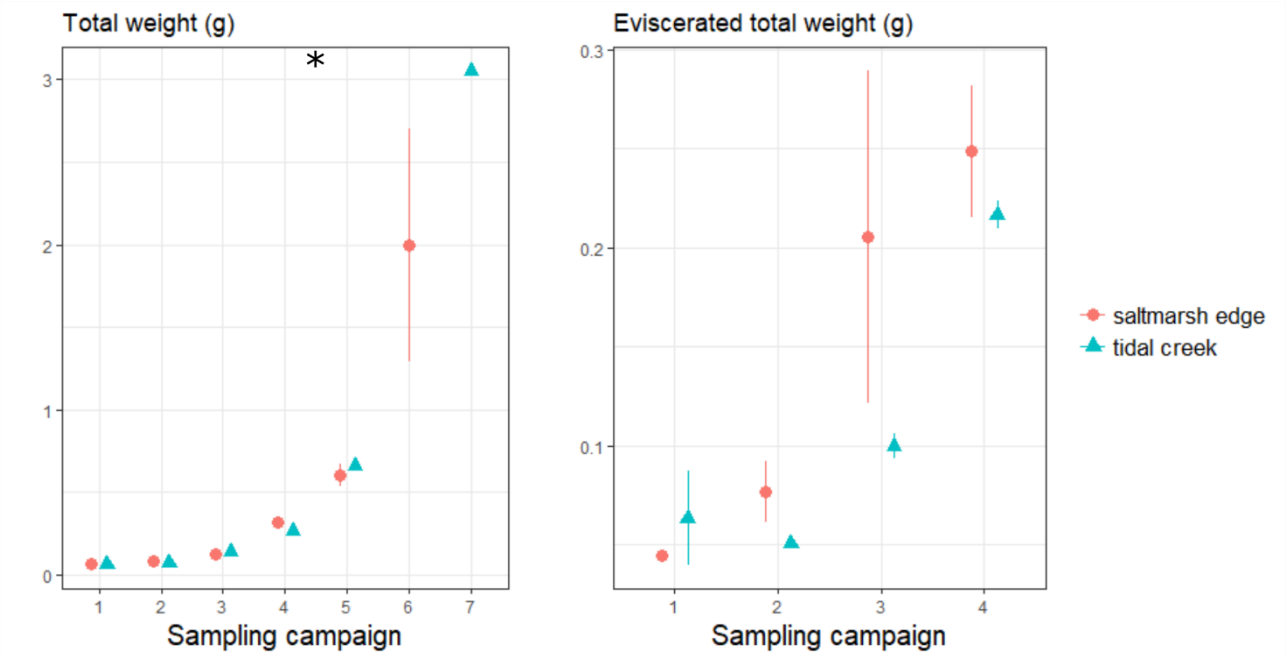


Figure 38 – Total weight (in g, left) and eviscerated total weight (in g, right) (mean ± St. Err.) of the S. aurata *collected in Dese station during sampling campaign. Significant differences ($p<0.05$) are reported with asterisk in the graph.*

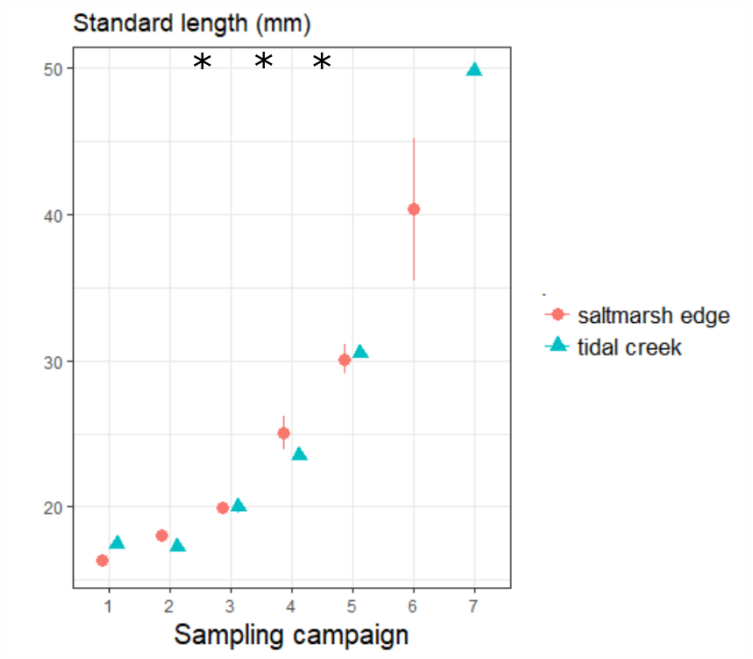


Figure 39 – Standard length (in mm, mean ± St. Err.) of the S. aurata *collected in Dese station during sampling campaign. Significant differences ($p<0.05$) are reported with asterisk in the graph.*

Difference in stomach content between sampling position were also tested (GLM) considering both total number of ingested prey and ingested biovolume.

Considering the smaller *S. aurata* (size class 1) (fig. 40), differences between sampling positions was observed in March (second sampling campaign) considering both the total ingested biovolume and the total number of ingested preys. The total ingested biovolume was greater in *S. aurata* collected in saltmarsh edge while in tidal creek individuals ate a greater number of prey (fig. 40). Comparing number of ingested prey (fig. 40, right) with their biovolume (fig. 40, left), size class 1 *S. aurata* in saltmarsh edge ate a smaller number of prey but larger. During the third sampling campaign however (fig. 40), even if no significant differences were observed, the trend had reversed: in tidal creek *S. aurata* ate a low number of prey with bigger size than the saltmarsh edge.

Differences between sampling campaigns were observed in tidal creek between the second and third sampling campaign, considering total ingested biovolume, and between the first and second sampling campaign considering total number of ingested prey (fig. 40). In saltmarsh edge, differences between sampling campaigns were observed in total ingested biovolume between first and second sampling campaigns (fig. 40, left).

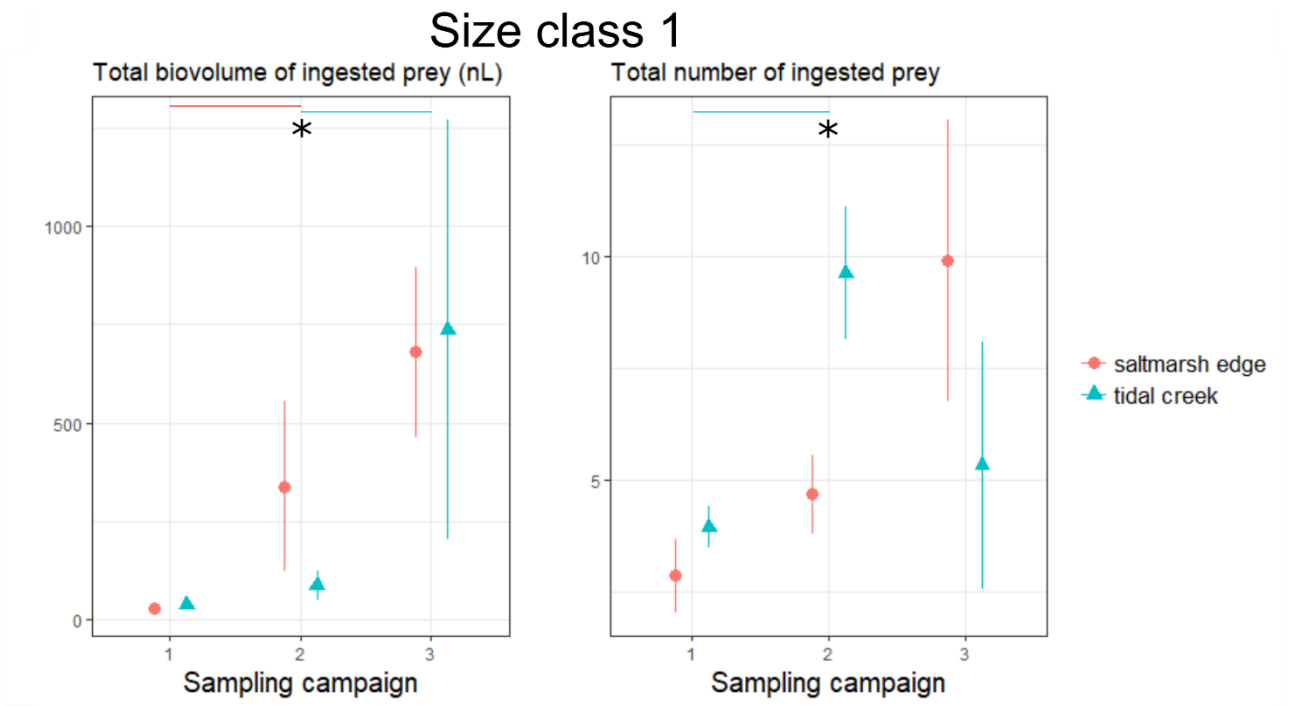


Figure 40 – Total biovolume (in nL, mean ± St. Err.) of ingested prey (left) and total number of ingested prey (right) (mean ± St. Err.) of the size class 1 (15mm<SL<20mm) S. aurata *collected during sampling campaigns. Significant differences (p<0.05) between positions or sampling campaigns are reported with asterisk and horizontal bars in the graph. The color of the horizontal bar indicates for which sampling position the difference between sampling campaigns were observed.*

Considering size class 2 *S. aurata* (fig. 41), differences between sampling campaigns were observed both for total number of ingested prey, in the tidal creek, and for total ingested biovolume of prey, in saltmarsh edge. The total ingested biovolume remained with low values during both sampling campaigns in the tidal creek, however increased significantly in the fourth campaign in the saltmarsh edge. Contrarily, the total number of ingested prey remained low in the saltmarsh edge during both sampling campaigns but increased significantly in the fourth campaign in the tidal creek.

Differences between sampling positions were observed for both number and biovolume of ingested prey during the fourth sampling campaign (fig. 41). As observed in March for *S. aurata* size class 1 (fig. 40), at the end of April, *S. aurata* size class 2 collected in the tidal creek ate a higher number of prey. However, in tidal creek the biovolume of prey was lower to those in saltmarsh edge. Considering size class 2, during the fourth sampling campaign the number of ingested prey was higher in the tidal creek but the total ingested biovolume was higher in the saltmarsh edge (fig. 41).

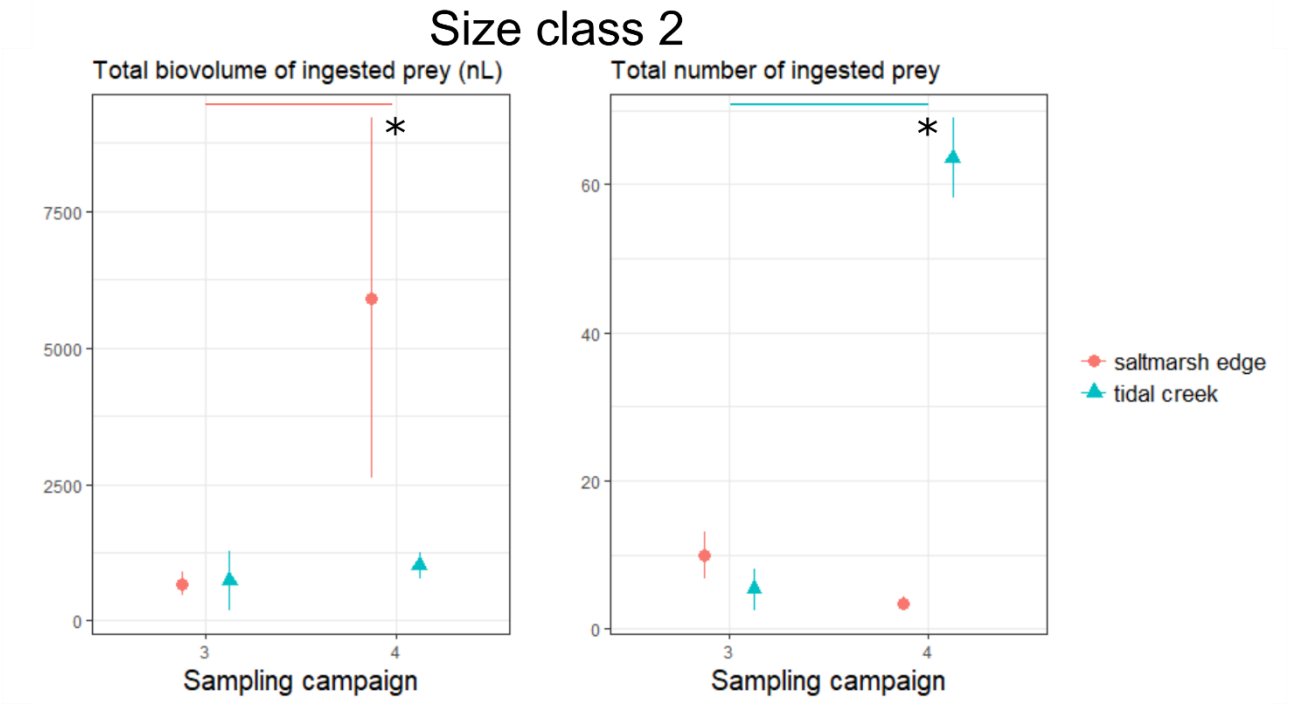


Figure 41 – Total biovolume (in nL, mean ± St. Err.) of ingested prey (left) and total number of ingested prey (right) (mean ± St. Err.) of the size class 2 (20mm <SL<35mm) S. aurata *collected during sampling campaigns. Significant differences (p<0.05) between positions or sampling campaigns are reported with asterisk and horizontal bars in the graph. The color of the horizontal bar indicates for which sampling position the difference between sampling campaigns were observed.*

Inside the stomachs of all analyzed *S. aurata* (tab. 17), 4194 prey specimens were collected and identified, mainly zooplankton and zoobenthos, belonging to five higher taxa: Amphipoda, Copepoda (Calanoida, Cyclopoida, Harpacticoida), Decapoda, Mysidacea and Polychaeta. For some prey, identification was possible up to the Family level, but data elaboration was performed considering just these taxa: Amphipoda, Calanoida, Cyclopoida, Harpacticoida, Mysidacea, Decapoda, and Polychaeta.

Dietary indices (%N, %V, %F) for each prey category were calculated for each date, station, position and *S. aurata* size class. Dietary indices are reported in Appendix B (tab. B1). Combining the three previous indices, the Index of Relative Importance (IRI), then expressed as % (%IRI) (tab. 18, 19, 20), was calculated to assess the contribution of each prey category to fish diets, in order to highlight differences between sampling campaigns, stations, size classes and sampling positions (saltmarsh edge and tidal creek). To observe the different trophic role of tidal creek and saltmarsh edge, only sampling campaigns during which *S. aurata* were collected in both positions were considered (tab. 17): 19 February, 21 March, 7 and 15 April. To observe differences between stations located at the ends of the sea-lagoon gradient, the %IRI values of *S. aurata* collected on 4 May, which is the date on which *S. aurata* was also collected at BA station, are also reported.

Index of Relative Importance show that smaller *S. aurata* (size class 1) (tab. 18) ate mostly Harpacticoida, both in the saltmarsh edge and tidal creek. Only during the third sampling campaign (7 April 2016), inside the tidal creek, Amphipoda had a %IRI similar to Harpacticoida. After Harpacticoida, also iper-benthonic Calanoida and Cyclopoida were important in the diet of *S. aurata* size class 1. In detail, Calanoida were

important in diet of *S. aurata* size class 1 just during February inside the tidal creek. Cyclopoida were prominent in the diet of size class 1 in February, especially in the saltmarsh edge, and in March with comparable values between the two sampling positions. Lastly, note the non-negligible importance of Polychaetes in the diet in saltmarsh edge, in March and April (tab. 18).

In *S. aurata* size class 2 individuals, collected in April and first day of May (tab. 18), iper-benthic prey such as Calanoida and Cyclopoida almost disappeared from the diet (tab. 19); Cyclopoida were important in the diet of size class 2 specimens only in March and only for individuals collected inside the tidal creek. Bigger prey such as Amphipoda, Polychaeta, Mysidacea and Decapoda contributed as well as Harpacticoida to the diet of class 2 specimens. In fact, Harpacticoida also remained important in the diet of *S. aurata* size class 2 (tab. 19). Analyzing differences between sampling position, bigger prey such as Mysidacea and Decapoda were more important in diet of specimens collected in saltmarsh edge (tab. 19). Other prey taxa, Amphipoda, Polychaeta and Harpacticoida in general did not show difference in the diet of *S. aurata* size class 2, having %IRI values comparable between the two sampling positions. In May, class 2 specimens were collected in BA saltmarsh edge and DE tidal creek: in both Amphipoda and Harpacticoida represented the main stomach content of class 2 individuals (tab. 19).

S. aurata size class 3 individuals ate almost exclusively prey items larger than Copepoda (tab. 20): Amphipoda, Polychaeta, Mysidacea and Decapoda. Unfortunately, collected specimens of size class 3 were too few to compare diet between sampling position or stations.

Table 18 – Index of Relative Importance (%IRI) calculated for the size class 1 S. aurata *for the selected sampling campaigns. (s.e. = saltmarsh edge; t.c.= tidal creek)*

date	**19/02**	**19/02**	**21/03**	**21/03**	**07/04**	**07/04**
station	**DE**	**DE**	**DE**	**DE**	**DE**	**DE**
position	**s.e.**	**t.c.**	**s.e.**	**t.c.**	**s.e.**	**t.c.**
size class	**CL 1**	**CL 1**	**CL 1**	**CL 1**	**CL 1**	**CL 1**
n° of *S. aurata*	**8**	**22**	**30**	**32**	**4**	**3**
Harpacticoida	80.8	73.3	62.4	73.8	65.9	47.4
Calanoida	2.8	25.3	0.0	0.0	0.0	0.0
Cyclopoida	14.4	1.3	16.7	23.4	7.6	8.3
Amphipoda	1.9	0.0	0.3	0.0	8.7	44.3
Polychaeta	0.0	0.0	20.6	2.8	17.8	0.0
Mysidacea	0.0	0.0	0.0	0.0	0.0	0.0
Decapoda	0.0	0.1	0.0	0.0	0.0	0.0

Table 19 - Index of Relative Importance (%IRI) calculated for the size class 2 S. aurata *for the selected sampling campaigns. (s.e. = saltmarsh edge; t.c.= tidal creek).*

date	**07/04**	**07/04**	**15/04**	**15/04**	**04/05**	**04/05**
station	**DE**	**DE**	**DE**	**DE**	**DE**	**BA**
position	**s.e.**	**t.c.**	**s.e.**	**t.c.**	**t.c.**	**s.e.**
size class	**CL 2**	**CL 2**	**CL 2**	**CL 2**	**CL 2**	**CL 2**
n° of *S. aurata*	**6**	**3**	**4**	**46**	**24**	**24**
Harpacticoida	69.9	54.2	31.5	87.7	43.0	27.7
Calanoida	0.0	0.0	0.0	0.0	0.0	0.0
Cyclopoida	0.0	10.4	0.0	0.3	0.0	0.2
Amphipoda	0.6	0.0	10.8	11.0	49.1	72.0
Polychaeta	17.0	30.4	0.0	0.7	7.5	0.0
Mysidacea	7.2	0.0	27.3	0.0	0.0	0.0
Decapoda	5.2	5.0	30.4	0.4	0.4	0.0

Table 20 - Index of Relative Importance (%IRI) calculated for the size class 3 S. aurata *for the selected sampling campaigns. (s.e. = saltmarsh edge; t.c.= tidal creek)*

date	**04/05**	**04/05**	**26/05**	**21/06**
station	**DE**	**BA**	**DE**	**DE**
position	**t.c.**	**s.e.**	**s.e.**	**t.c.**
size class	**CL 3**	**CL 3**	**CL 3**	**CL 3**
n° of *S. aurata*	**2**	**6**	**2**	**1**
Harpacticoida	0.0	0.7	0.0	0.0
Calanoida	0.0	0.0	0.0	0.0
Cyclopoida	0.0	0.0	0.0	0.0
Amphipoda	0.0	96.0	100.0	25.5
Polychaeta	100.0	0.0	0.0	55.1
Mysidacea	0.0	0.0	0.0	19.4
Decapoda	0.0	3.3	0.0	0.0

A non-multimetric multidimensional scaling analysis was performed to highlight differences in diet between sampling positions and size classes (fig. 42). Results show that diet changed strongly between the three size classes (ADONIS, $p<0.01$) indicating an effect related to sampling period and size ontogenetic changes, while no differences were observed between sampling positions (ADONIS, $p>0.05$). Size class 1 individuals were related to small prey (Cyclopoida, Calanoida, and Harpacticoida). Bigger individuals changed their diet toward large prey, as Mysidacea, Polychaeta and Decapoda, while size class 2 individuals were found to have a more intermediate size prey items (large Harpacticoida and Amphipoda).

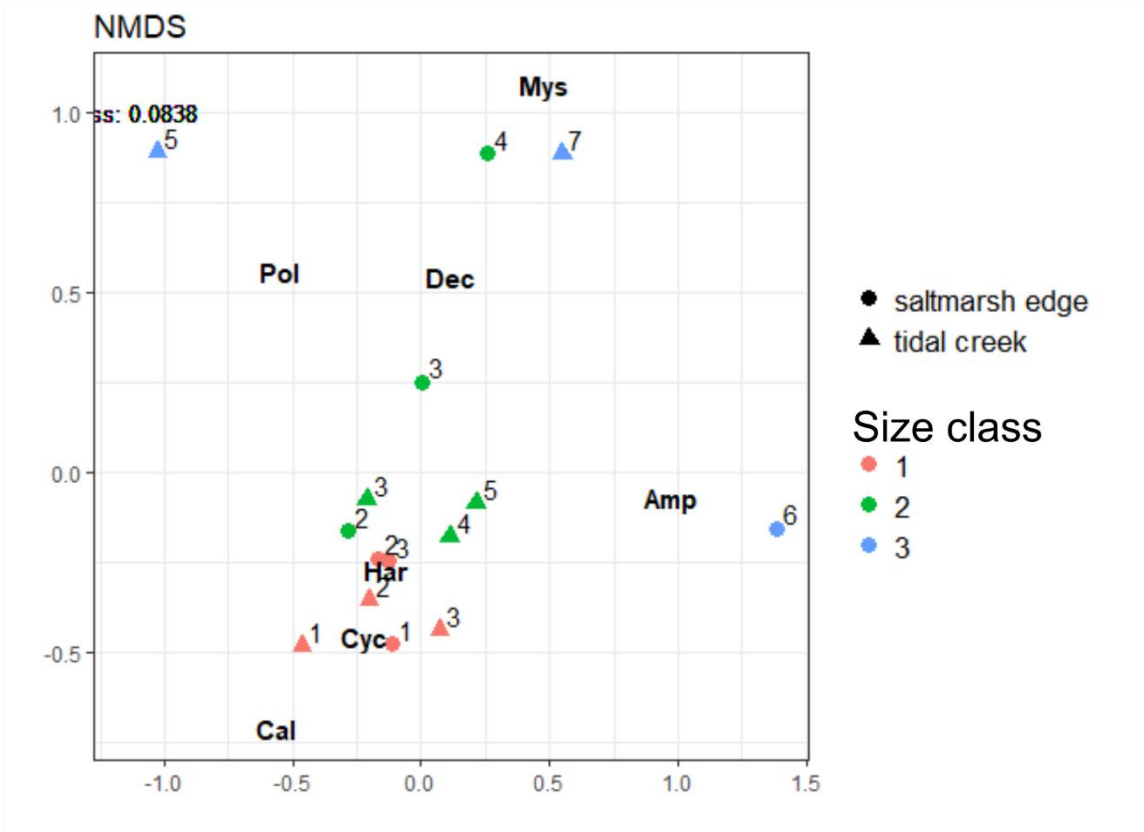


Figure 42 - Non-Multimeric Dimensional Scaling performed on diet data (%IRI) of S. aurata *individuals collected in Dese stations. Label of points represent the different sampling campaign. Cal = Calanoida, Cyc = Cyclopoida, Har = Harpacticoida, Amp = Amphipoda, Dec = Decapoda, Pol = Polychaeta, Mys = Mysidacea. Ss = stress factor.*

A graphic method (from Amundsen et al. 1996, modified) was used to observe the feeding and foraging strategy of *S. aurata* in the different sampling positions (fig. 43 to 47). The graph in Figure 36 can help to interpretate the results. Both number and biovolume of ingested prey were used to evaluate the different contribution and importance of prey in the diets. Again, to observe any differences in feeding strategy of *S. aurata* juveniles between saltmarsh edge and intertidal creek, only 4 sampling dates were considered, those in which individuals were collected in both positions.

Results of feeding strategy analysis suggest that diet of *S. aurata* size class 1, especially in number of prey rather than biovolume, was dominated by Harpacticoida, located in the upper right part of the graphs, during all sampling data and in both tidal creek and saltmarsh edge (fig. 43, 44, 45). Using this approach is however difficult to evaluate if Harpacticoida dominated the feeding strategy and the diet of *S. aurata* size class 1 because all individuals were specialized toward that prey, or because they were the most abundant in the environment. Differences between sampling position in foraging strategy for *S. aurata* size class 1 were observed considering Calanoida, during first sampling date (fig. 43) and Amphipoda and Polychaeta during second and third sampling campaigns (fig. 44, 45). In February (fig. 43), the diet of individuals collected in saltmarsh edge, considering both the number of prey and biovolume, was dominated by Harpacticoida but some individuals had preyed almost exclusively on Calanoida. In March (fig. 44) and the beginning of April

(fig. 45), the feeding strategy of some class 1 individuals was characterized, especially in biovolume, by large prey, Amphipoda and Polychaeta. In March, a low number of individuals were feeding on Amphipoda in saltmarsh edge and Polychaeta in both sampling positions (fig. 44). In April (fig. 45), again, a low number of class 1 individuals fed in a specialized way Amphipoda and Polychaeta in saltmarsh edge and Amphipoda in intertidal creek.

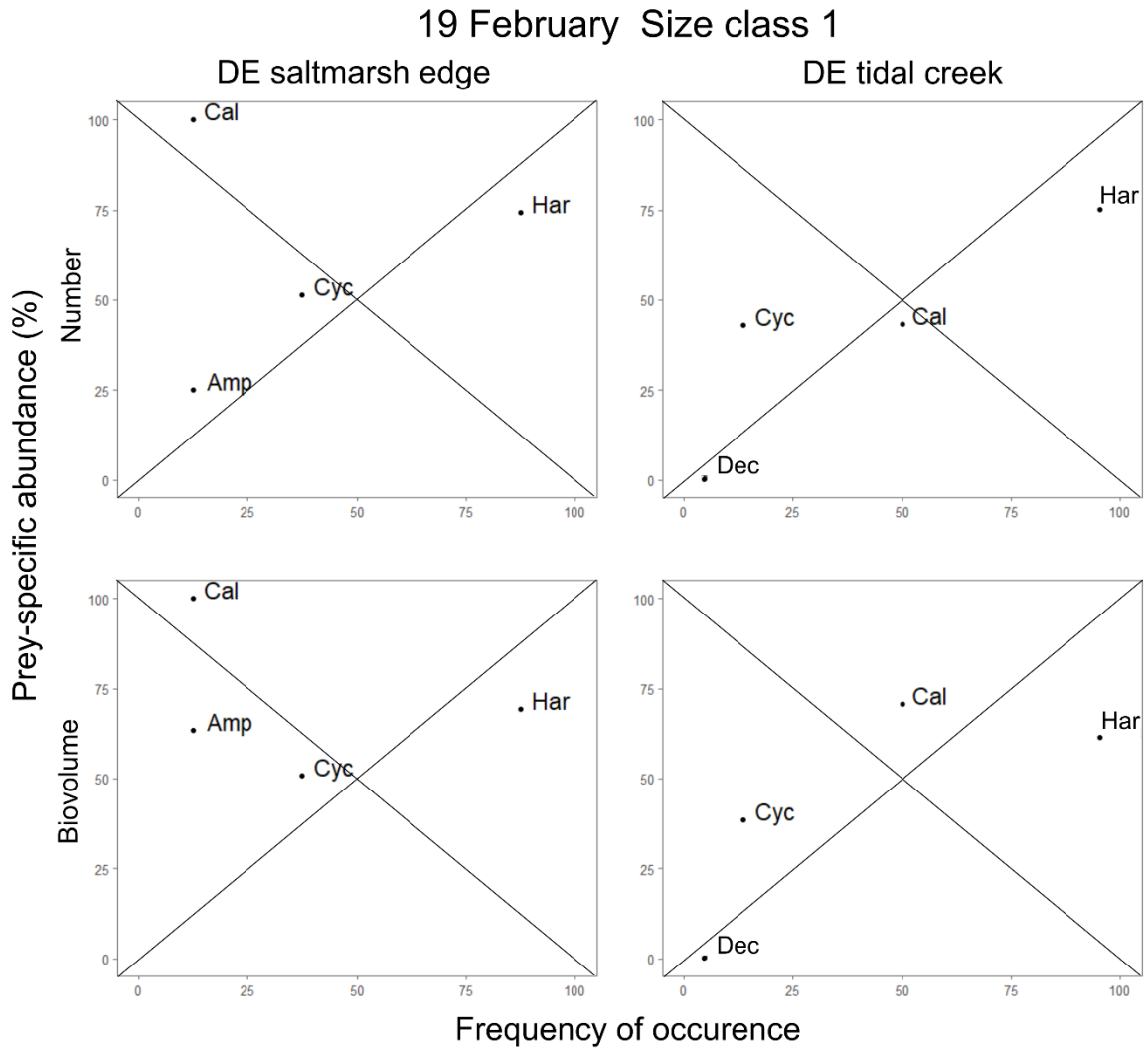


Figure 43 – Modified Amundsen graphical method for interpretation of feeding strategy of size class 1 S. aurata *during the first sampling campaign. N = Amundsen calculated on prey number. V = Amundsen calculated on prey biovolume. (Cal = Calanoida, Cyc = Cyclopoida, Har = Harpacticoida, Amp = Amphipoda, Dec = Decapoda, Pol = Polychaeta).*

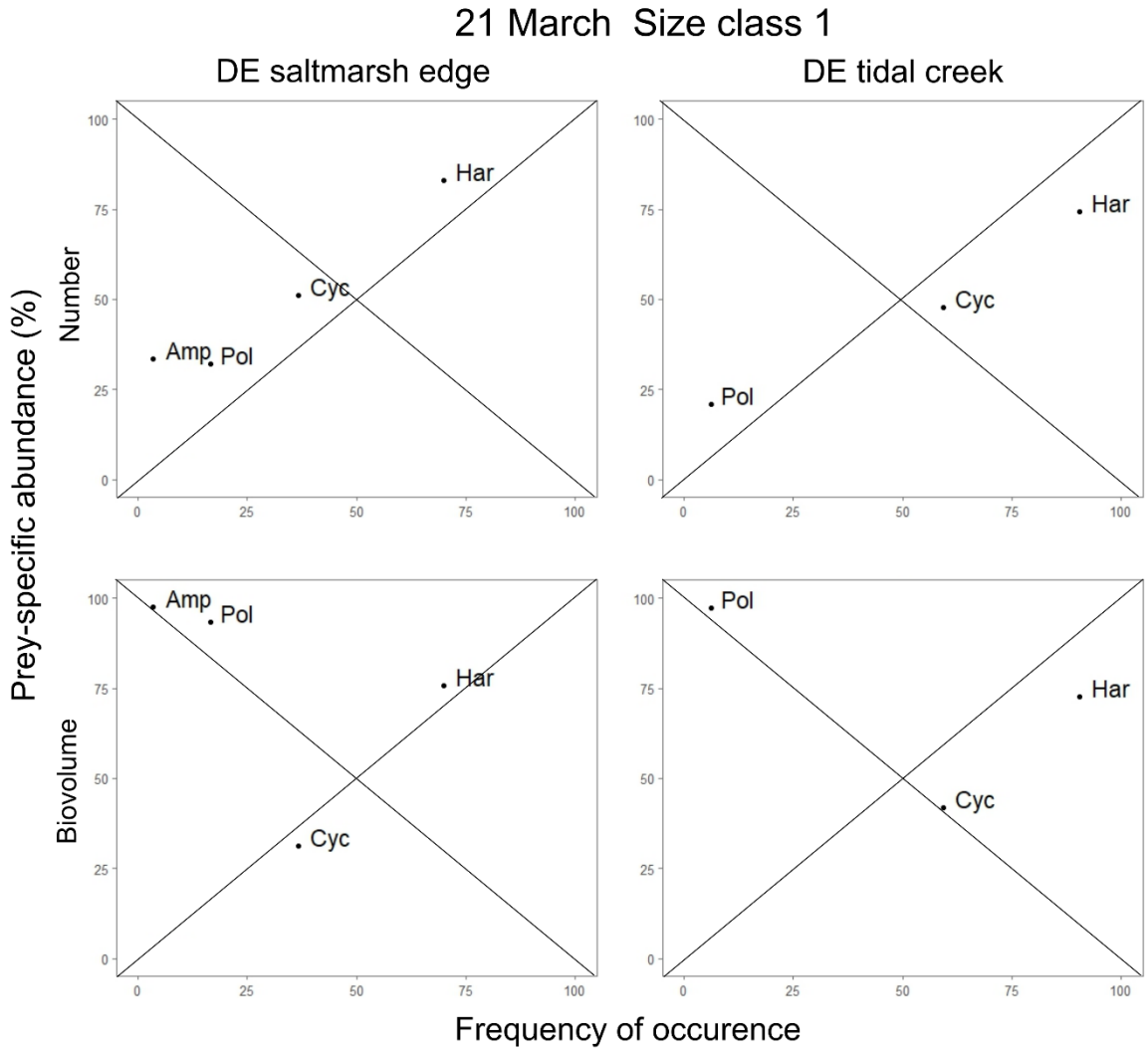


Figure 44 - Modified Amundsen graphical method for interpretation of feeding strategy of size class 1 S. aurata *during the second sampling campaign. N = Amundsen calculated on prey number. V = Amundsen calculated on prey biovolume. (Cal = Calanoida, Cyc = Cyclopoida, Har = Harpacticoida, Amp = Amphipoda, Dec = Decapoda, Pol = Polychaeta).*

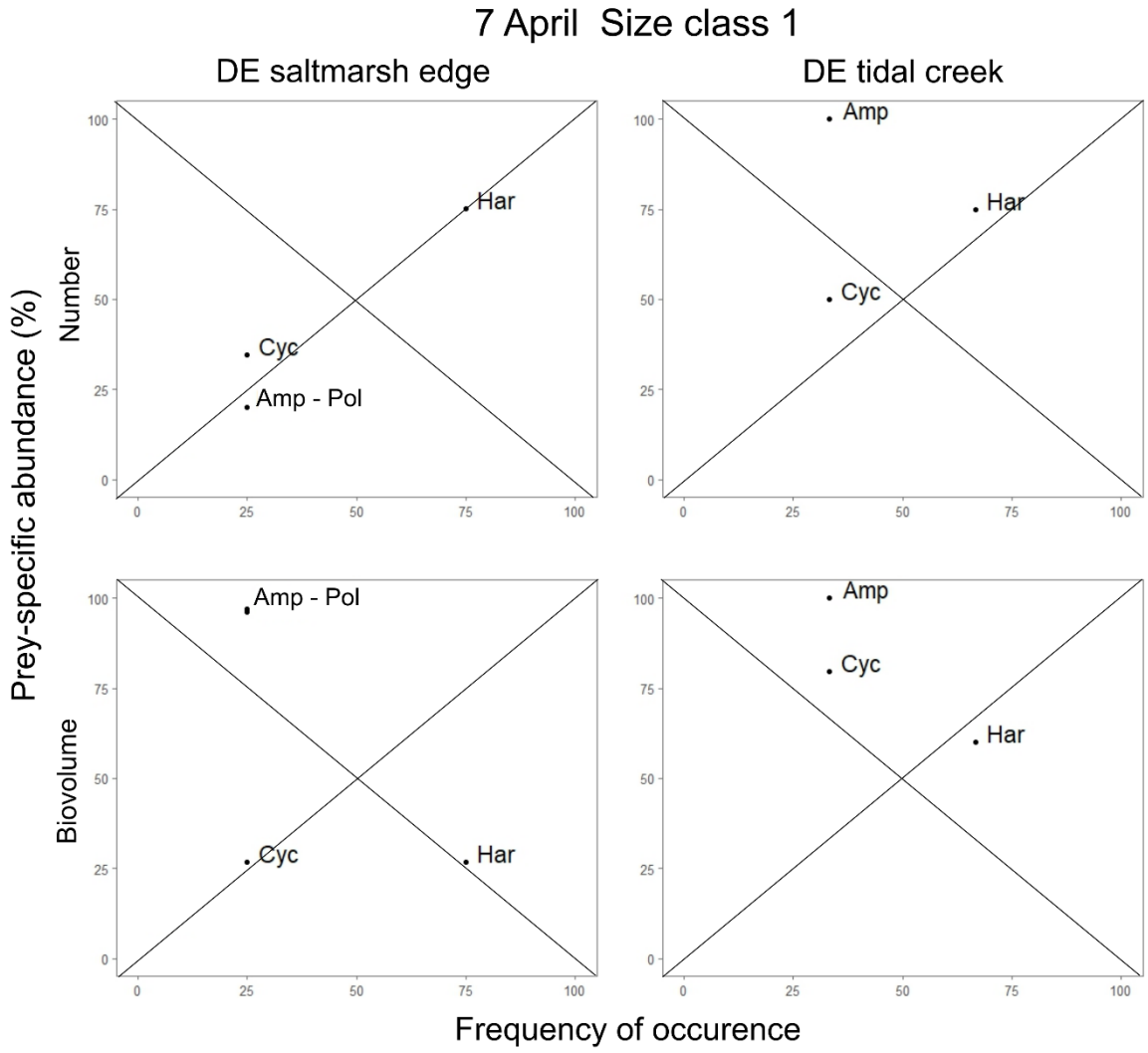


Figure 45 - Modified Amundsen graphical method for interpretation of feeding strategy of size class 1 S. aurata *during the third sampling campaign. N = Amundsen calculated on prey number. V = Amundsen calculated on prey biovolume. (Cal = Calanoida, Cyc = Cyclopoida, Har = Harpacticoida, Amp = Amphipoda, Dec = Decapoda, Pol = Polychaeta).*

Differences in foraging strategy between sampling positions (tidal creek and saltmarsh edge) were also observed among class 2 individuals (fig. 46, 47), especially during the second sampling campaign of April. Moreover, for size class 2 individuals, foraging strategy, calculated by considering number of prey, was different from the one calculated considering biovolumes of ingested preys. These differences in results of feeding strategy of size class 2 individuals is probabably related to the large difference in biovolume of the main considered taxa (e.g. Harpacticoida vs. Mysidacea and Polychaeta).

During the first sampling campaign of April (fig. 46), considering the number of ingested prey, the feeding strategy were dominated by Harpacticoida in both sampling positions, indicating no differences. The dominance of Harpacticoida in the feeding strategy also occurred considering the number and biovolume of ingested prey of *S. aurata* individuals collected in the tidal creek during the second campaign (fig. 47). Among *S. aurata* size class 2 collected in saltmarsh edge a small portion of individuals fed almost exclusively on

Mysidacea or Decapoda during both sampling campaigns (fig. 46, 47). In first sampling date, the feeding strategy of class 2 *S. aurata* individuals collected in tidal creek was dominated by Harpacticoida. However, some individuals had preyed almost exclusively Polychaeta (fig. 46).

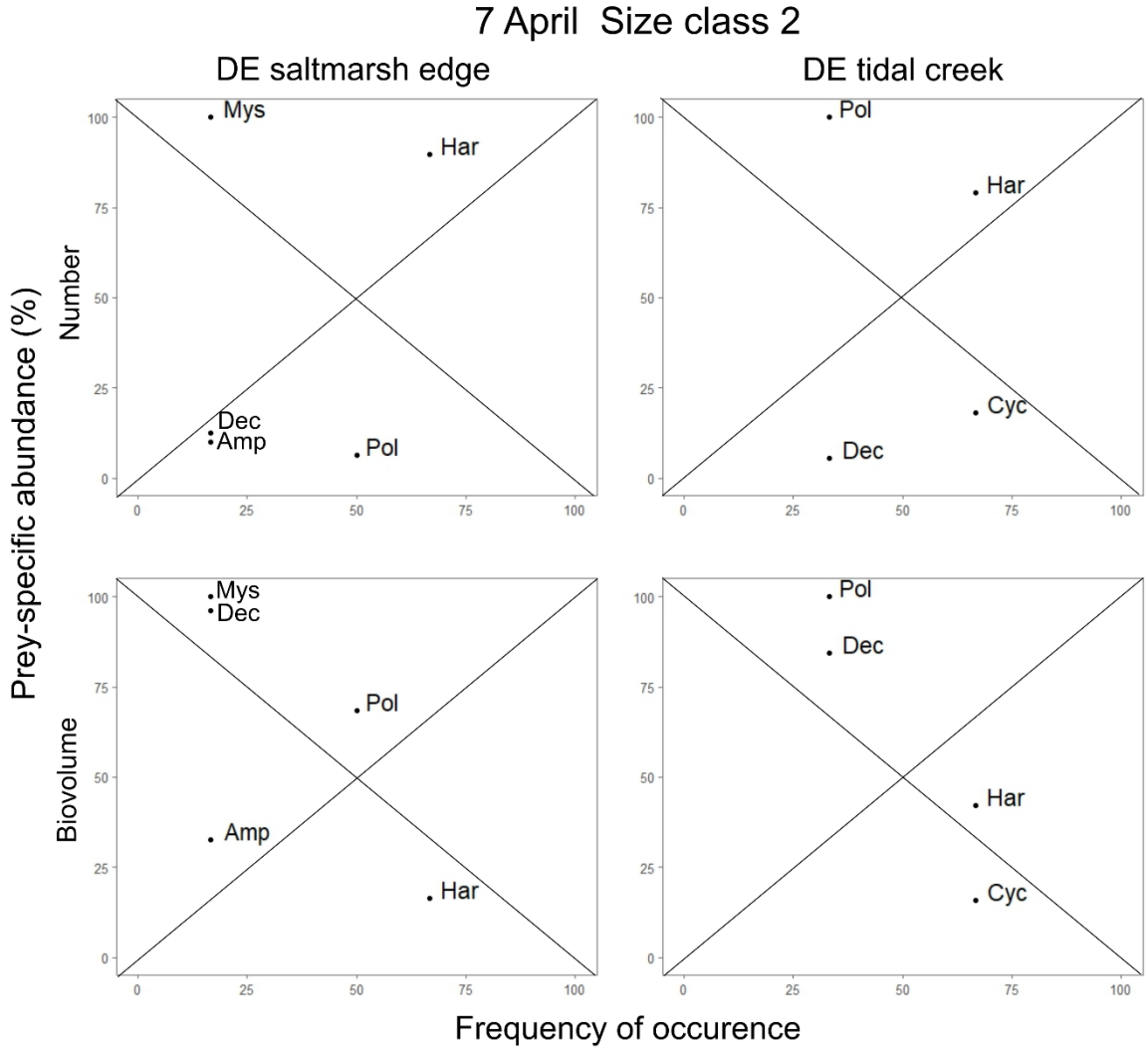


Figure 46 - Modified Amundsen graphical method for interpretation of feeding strategy of size class 2 S. aurata *during the fourth sampling campaign. N = Amundsen calculated on prey number. V = Amundsen calculated on prey biovolume. (Cal = Calanoida, Cyc = Cyclopoida, Har = Harpacticoida, Amp = Amphipoda, Dec = Decapoda, Pol = Polychaeta).*

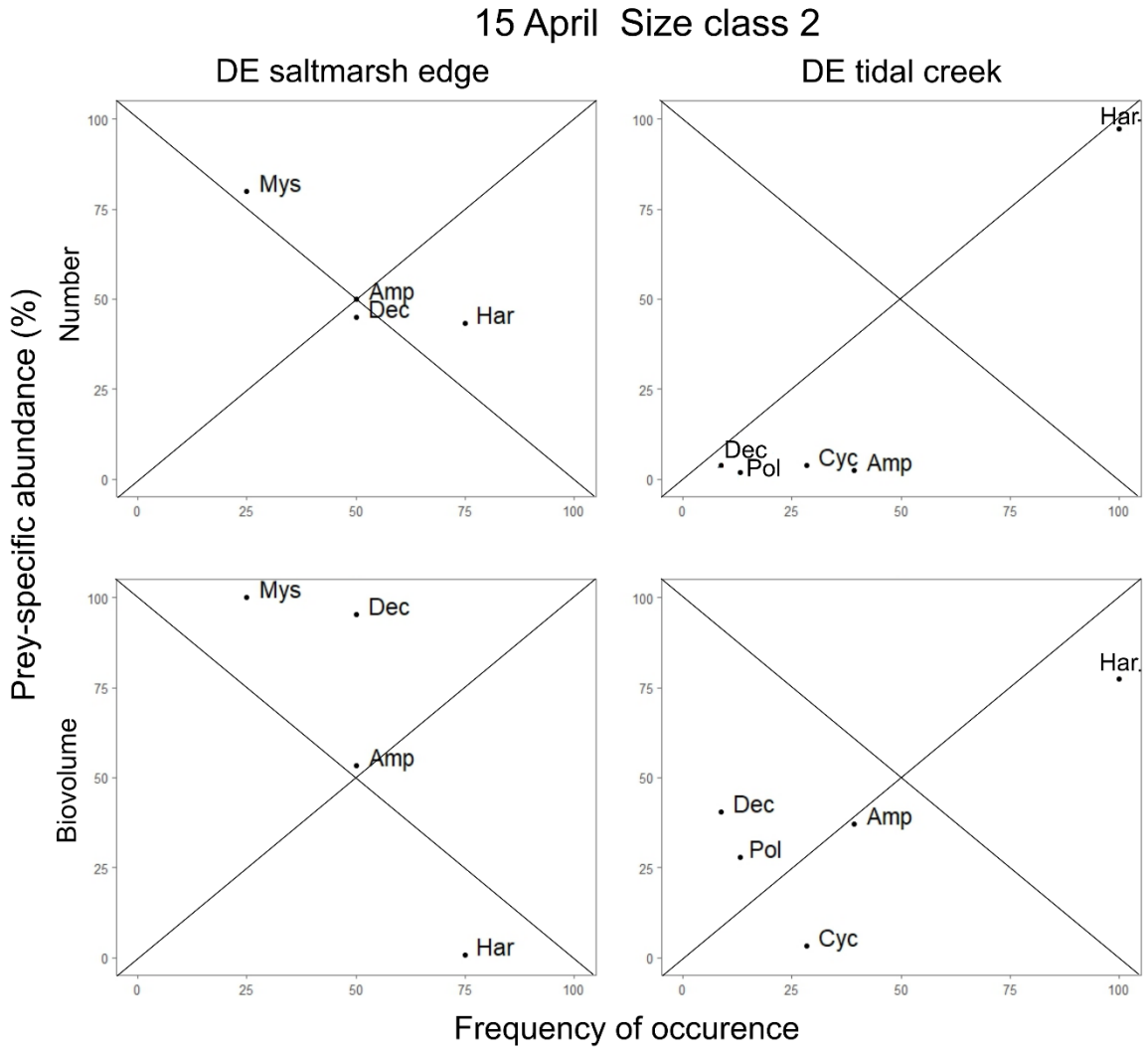


Figure 47 - Modified Amundsen graphical method for interpretation of feeding strategy of size class 2 S. aurata *during the fifth sampling campaign. N = Amundsen calculated on prey number. V = Amundsen calculated on prey biovolume. (Cal = Calanoida, Cyc = Cyclopoida, Har = Harpacticoida, Amp = Amphipoda, Dec = Decapoda, Pol = Polychaeta).*

The analysis of planktonic-benthic samples collected immediately after fish sampling in station DE, allowed identification and quantified the presence and abundance of different taxa in the saltmarsh aquatic environment. These samples were collected in order to compare the ingested prey with the prey's availability in the aquatic environment of two sampled positions (saltmarsh edge and tidal creek). Each planktonic or benthic specimen collected was identified, counted and photographed to obtain its biovolume. The two sampling replicates performed in both sampling positions were then cumulated (sum) to show total density and biovolume of different organisms collected (tab. 21, 22).

Results of plankton-benthos samples, both in terms of number of individuals (tab. 21) and biovolume (tab. 22), show that the higher densities of zoobenthic-zooplanktonic organisms were present in May rather than in March, indicating that secondary production exploded and increased greatly during spring months. Higher values in May rather than in March were observed both for number of individuals (tab. 21) and for biovolume

(tab. 22). In some cases, values in May increased almost ten times compared to March. For examples, Harpacticoida in the intertidal creek increased from 456 individuals/m^2 in March to 1654 individuals/m^2 in May (tab. 21), and thus in biovolume from 4676 nL/m^2 to 44610 nL/m^2 (tab. 22). This higher increase in biovolume rather than individuals should be related to a different species composition of Harpacticoida communities during the two sampling dates. Even for larger prey items such as Amphipoda and Polychaeta both the densities and biovolumes increased greatly both in saltmarsh edge and in intertidal creek in May. It is also important to note that for Amphipoda, Polychaeta and Mysidacea, especially in March, low densities of individuals with high biovolumes were present, indicating that individuals of these taxa, even if less numerous, were very large sized. Observing the differences between the two sampling positions, results of possible prey distribution show that Harpacticoida, Cyclopoida and Amphipoda, during the two sampling dates, were more abundant (higher density and biovolume) in tidal creek rather than saltmarsh edge, especially in March, when generally density exceeded from five to ten times the one in saltmarsh edge (tab. 21). Also, in March the density and biovolume of Calanoida were higher in tidal creek rather than saltmarsh edge (tab. 21, 22). Conversely, Mysidacea and Ostracoda were found mostly in saltmarsh edge both considering densities and biovolumes. More complex pattern occurred in Polychaeta, Oligochaeta and Nematoda densities and biovolumes distributions (tab. 21, 22): these taxa in March were more concentrated in saltmarsh edge while in May in tidal creek. Finally, it must be considered that in May no *S. aurata* specimens were collected in DE saltmarsh edge (tab. 17).

Table 21 – Density (n. individuals/m²) of organisms collected with plankton/benthos net for the analysis of possible available preys. (s.e. = saltmarsh edge; t.c.= tidal creek).

Station	DE			
Date	21/03/2016		04/05/2016	
Taxon	s.e.	t.c.	s.e.	t.c.
Harpacticoida	80.71	455.71	1470.71	1654.29
Calanoida	5.00	27.14	49.29	3.57
Cyclopoida	2.86	32.14	27.14	38.57
Amphipoda	3.57	27.14	54.29	170.71
Polychaeta	90.00	37.14	156.43	275.71
Mysidacea	8.57	2.14	12.14	5.00
Decapoda	0.00	0.00	0.00	0.00
Bivalvia	0.00	0.71	0.00	0.00
Oligochaeta	25.71	14.29	51.43	164.29
Ostracoda	32.14	0.00	170.71	12.14
Nematoda	15.71	14.29	67.14	84.29

Table 22 – Biovolume (nL/m²) of organisms collected with plankton/benthos net for the analysis of possible available preys. (s.e. = saltmarsh edge; t.c.= tidal creek)

Station	DE			
Date	21/03/2016		04/05/2016	
Taxon	s.e.	t.c.	s.e.	t.c.
Harpacticoida	709.32	4676.09	47020.85	44610.39
Calanoida	337.82	1833.85	3329.89	241.30
Cyclopoida	28.01	315.08	291.43	414.14
Amphipoda	14534.44	56182.42	92349.05	427956.41
Polychaeta	45743.99	54867.45	49993.91	100429.21
Mysidacea	35085.51	8771.38	49704.47	20466.55
Decapoda	0.00	0.00	0.00	0.00
Bivalvia	0.00	2371.24	0.00	0.00
Oligochaeta	3323.15	1846.19	9382.05	29970.45
Ostracoda	5306.76	0.00	28184.80	2004.78
Nematoda	115.04	104.58	491.52	617.02

To assess the dietary preferences of *S. aurata* juveniles, in terms of relationships between ingested prey and the availability of potential prey in the environment, two selection indices (Strauss' index, tab. 23, and Vanderploeg and Scavia's index, tab. 24) were calculated for each size class and sampling position. Biovolumes were used to make the results comparable and to avoid the underestimation of the prey importance in the predator diet deriving from the use of the number of prey individuals (see also "Material and Methods"). The indices could only be calculated for two dates, March and the beginning of May. Generally, *S. aurata* size class 1 were found in March while size class 2 in May and only in tidal creek. Results of Strauss' index (L) (tab. 23), which only confront the abundance of organism in diet and environment, and those of Vanderploeg and Scavia's index (E*) (tab. 24), which consider the different available prey abundance and thus the electivity of *S. aurata* individuals, were sometimes different.

Generally, using L index considering biovolume of ingested prey (tab. 23), it seems that smaller *S. aurata* (size class 1), among all the different possible prey, had a small positive selection towards Harpacticoida and Cyclopoida especially in tidal creek (Harpacticoida L = 0.26; Cyclopoida L = 0.17). Contrarily, individuals collected in saltmarsh edge showed a positive selection only towards Polychaeta (L = 0.45) (tab. 23). This selection behavior toward Harpacticoida, which were weakly selected only in tidal creek, it was probably due to their low biovolume in saltmarsh edge in March (tab. 22). In general, class 1 individuals did not show a preference for Amphipoda and Mysidacea but in some cases they avoided them (tab. 23). Considering *S. aurata* size class 2, Harpacticoida were preferred especially by those individuals collected in March in saltmarsh edge (L= 0.23), coupled with Polychaeta (L = 0.32) (tab. 23). Size class 2 individuals collected in May, using L index, did not show any selectivity in tidal creek. Finally, size class 3 individuals, among the different possible prey present in environment in tidal creek in May, selected Polychaeta (L = 0.84).

Table 23 – Strauss' linear selection index (L) calculated for each size class and position during the two sampling dates. (s.e. = saltmarsh edge, t.c.= tidal creek). In bold the values above +0,10.

Station	DE				
Date	21/03/2016			04/05/2016	
Position	t.c.	s.e.	s.e.	t.c.	t.c.
size class	CL 1	CL 1	CL 2	CL 2	CL 3
n° of *S. aurata*	32	30	3	24	2
Harpacticoida	**0.26**	0.04	**0.23**	0.01	-0.07
Calanoida	-0.01	0.00	0.00	0.00	0.00
Cyclopoida	**0.17**	0.02	0.01	0.00	0.00
Amphipoda	-0.43	-0.08	-0.14	-0.05	-0.68
Polychaeta	**0.11**	**0.45**	**0.32**	0.09	**0.84**
Mysidacea	-0.07	-0.33	-0.33	-0.03	-0.03
Decapoda	0.00	0.00	0.00	0.04	0.00
Bivalvia	-0.02	0.00	0.00	0.00	0.00
Oligochaeta	-0.01	-0.03	-0.03	-0.05	-0.05
Ostracoda	0.00	-0.05	-0.05	0.00	0.00
Nematoda	0.00	0.00	0.00	0.00	0.00

Table 24 – Vanderploeg and Scavia's relativized selectivity index (E) calculated for each size class and position during the two sampling dates (s.e. = saltmarsh edge, t.c.= tidal creek). In bold the values above +0,10.*

Station	DE				
Date	21/03/2016			04/05/2016	
Position	t.c.	s.e.	s.e.	t.c.	t.c.
size class	CL 1	CL 1	CL 2	CL 2	CL 3
n° of *S. aurata*	32	30	3	24	2
Harpacticoida	0.06	0.01	**0.74**	**0.56**	-1.00
Calanoida	-1.00	-1.00	-1.00	-1.00	-1.00
Cyclopoida	**0.81**	**0.81**	**0.61**	-1.00	-1.00
Amphipoda	-1.00	-0.87	-1.00	**0.46**	-1.00
Polychaeta	**-0.71**	**-0.50**	**-0.51**	**0.64**	**0.83**
Mysidacea	-1.00	-1.00	-1.00	-0.82	-1.00
Decapoda	-1.00	-1.00	-1.00	-1.00	-1.00
Bivalvia	-1.00	-1.00	-1.00	-1.00	-1.00
Oligochaeta	-1.00	-1.00	-1.00	-1.00	-1.00
Ostracoda	-1.00	-1.00	-1.00	-1.00	-1.00
Nematoda	-1.00	-1.00	-1.00	-1.00	-1.00

Relativized Selectivity Index (Vanderploeg and Scavia's index, E*) was also calculated. Considering Vanderploeg and Scavia's index (E*) for *S. aurata* class 1 in March (tab. 24), Cyclopoida was the only prey taxon positively selected (tab. 24). In particular, for size class 1, the same values of preference towards Cyclopoida (E* = 0.81) were observed in both saltmarsh edge and tidal creek (tab. 24), indicating that this prey was strongly selected by class 1 individuals in both positions, despite the availability of these prey in the

two saltmarsh positions was different (tab. 22). Then, using E* index, for size class 1 individuals, Polychaeta were strongly avoided both in saltmarsh edge (E* = -0.50) and tidal creek (E* = -0.71) (tab. 24), contrarily to what was observed with Strauss' linear selection index (L, tab. 23). Size class 2 individuals in March they selected Harpacticoida and Cyclopoida, while avoiding Polychaeta (tab. 24); in May, they selected Polychaeta, Harpacticoida and Amphipoda, while avoiding Mysidacea (tab. 24). The electivity toward Cyclopoida remained high in size class 2 individuals collected in March while disappear in the ones collected in May (tab. 24), even if this prey had increased its environmental biovolume in May (tab. 22). In general, these results indicate that probably, while they grow up, juvenile *S. aurata* stop looking for Cyclopoida and select Harpacticoida and large prey as Amphipoda and Polychaeta. Finally, also using E* index, *S. aurata* size class 3 individuals had selected Polychaeta and avoided the other prey.

3.3.1.1 Differences in ontogenetic shift of diet

Since there were no strong differences in the diet composition of *S. aurata* individuals between the two sampling positions, analyzing the ontogenetic shift of diet, samples collected in the two positions and in the two stations were combined.

Results showed that the mean length value of individuals had a small standard error, indicating that individuals, within each campaign, were very similar to each other (fig. 48). The differences in the average length of individuals between one campaign and the next were not significant except between the fourth and fifth sampling campaign (fig. 48). In fact, *S. aurata* individuals collected, even if increased in standard length since their entrance in lagoon in February, showed significant growth in length only between April and May (fig. 48).

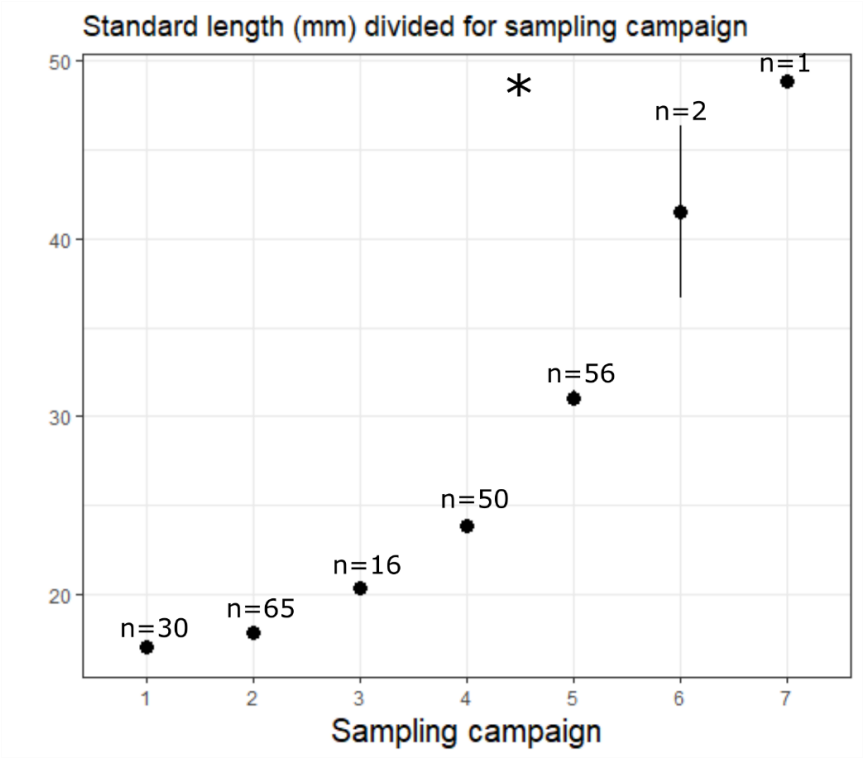


Figure 48 - Standard length (in mm, mean ± St. Err.) of the S. aurata *collected in all sampling stations during sampling campaigns. Significant differences (ANOVA p<0.05) between sampling campaigns are reported with asterisk in the graph. n = number of* S. aurata *collected during each sampling campaign. Significant differences (p<0.05).*

To analyze the ontogenetic shift of diet, individuals were divided in size classes with a dimensional range smaller than the one previously used: A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30mm; D = 30≤SL<35; E = 35≤SL<50mm. Because of this, within each dimensional class there was a low variability and all the individuals within each size class were very similar to each other (fig. 49). Only the E size class had a higher variability because characterized by a longer range of lengths (15 mm instead of 5 mm of the other dimensional classes considered). The most abundant size class (99 individuals) were the one represented by the smaller individuals (size class A, 15mm<SL<20mm). Gradually the other size classes were represented by less individuals (fig. 49).

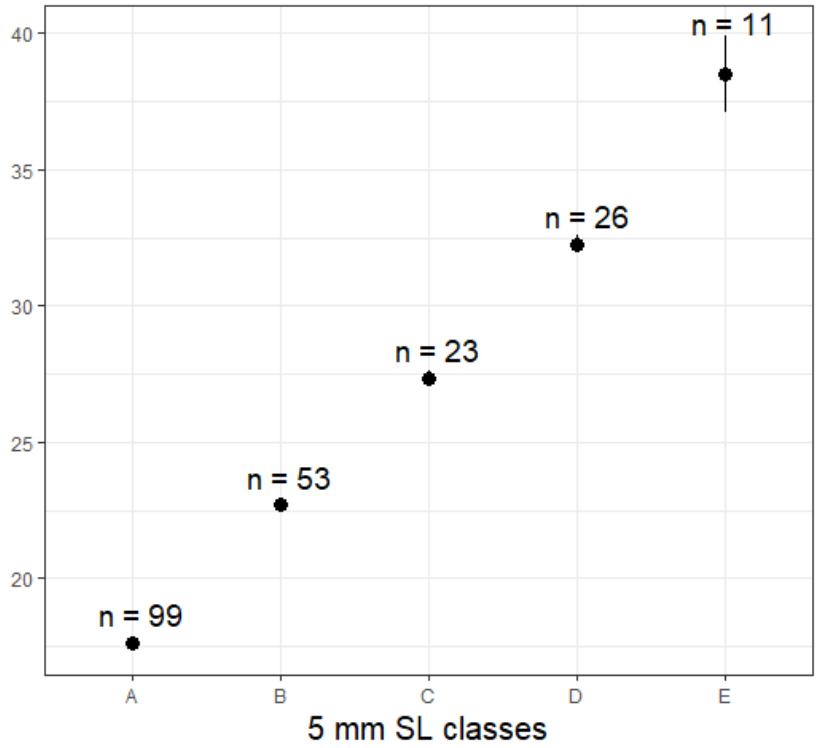


Figure 49 - Standard length (in mm, mean ± St. Err.) of the S. aurata *collected in all sampling stations and divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). n = number of* S. aurata *collected for each size class.*

The mean total biovolume (nL) of ingested prey (fig. 50) increased with the size of individuals, indicating that the stomachs of big individuals contained a large biovolume of prey. At the same time, as well as the size, there was also an increase in total ingested biovolume. Statistical differences in ingested biovolume were found between the smaller size class: between size class A and B and between size class B and C (fig. 50).

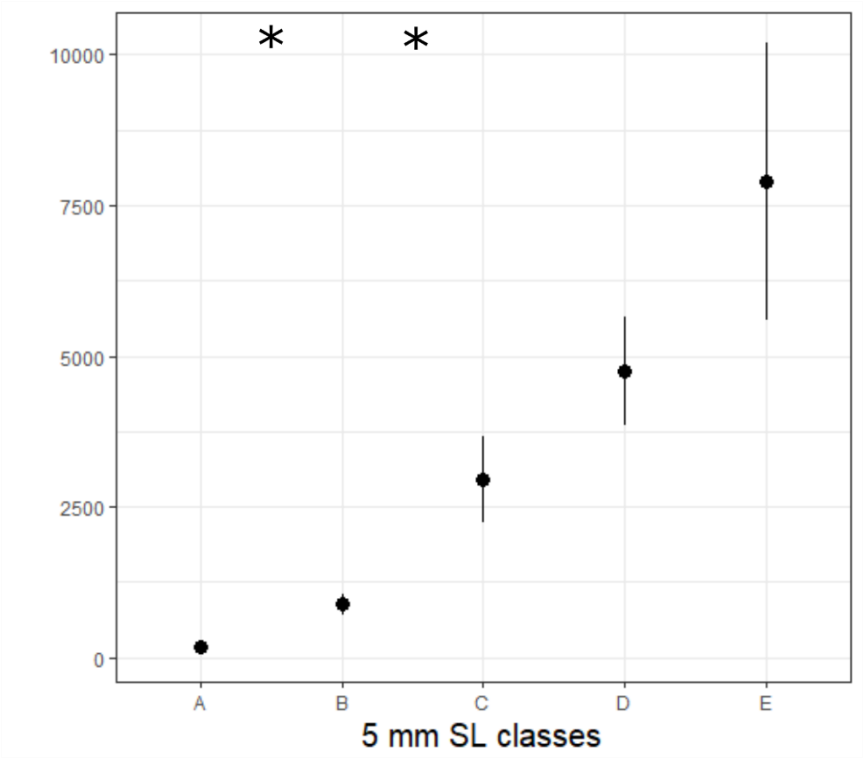


Figure 50 – Total biovolume (nL) of ingested prey (mean ± St. Err.) of the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

Total numbers of ingested prey were also tested to observe the difference between size classes and to compare the number of prey with ingested biovolume (fig. 51). Smaller *S. aurata* (size class A) ate on average less than 10 prey per individual. Individuals belonging to size class B ate the largest number of prey: on average more than 45 prey per stomach. The number of ingested prey decreased with the further increase in the size of individuals (fig. 51). Statistical differences therefore were observed between size class A and B, when ingested prey increased, between size class C and D instead the ingested prey decreased drastically (fig. 51). Observing the mean numbers of ingested prey (fig. 51) and confronting them with mean ingested biovolumes (fig. 50) it was possible to speculate on the size of ingested prey. Results show that small *S. aurata* (size class A) ate a lower number of small prey. Size class B individuals ate a great number of prey, but each prey had a small biovolume. Large *S. aurata* individuals (size class D and E) ate few prey, but each prey was characterized by a large biovolume. The *S. aurata* individuals belonging to size class C had halfway characteristics: they ate a large number of medium-sized prey (fig. 50, 51).

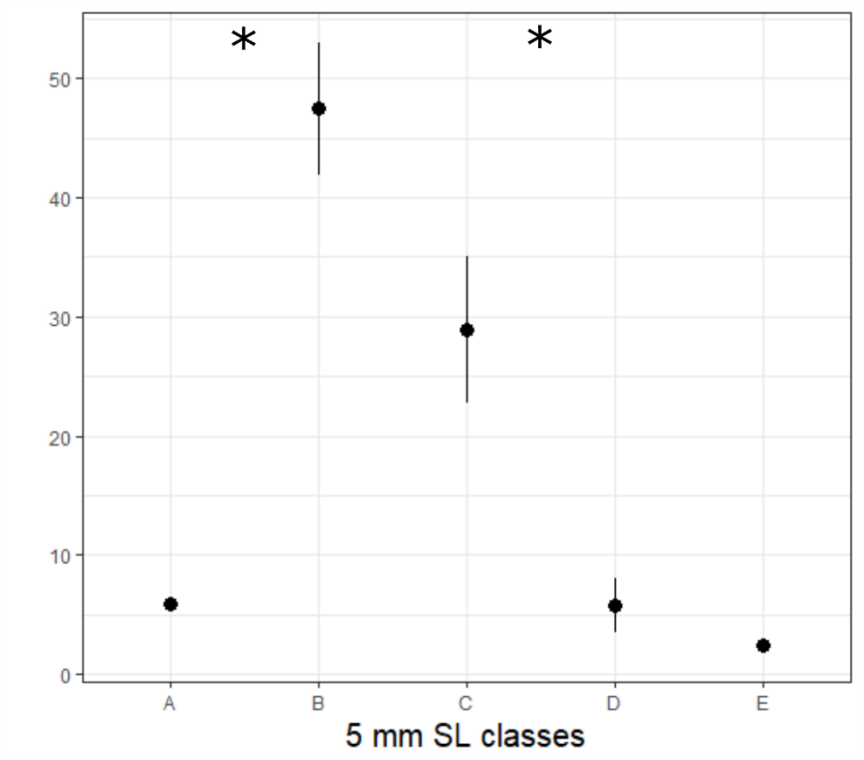


Figure 51 - Total number of ingested prey (mean ± St. Err.) of the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

The total biovolume ingested by each *S. aurata* were then divided for the number of ingested prey in order to study the mean prey-size (as individual biovolume) for each size class (fig. 52). Statistical differences were observed between size class A and B and between size class B and C, indicating that mean prey-size increased rapidly during the first entrance in saltmarsh habitats. Mean prey-size and its variability in general increased with the *S. aurata* length, from size A to size E (fig. 52): size class A individuals ate prey having on average a small size while the largest prey-size were eaten by *S. aurata* belonging to size class E (fig. 52). The highest variability observed in size classes C, D and E probably correspond to the period in which individuals choose to eat many small prey or few large prey.

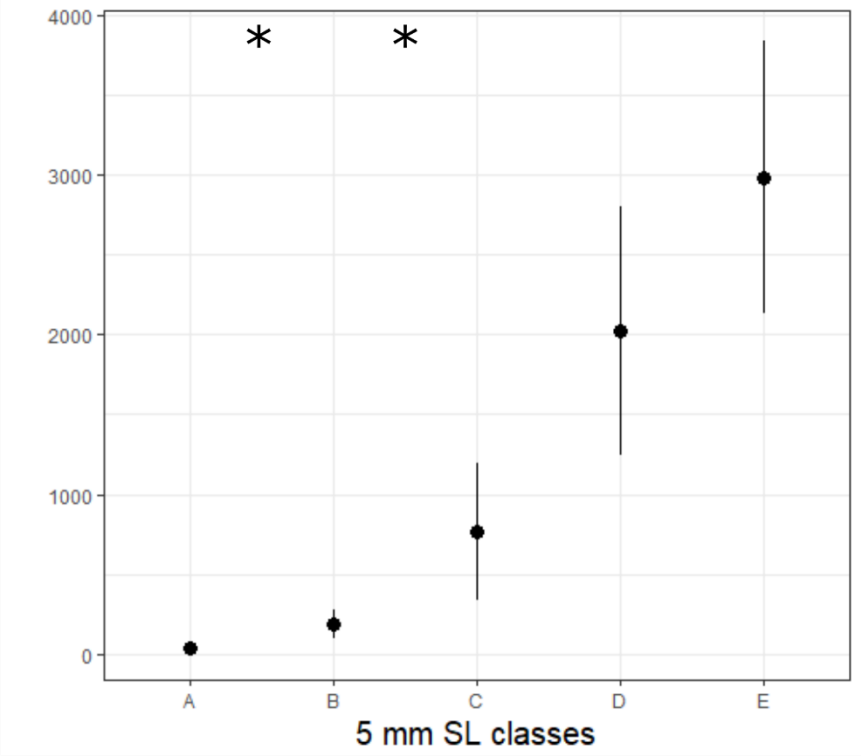


Figure 52 – Mean prey-size (in nL, mean ± St. Err.) for the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

The number and the biovolume of the most represented prey taxa (Harpacticoida and Amphipoda) in the stomach were statistically analyzed. Results concerning Harpacticoida (fig. 53, 54), the most abundant prey taxon, were similar to those of the total stomach content (fig. 51, 52). The mean number of ingested Harpacticoids increased significantly from size class A to B and then decreased (fig. 53, left). At the same time, the mean volume of ingested Harpacticoida increased up to size class C (fig. 53, right). Significant differences were observed between the same size classes both for number of Harpacticoida and their biovolume (fig. 53). These results indicate that probably the small *S. aurata* ate few and small Harpacticoida. Then, size class C individuals did start eating less Harpacticoida but with greater mean unit biovolume (fig. 54).

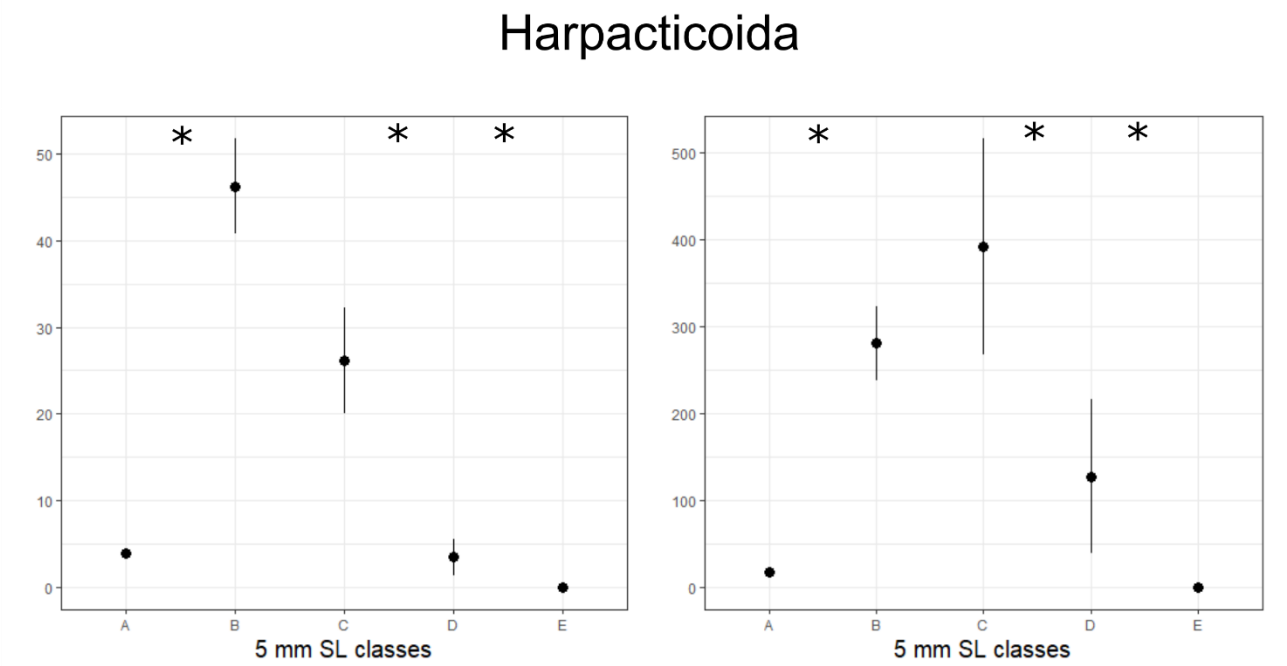


Figure 53 - Total number (left) and total volume (in nL, right) of ingested Harpacticoida (mean ± St. Err.) of the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

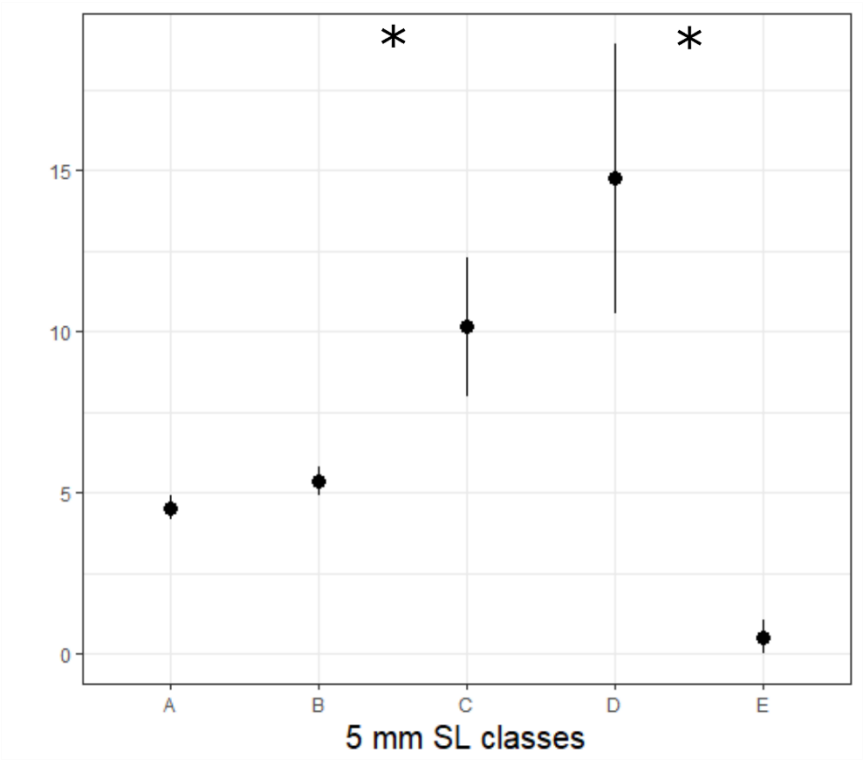


Figure 54 - Mean-size of ingested Harpacticoida-size (in nL, mean ± St. Err.) for the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

Considering the ingested Amphipoda (fig. 55, 56) results were different from what was observed with Harpacticoida probably because Amphipoda are larger prey that small *S. aurata* initially avoid. Statistical

differences were observed between size class A and B and between size class B and C, considering total number of ingested Amphipoda, while only from size class A and B considering biovolumes (fig. 55). In general, Amphipoda start to be eaten plenty when *S. aurata* reach 25mm in Standard Length (fig. 55, left). Even if the number of ingested Amphipoda was high still from *S. aurata* size class C, the total volume of ingested Amphipoda increased from size class D (fig. 55). Size class D individuals preyed on Amphipoda with a small unit biovolume; starting from size class D, *S. aurata* juveniles ate larger sized Amphipoda (fig. 56).

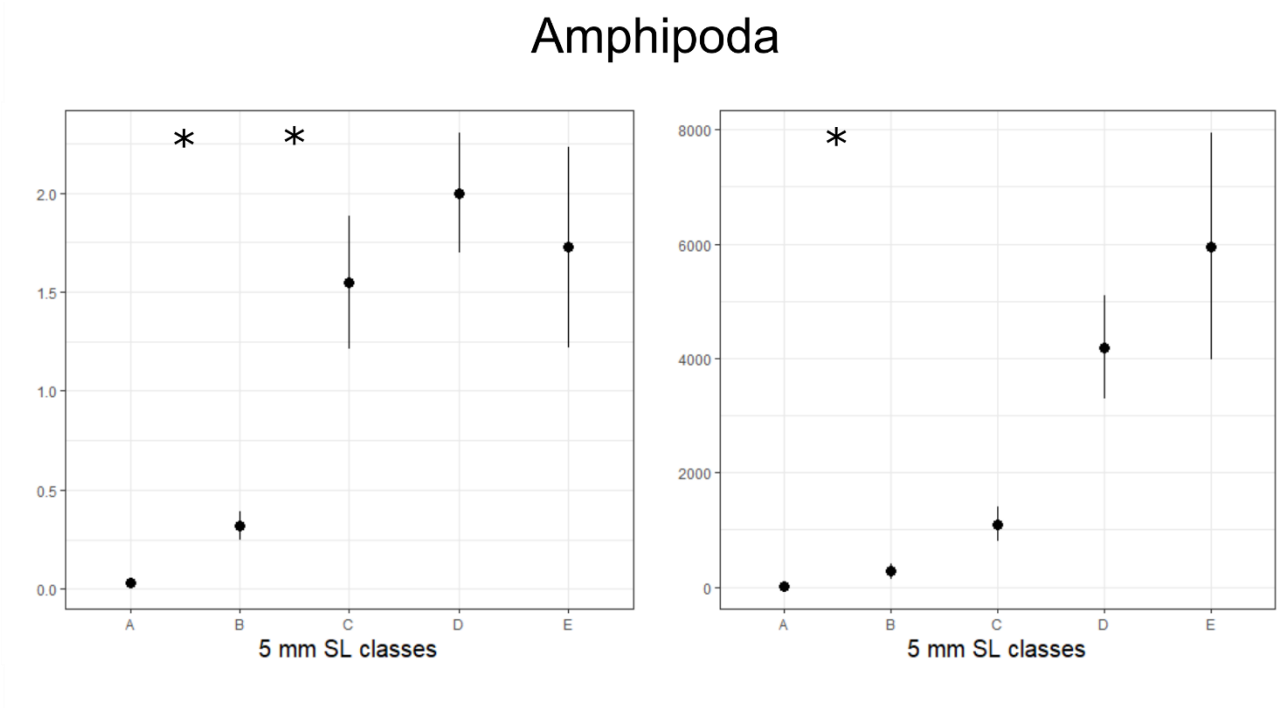


Figure 55 - Total number (left) and total volume (in nL, right) of ingested Amphipoda (mean ± St. Err.) of the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

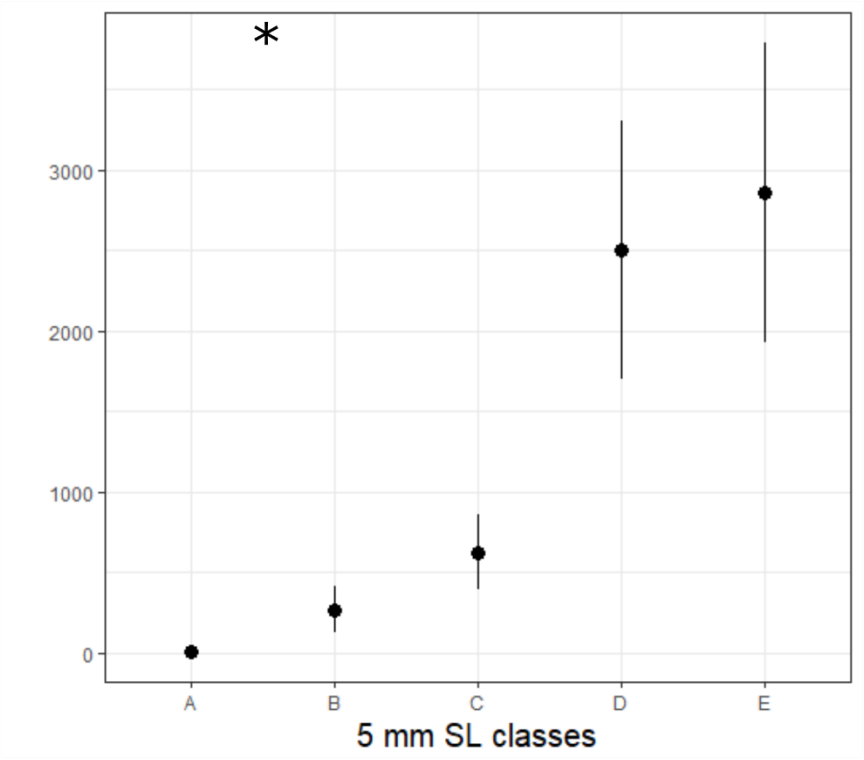


Figure 56 - Mean-size of ingested Amphipoda (in nL, mean ± St. Err.) for the different S. aurata *divided in 5 mm standard length classes. (A = SL<20 mm; B = 20≤SL<25 mm; C = 25≤SL<30 mm; D = 30≤SL<35 mm; E = 35≤SL<50 mm). Significant differences (p<0.05) between size classes are reported with asterisk in the graph.*

Results of %IRI calculated on 5 mm size classes (tab. 25) showed that Harpacticoida were important in the diet of all individuals smaller than 35 mm in standard length. Smallest *S. aurata* (class A) in addition to Harpacticoida ate small preys as Cyclopoida. The diet of juvenile sea breams belonging to class B was focused almost exclusively on Harpacticoida (%IRI = 90,4%), indicating a shift in diet towards only benthic prey. Gradually (size classes C and D) the contribution of Harpacticoida on *S. aurata* diet decreased and at the same time increased the presence of Decapoda, Amphipoda and Polychaeta in the stomach contents (tab. 25). The diet of class E individuals was completely different from that of the sea breams of the smaller size classes. Finally, the diet of the larger juveniles was characterized by larger benthic preys like Decapoda, Amphipoda and Polychaeta (tab. 25).

Table 25 - Index of Relative Importance (%IRI) calculated for all the S. aurata *collected for stomach content analysis, divided in 5 size classes.*

size range	**SL<20mm**	**20<SL<25mm**	**25<SL<30mm**	**30<SL<35mm**	**35<SL<50mm**
size class	**A**	**B**	**C**	**D**	**E**
N° of *S. aurata*	**99**	**53**	**23**	**26**	**11**
Harpacticoida	77.5	90.4	83.0	74.9	1.0
Calanoida	0.9	0.0	0.0	0.0	0.0
Cyclopoida	14.2	0.3	0.2	0.0	0.0
Decapoda	0.0	4.8	2.9	11.0	36.8
Amphipoda	0.5	2.8	4.4	11.2	38.5
Polychaeta	6.9	1.7	8.3	2.9	22.4
Mysidacea	0.0	0.1	1.2	0.0	1.3

Changes in diet have also been compared with change in head shape morphology (fig. 57) to observe in detail any common factor during ontogeny. An Elliptic Fourier analysis was performed on a subsample of 99 individuals divided in 4 size classes with 5 mm range. Size classes considered were A (SL<20mm), B (20≤SL<25mm), C (25≤SL<30mm) and D (30≤SL<35mm). Statistical differences in mean head shapes was observed between individuals belonging to size class A and B (fig. 57, top right), indicating that the most changes probably take place during the first period of colonization of saltmarsh lagoon habitats, with the transition from a pelagic habit to a more markedly benthic habit. No significant differences were observed between size class B and C individuals or between size class C and D individuals, even if changes in head shape could be easily observed in the graph (fig. 57, down right).

Between the first two size classes (fig. 57, top right) it was possible to observe that the areas of major changes are the top of the head and the lower part of the head, namely the areas between the mouth and the operculum. These two areas increased even between size class B and C (fig. 57, down left), where, however, the rear part of the head, behind the operculum, started to increase as well. Finally, between size class C and D (fig. 57, down right) the largest changes in head shape morphology occurred in the front part of the head, near the nares, and, again, in the lower part of the head, near the mouth.

In general, during growth it is possible to observe that juveniles *S. aurata* increased in head dimension, especially in the upper part above the eyes. The rounding of the head and the tapering of the snout were the most evident changes. Furthermore, with the growth the snout area was pressed and moves downwards. These changes can be summarized in a downward displacement of the mouth, bringing the head from a longer and tapered to a squatter and flattened shape. The movement of the mouth downwards seems to be linked to the passage from a planktonic to a benthic diet, while the swelling of the jaw, combined with the development of the musculature, could help to make powerful bites and increase the size of prey.

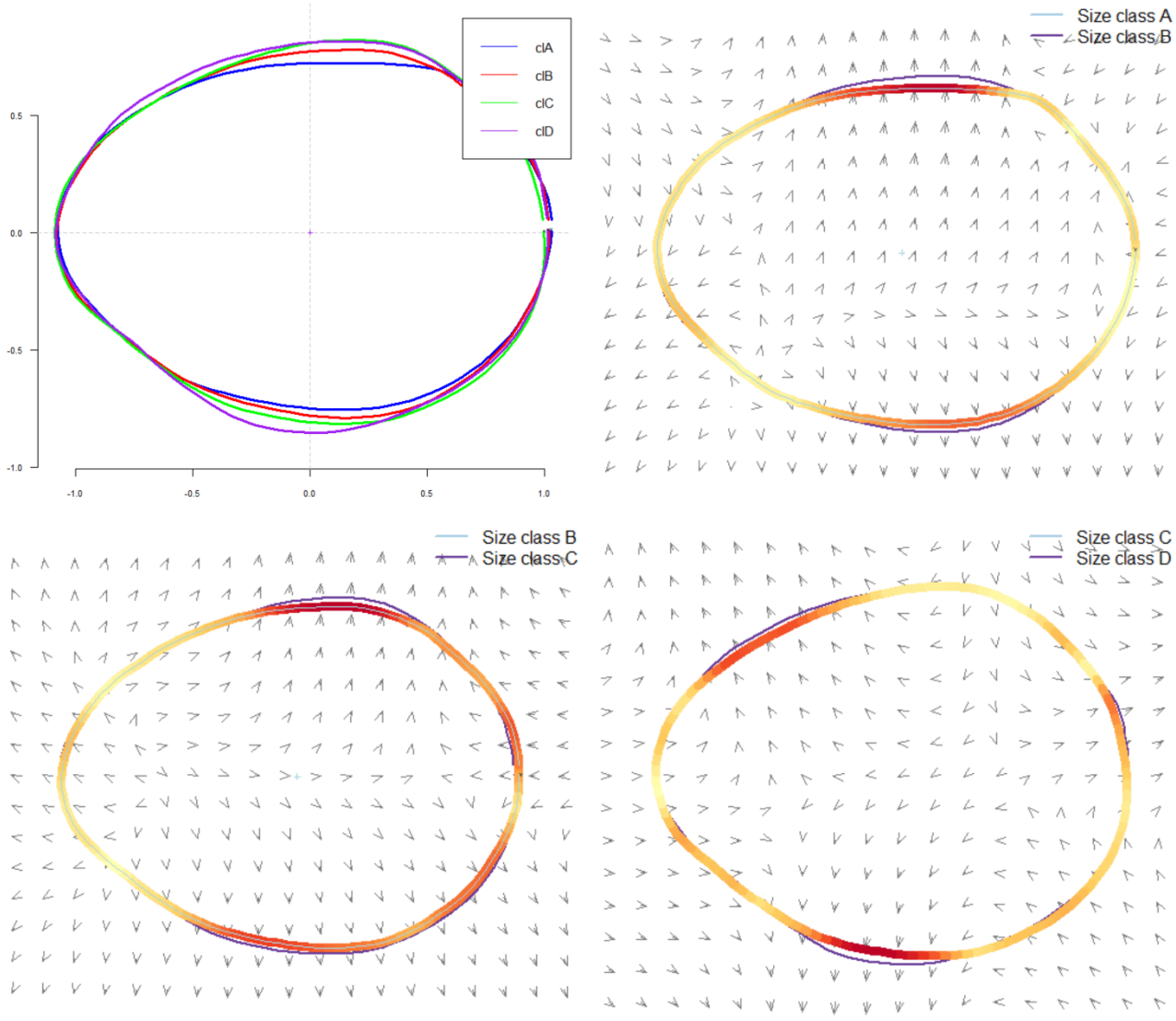


Figure 57 – Maps showing the mean head shape changes of juveniles S. aurata. *Top left: changes in head shapes between all considered size classes (cl = size class). Top right and down: detail of changes in head shapes between size class A and B, B and C, C and D. Arrow indicate where and in which direction deformation occur and then color indicate (from yellow to red) the intensity of the changes between the smaller size class and the other considered.*

3.3.2 Stable isotope analysis

Analysis of carbon and nitrogen stable isotope ratios were conducted using subsamples of *S. aurata* (tab. 17) to observe and test any differences between sampling station and sampling position (saltmarsh edge and tidal creek). As for stomach content analysis, the differences in isotopic signature between size classes were also analyzed.

All individuals of *S. aurata* analyzed for stable isotope were included within the range between –22.233 and -14.095‰ for $\delta^{13}C$ and between 8.316 and 16.077‰ for $\delta^{15}N$ (fig. 58, 59). The linear regression between standard length and isotopic signature ($\delta^{13}C$ or $\delta^{15}N$) were tested with t-test of the slopes to analyze any

differences between sampling stations, positions and size classes (tab. 26). No significant differences have been detected considering $\delta^{13}C$, but they have been observed for $\delta^{15}N$ (tab. 26, fig. 59). Differences in $\delta^{15}N$ were observed only in BA station between size class 3 and smaller individuals (size class 1 and 2) (tab. 26) and between size class 2 individuals collected in BA saltmarsh edge and DE saltmarsh edge. No differences were observed between size classes among tidal creek and saltmarsh edge in DE station.

In general, except in some cases in BA station, individuals showed an isotopic signature enrichment, shifting towards higher values of both $\delta^{13}C$ and $\delta^{15}N$ as they grew (fig. 58, 59). Especially in BA and DE intertidal creek, small *S. aurata* (size class 1) had an isotopic signature completely different from the bigger *S. aurata*, for both $\delta^{13}C$ and $\delta^{15}N$ (fig. 58, 59). In BA station, small *S. aurata* (size class 1) had a lower isotopic signature (both $\delta^{13}C$ and $\delta^{15}N$) compared to other sites. In DE saltmarsh edge, some *S. aurata* size class 1 individuals had values of $\delta^{13}C$ and $\delta^{15}N$ comparable to bigger ones, indicating a wide range of isotopic signature (fig. 58, 59). Considering size class 2 individuals, those collected in DE stations had a rapid enrichment in $\delta^{15}N$, more than those observed in BA station. Finally, size class 3 individuals, collected only in BA station, had values of $\delta^{13}C$ and $\delta^{15}N$ similar to size class 2 individuals collected in the same station (tab. 26, fig. 58, 59).

Table 26 – t test of the slopes to analyze differences in $\delta^{13}C$ and $\delta^{15}N$ between sampling stations, position or size class. n.s. = not significant.

Stable isotope	test	p-value
$\delta^{13}C$	BA, saltmarsh edge, size class 1 vs 2	n.s.
	BA, saltmarsh edge, size class 2 vs 3	n.s.
	BA, saltmarsh edge, size class 1 vs 3	n.s.
	DE, saltmarsh edge, size class 1 vs 2	n.s.
	DE, tidal creek, size class 1 vs 2	n.s.
	DE, saltmarsh edge vs tidal creek, size class 1	n.s.
	DE, saltmarsh edge vs tidal creek, size class 2	n.s.
	BA vs DE, saltmarsh edge, size class 1	n.s.
	BA vs DE, saltmarsh edge, size class 2	n.s.
$\delta^{15}N$	BA, saltmarsh edge, size class 1 vs 2	n.s.
	BA, saltmarsh edge, size class 2 vs 3	<0.05
	BA, saltmarsh edge, size class 1 vs 3	<0.05
	DE, saltmarsh edge, size class 1 vs 2	n.s.
	DE, tidal creek, size class 1 vs 2	n.s.
	DE, saltmarsh edge vs tidal creek, size class 1	n.s.
	DE, saltmarsh edge vs tidal creek, size class 2	n.s.
	BA vs DE, saltmarsh edge, size class 1	n.s.
	BA vs DE, saltmarsh edge, size class 2	<0.05

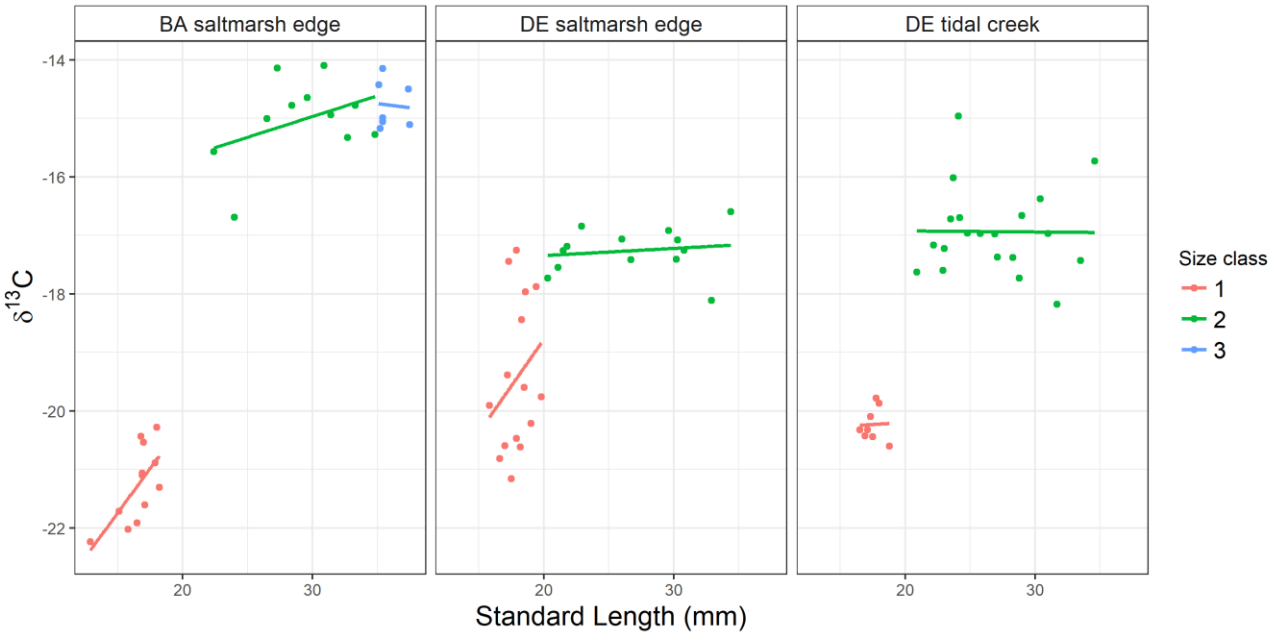


Figure 58 - Relationship between standard length and isotopic signatures ($\delta^{13}C$, ‰) relative to the overall fish sampled, divided for size classes and sampling station and position.

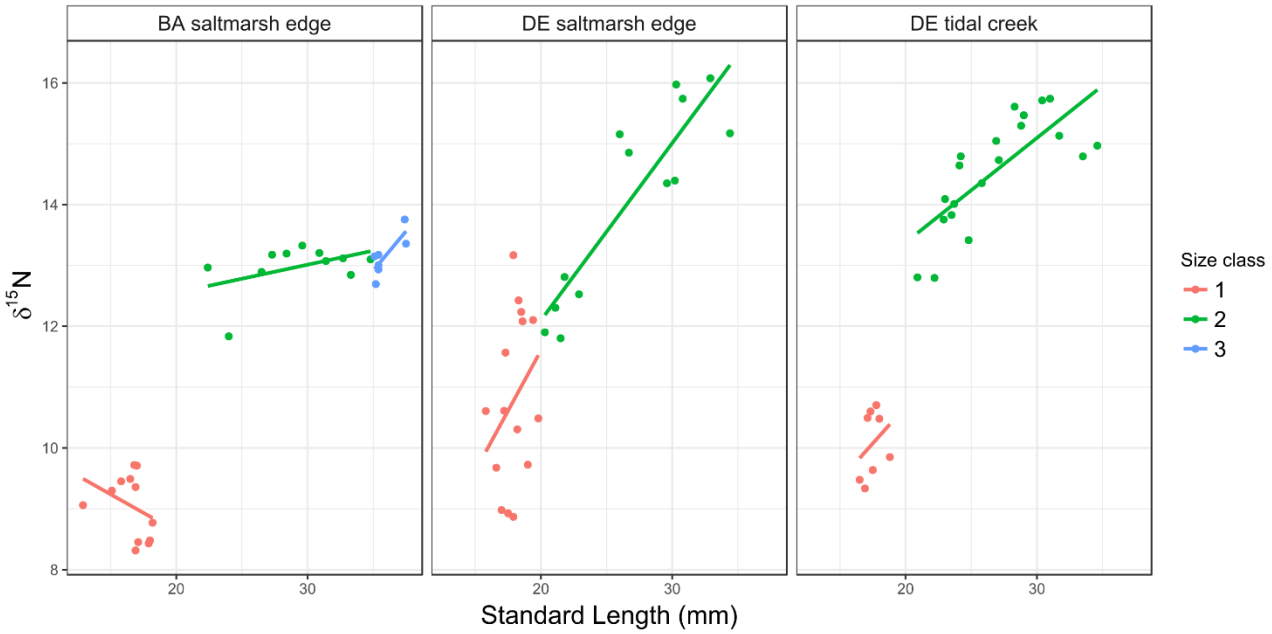


Figure 59 – Relationship between standard length and isotopic signatures ($\delta^{15}N$, ‰) relative to the overall fish sampled, divided for size classes and sampling station and position.

PERMANOVA was used to assess statistical differences in $\delta^{13}C$ and $\delta^{15}N$ signature among sampling sites, positions and size classes but cumulating sampling dates; Pair-Wise test was then conducted to analyze in detail the differences (tab. 27, 28, 29, 30). Sampling site (BA and DE), sampling position (saltmarsh edge and tidal creek) and size class (size class 1, 2 and when present size class 3) were used as factors in PERMANOVA.

Results of carbon stable isotope ratios ($\delta^{13}C$) (tab. 27, 28) showed that no statistical differences in isotopic signature were present between sampling positions, both for first and second size class (tab. 27). Indeed, the differences considering the interaction between sampling position and size class (tab. 27) could be attributed to the absence of size class 3 individuals in DE station rather than to differences between tidal creek and saltmarsh edge (tab. 28). However, strong differences in isotopic signature were always found between the smaller individuals (size class 1) and the bigger ones (size classes 2 and 3), both in DE and BA station and both in saltmarsh edge and tidal creek (tab. 28).

Table 27 - PERMANOVA table of results conducted on carbon stable isotope ratios ($\delta^{13}C$) on dorsal muscle of S. aurata. *In red bold the statistical difference p<0.05.*

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Sampling site	1	1.313	1.313	1.613	0.214	998
Sampling position	1	0.504	0.504	0.619	0.454	996
Size class	**2**	**318.41**	**159.210**	**195.600**	**0.001**	**999**
Sampling site x Position	0	0.000		No test		
Sampling site x Size class	**1**	**58.951**	**58.951**	**72.427**	**0.001**	**999**
Sampling position x Size class	**1**	**4.676**	**4.676**	**5.745**	**0.018**	**996**
Sampling site x Sampling position x Size class	0	0.000		No test		
Res	81	65.929	0.814			
Total	87	455.43				

Table 28 – Pair-Wise tests on carbon stable isotope ratios ($\delta^{13}C$) on dorsal muscle of S. aurata. *In red the statistical difference p<0.05. (s.e. = saltmarsh edge, t.c. = tidal creek).*

Term 'Size class'			
Groups	**t**	**P(perm)**	**perms**
1, 2	17.122	0.001	989
1, 3	15.313	0.001	998
2, 3	0.5944	0.563	996

Term 'Sampling site x Size class' for pairs of levels of factor 'size class'			
Within level 'BA' of factor 'Sampling site'			
Groups	**t**	**P(perm)**	**Unique perms**
1, 2	21.89	0.001	979
1, 3	23.687	0.001	957
2, 3	0.833	0.433	876
Within level 'DE' of factor 'Sampling site'			
Groups	**t**	**P(perm)**	**Unique perms**
1, 2	9.0387	0.001	998

Term 'Sampling position x Size class' for pairs of levels of factor 'Size class'			
Within level 's.e.' of factor 'Sampling position'			
Groups	**t**	**P(perm)**	**Unique perms**
1, 2	15.138	0.001	997
1, 3	13.967	0.001	998
2, 3	0.66714	0.508	997
Within level 't.c. ' of factor 'Sampling position'			
Groups	**t**	**P(perm)**	**Unique perms**
1, 2	8.7864	0.001	962

Term 'Sampling site x Size class' for pairs of levels of factor 'Sampling site'			
Within level '1' of factor 'size class'			
Groups	t	P(perm)	Unique perms
BA, DE	4.9393	0.001	996
Within level '2' of factor 'Size class'			
Groups	t	P(perm)	Unique perms
BA, DE	6.7058	0.001	997

Term 'Sampling position x Size class' for pairs of levels of factor 'Sampling position'			
Within level '1' of factor 'size class'			
Groups	t	P(perm)	Unique perms
s.e., t.c.	1.9162	0.072	995
Within level '2' of factor 'Size class'			
Groups	t	P(perm)	Unique perms
s.e., t.c.	1.2859	0.243	998

Analyzing the nitrogen stable isotope ratios ($\delta^{15}N$) (tab. 29, 30), differences were found only between sampling sites and between size classes while, as observed for $\delta^{13}C$ (tab. 27, 28), no differences were detected among saltmarsh edge and tidal creek (tab. 29). Again, as observed with carbon stable isotope ratios, differences were detected between DE and BA and between size class 1 and bigger individuals (size class 2 and 3) (tab. 30).

Table 29 - PERMANOVA table of results conducted on nitrogen stable isotope ratios ($\delta^{15}N$) on dorsal muscle of S. aurata. *In red bold the statistical difference p<0.05.*

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Sampling site	**1**	**20.359**	**20.359**	**13.672**	**0.001**	**998**
Sampling position	1	6.48E-02	6.48E-02	4.35E-02	0.84	997
Size class	**2**	**219.91**	**109.96**	**73.844**	**0.001**	**999**
Sampling site x Sampling position	0	0		No test		
Sampling site x Size class	1	2.9332	2.9332	1.9699	0.185	997
Sampling position x Size class	1	5.2943	5.2943	3.5555	0.067	998
Sampling site x Sampling position x Size class	0	0		No test		
Res	81	120.61	1.489			
Total	87	448.01				

Table 30 – Pair-Wise tests on nitrogen stable isotope ratios on dorsal muscle of S. aurata. *In red the statistical difference p<0.05.*

Term 'Sampling site'			
Groups	t	P(perm)	Unique perms
BA, DE	3.6976	0.001	999

Term 'Size class'			
Groups	t	P(perm)	Unique perms
1, 2	10.992	0.001	998
1, 3	9.1919	0.001	998
2, 3	0.27327	0.782	997

Marked ontogenetic shift was observed analyzing the isotopic niche (biplot $\delta^{13}C$-$\delta^{15}N$) of each size class (fig. 60, tab. 31). The isotopic niche of *S. aurata* varied in width, shape and position especially between size class, also within the same site or sampling position (fig. 60, tab. 31). As a general trend, as the size class increased from 1 to 2, the isotopic niche of *S. aurata*, represented by the SEA_C, moved along both axes (fig. 60). Instead, no increase was observed between size class 2 and 3 in BA station (fig. 60).

The smallest and the biggest isotopic niche (SEA_B) was recorded for size class 1 respectively in DE tidal creek and DE saltmarsh edge (probability of difference > 0.95) (tab. 31). Among size class 2 individuals, SEA_B of DE tidal creek and DE saltmarsh edge were similar (tab. 31) and no differences were detected (probability of difference < 0.95), however both were bigger than SEA_B of BA station (probability > 0.95). Within each sampling station, differences of SEA_B between size class 1 and 2 were found only in DE tidal creek (probability > 0.95), when size class 1 had an isotopic niche smaller than size class 2 (fig. 60, tab. 31). Considering the community wide metrics, for NND_B and $SDNND_B$ no differences (probability < 0.95) were observed both among stations, positions or size classes. In DE and BA saltmarsh edge, from size class 1 to size class 2, decreased (probability > 0.95) only the values of Carbon Range (CR_B) while in DE tidal creek from size class 1 to 2 increased (probability > 0.95) both Nitrogen Range (NR_B), Carbon Range (CR_B) and Centroid Distance (CD_B). Within the same size class, differences in community metrics between sampling station and position were detected. For size class 1 individuals, the bigger NR_B, CR_B and CD_B (tab. 31) were found in DE saltmarsh edge and these values were significantly higher (probability > 0.95) than those of DE tidal creek and BA station. Between DE tidal creek and BA differences in community metrics were detected only for CR_B, which was higher in BA (probability > 0.95) (fig. 60). For size class 2 individuals the bigger differences between all sampling stations and positions (probability > 0.95) were found in for NR_B for which values were higher in DE saltmarsh edge and lower in BA (tab. 31). For size class 2, differences in CR_B were found between DE saltmarsh edge and tidal creek, when CR_B was higher in tidal creek (probability > 0.95) (tab. 31).

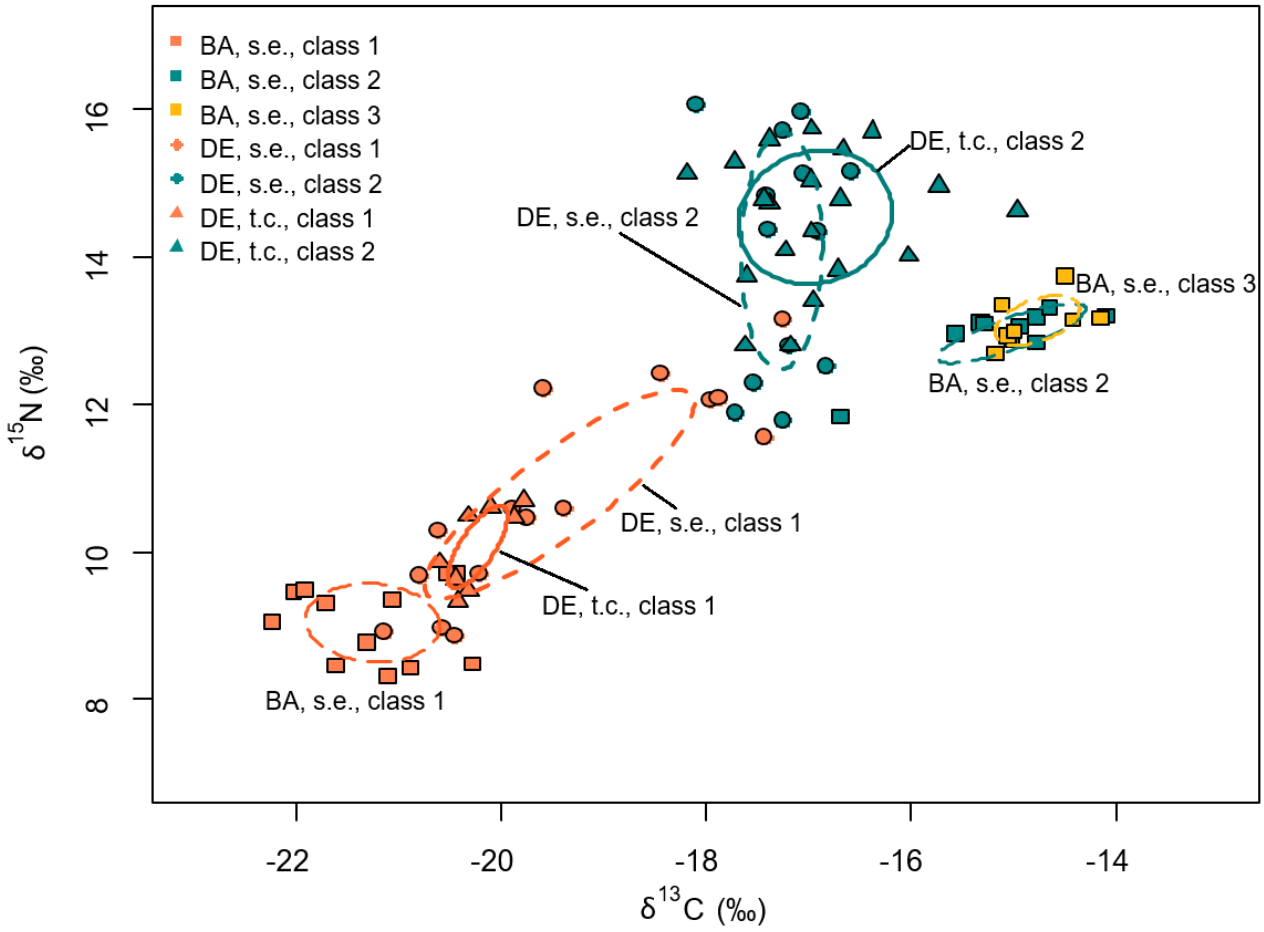


Figure 60 - $\delta^{13}C$ (‰) vs $\delta^{15}N$ (‰) of S. aurata *samples divided for groups (sampling station, position and size class). Different colours correspond to different size classes. The isotopic niche of each size class is represented by the bayesian corrected Standard Ellipse Areas (SEA_C). (s.e. = saltmarsh edge, t.c. = tidal creek).*

Table 31 – Community-wide metrics calculated for each size class of S. aurata *in different sampling stations/positions.*

Station/position	**DE s. e.**		**DE t. c.**		**BA s. e.**		
Size class	**1**	**2**	**1**	**2**	**1**	**2**	**3**
NR	4.30	4.27	1.37	2.95	1.40	1.49	1.06
CR	3.90	1.52	0.82	3.21	1.95	2.59	1.03
CD	1.65	1.45	0.57	1.03	0.76	0.58	0.47
NND	0.45	0.47	0.22	0.45	0.31	0.28	0.29
SDNND	0.35	0.15	0.04	0.19	0.20	0.44	0.18
SEA	2.80	1.98	0.34	2.09	1.08	0.52	0.38
SEA_C	3.01	2.16	0.39	2.20	1.18	0.58	0.46

To detect the relationship between *S. aurata* and habitats, all the environmental sources of organic matter collected in the different sampling stations (DE and BA) and sampling positions (saltmarsh edge and tidal creek) were analyzed. Being a short distance between tidal creek and saltmarsh edge in DE and BA station, and tue to no significant statistical differences occurring in values of sources (Andolina, 2017), only the Halophytes collected in tidal creek were used, even if they were collected also in saltmarsh edge. Among the

different species of Halophytes present and collected in the saltmarsh, isotope analyses were conducted only on *Sarcocornia fructicosa*, the species most abundant and always present. Among the different species of macroalgae, only *Ulva* sp. was analyzed because it was the species most abundant and always present. To deepen the topic about the relationship between individuals and environmental sources, Bayesian mixing models were also applied for DE station, for each sampling position and for the size class 1 and 2. Because in DE station sources were collected both in March and May, the values used in the models were the mean values.

PERMANOVA was used to assess statistical differences in $\delta^{13}C$ and $\delta^{15}N$ signature of environmental sources among sampling sites, positions and size classes, cumulating the two sampling dates; Pair-Wise test was then conducted to analyse in detail the differences (tab. 32, 33, 34, 35). Sampling station (BA and DE), sampling position (saltmarsh edge and tidal creek) and source typology were used as factors in PERMANOVA. Analyzing environmental sources, results showed that for both $\delta^{13}C$ and $\delta^{15}N$ differences were detected between sampling stations, typology of environmental sources and considering the interaction of these two factors (tab. 32, 33, 34, 35). Differences between BA and DE were observed for Plankton, POM, SOM and Halophyte considering $\delta^{13}C$ (tab. 33) and for Macroalgae, Plankton, SOM and Halophyte considering $\delta^{15}N$ (tab. 35). In DE stations, except POM and Halophytes, the environmental sources were different from each other and each had a distinct signature, both for $\delta^{13}C$ and $\delta^{15}N$ (tab. 33, 35). Instead, in BA stations, the difference between isotopic signature of the sources were always detected except between Macroalgae and Plankton (both $\delta^{13}C$ and $\delta^{15}N$), which had a similar signature (tab. 33, 35). Differences between sampling position were detected only for $\delta^{13}C$ (tab. 32), but differences did not appear considering the interaction between sampling position and station or habitat typology (tab. 32). In general, considering $\delta^{13}C$, higher values were detected in saltmarsh edge compared to tidal creek, especially in DE station.

Table 32 - PERMANOVA table of results conducted on carbon stable isotope ratios ($\delta^{13}C$) on environmental sources. In red bold the statistical difference $p<0.05$.

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Sampling station	1	65.05	65.054	72.721	**0.001**	996
Sampling position	1	5.047	5.0479	5.6427	**0.022**	998
Source typology	5	1163	232.64	260.05	**0.001**	999
Sampling station x Sampling position	1	3.497	3.4977	3.9099	0.048	995
Sampling station x Source typology	4	93.56	23.39	26.146	**0.001**	999
Sampling position x Source typology	4	1.466	0.3665	0.4097	0.784	999
Sampling station x Sampling position x Source typology	3	1.254	0.4181	0.4674	0.718	998
Res	60	53.68	0.8946			
Total	79	1907.1				

Table 33 – Pair-Wise tests on carbon stable isotope ratios ($\delta^{13}C$) on environmental sources. In red the statistical difference p<0.05. (s.e. = saltmarsh edge, t.c. = tidal creek).

Term 'Sampling station x Source typology' for pairs of levels of factor 'Sampling station'			
Within level 'Macroalgae' of factor 'Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	0.29675	0.76	997
Within level 'Plankton' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	6.9692	0.002	753
Within level 'POM' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	7.4539	0.001	998
Within level 'SOM' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	11.538	0.001	995
Within level 'Halophytes' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	4.0943	0.014	79
Factor 'Sampling position'			
Groups	**t**	**P(perm)**	**Unique perms**
s.e., t.c.	2.3754	0.026	998

Term 'Sampling station x Source typology' for pairs of levels of factor 'Source typology'			
Within level 'Dese' of factor 'Sampling station'			
Groups	**t**	**P(perm)**	**Unique perms**
Macroalgae, Plankton	8.9043	0.001	991
Macroalgae, POM	15.272	0.001	998
Macroalgae, SOM	20.438	0.001	999
Macroalgae, Halophytes	20.616	0.001	994
Plankton, POM	5.7534	0.001	997
Plankton, SOM	5.6537	0.001	995
Plankton, Halophytes	8.7422	0.001	988
POM, SOM	4.0808	0.001	997
POM, Halophytes	0.81786	0.407	998
SOM, Halophytes	11.195	0.001	999
Within level 'Baccan' of factor 'Sampling station'			
Groups	**t**	**P(perm)**	**Unique perms**
Macroalgae, Plankton	3,65E-02	0.955	460
Macroalgae, POM	6.7384	0.002	989
Macroalgae, SOM	8.3894	0.005	986
Macroalgae, Halophytes	15.362	0.002	569

Macroalgae, Fanerogame	15.971	0.004	970
Plankton, POM	15.434	0.009	464
Plankton, SOM	29.263	0.008	456
Plankton, Halophytes	468.91	0.009	81
Plankton, Seagrass	23.213	0.007	438
POM, SOM	3.4263	0.012	984
POM, Halophytes	29.362	0.001	573
POM, Seagrass	35.891	0.003	931
SOM, Halophytes	43.465	0.004	569
SOM, Seagrass	40.954	0.001	937
Halophytes, Seagrass	42.164	0.002	572

Table 34 - PERMANOVA table of results conducted on carbon stable isotope ratios ($\delta^{15}N$) on environmental sources. In red bold the statistical difference p<0.05.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**perms**
Sampling station	1	116.75	116.75	53.937	0.001	999
Sampling position	1	2.1474	2.1474	0.99207	0.325	996
Source typology	5	261.6	52.321	24.171	0.001	999
Sampling station x Sampling position	1	0.15588	0.15588	7.20E-02	0.765	996
Sampling station x Source typology	4	47.462	11.865	5.4816	0.003	998
Sampling position x Source typology	4	14.191	3.5477	1.6389	0.16	998
Sampling station x Sampling position x Source typology	3	5.3584	1.7861	0.82515	0.459	999
Res	60	129.88	2.1646			
Total	79	658.08				

Table 35 – Pair-Wise tests on carbon stable isotope ratios ($\delta^{15}N$) on environmental sources. In red the statistical difference p<0.05.

Term 'Sampling station x Source typology' for pairs of levels of factor 'Sampling station'			
Within level 'Macroalgae' of factor 'Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	4.9732	0.001	997
Within level 'Plankton' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	2.5058	0.068	981
Within level 'POM' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	0.9475	0.35	997
Within level 'SOM' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	16.053	0.001	996

Within level 'Halophytes' of factor "Source typology'			
Groups	**t**	**P(perm)**	**Unique perms**
Dese, Baccan	3.7403	0.018	84

Term 'Sampling station x Source typology' for pairs of levels of factor 'Source typology'			
Within level 'Dese' of factor 'Sampling station'			
Groups	**t**	**P(perm)**	**Unique perms**
Macroalgae, Plankton	3.0518	0.009	997
Macroalgae, POM	5.8156	0.001	998
Macroalgae, SOM	5.3644	0.001	995
Macroalgae, Halophytes	6.7987	0.001	997
Plankton, POM	2.9184	0.011	997
Plankton, SOM	1.6152	0.134	997
Plankton, Halophytes	5.6529	0.001	989
POM, SOM	3.2737	0.003	997
POM, Halophytes	0.10045	0.915	995
SOM, Halophytes	8.2156	0.001	997
Within level 'Baccan' of factor 'Sampling station'			
Groups	**t**	**P(perm)**	**Unique perms**
Macroalgae, Plankton	0.15592	0.873	929
Macroalgae, POM	17.824	0.005	977
Macroalgae, SOM	13.181	0.001	975
Macroalgae, Halophytes	24.935	0.003	560
Macroalgae, Seagrass	6.0542	0.005	987
Plankton, POM	15.167	0.007	922
Plankton, SOM	10.456	0.004	921
Plankton, Halophytes	26.091	0.006	208
Plankton, Seagrass	5.363	0.005	921
POM, SOM	4.2855	0.007	984
POM, Halophytes	9.3867	0.002	571
POM, Seagrass	11.037	0.002	971
SOM, Halophytes	1.6049	0.147	572
SOM, Seagrass	10.235	0.003	980
Halophytes, Seagrass	13.02	0.001	593

In Table 36 and Figure 61 is possible to observe the values and the localization in the biplot $\delta^{13}C$- $\delta^{15}N$ of the environmental sources collected in BA and DE, both in saltmarsh edge and tidal creek. For Halophyte, as previously stated, only the samples collected in saltmarsh edge were analyzed, therefore the same values were observed between position (tab. 36). Instead, seagrasses were found and collected only in BA station.

The distribution of the basal sources over the $\delta^{13}C$ axis of the isotope space (the biplot $\delta^{13}C$- $\delta^{15}N$) followed almost the same order in DE and BA, with Halophytes showing the most ^{13}C-depleted values, the POM, SOM and plankton the intermediate ones. Finally, *Ulva* sp. and seagrass show the most enriched values respectively in DE and BA station. As for the $\delta^{13}C$, the $\delta^{15}N$ of all sources ranged similarly between the two sampling positions (fig. 61, tab. 36). Halophyte was the source characterized by lower values of $\delta^{15}N$ both in BA and DE while *Ulva* sp. own the higher values (tab. 36). In DE station, considering $\delta^{15}N$, intermediate values were observed for plankton, SOM and POM. In general, no large differences were observed between position both in DE and in BA and both considering $\delta^{13}C$ and $\delta^{15}N$.

As observed with statistical tests (tab. 32-35), the major differences in values of each environmental source occurred between sampling stations: sources of BA station had generally higher values of $\delta^{13}C$. Moreover, along the $\delta^{13}C$ axis it was possible to observe differences between sampling position: especially in DE station, saltmarsh edge had generally higher values compared to tidal creek (fig. 61, tab. 36). However, in general, the distribution of the basal sources was similar between sampling position inside the same sampling station, and each environmental source showed similar values between saltmarsh edge and tidal creek (fig. 61, tab. 36).

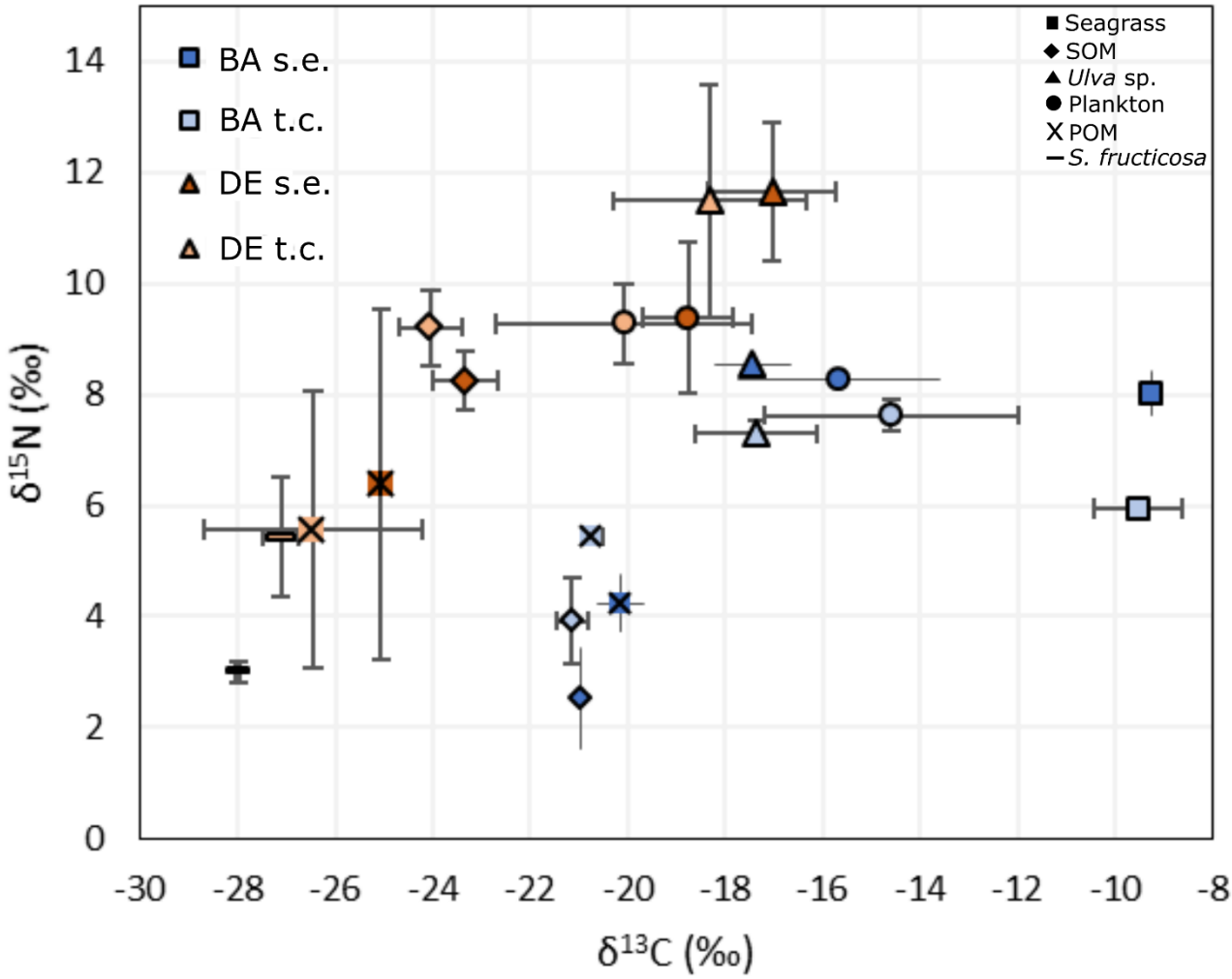


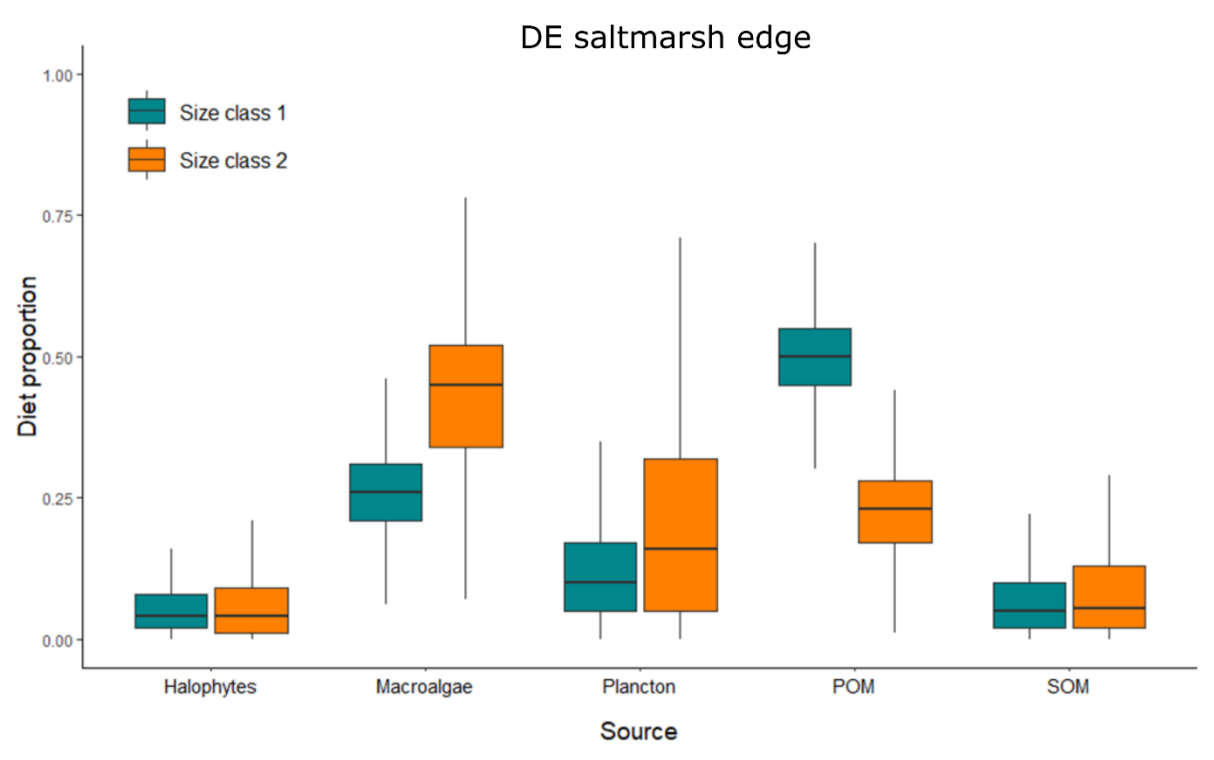
Figure 61 – Mean (± s.d.) $\delta^{13}C$ (‰) vs. $\delta^{15}N$ (‰) of sources of organic matter sampled in each DE and BA sampling positions (s.e. = saltmarsh edge, t.c. = tidal creek).

Table 36 - Mean (± s.d.) $\delta^{13}C$ (‰) vs. $\delta^{15}N$ (‰) of sources of organic matter sampled in DE and BA sampling positions. (s.e. = saltmarsh edge, t.c. = tidal creek).

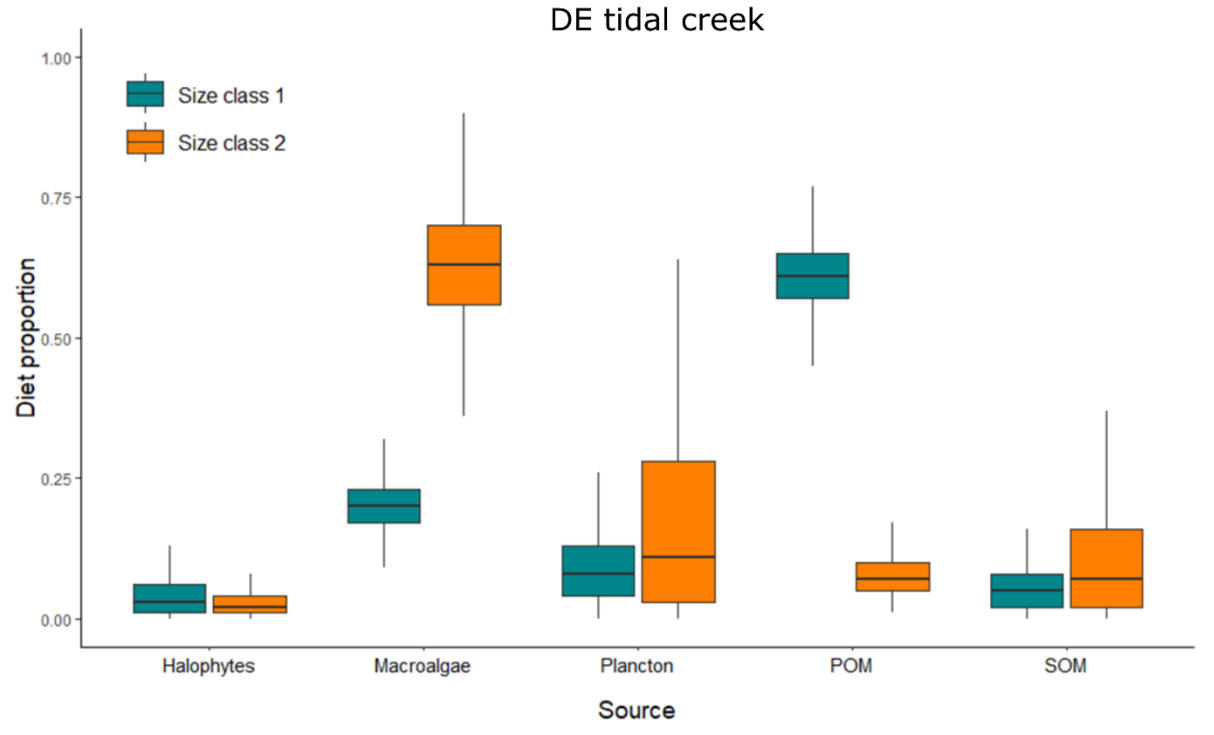
Station	environmental sources	position	mean δC ‰	s.d. dC	mean δN ‰	s.d. dN
DE	Macroalgae (*Ulva* sp.)	s.e.	-17,04	0,67	11,65	1,27
		t.c.	-18,33	1,97	11,48	2,09
	Halophytes (*Sarcocornia fructicosa*)	s.e.	-27,11	0,36	5,43	1,08
		t.c.	-27,11	0,36	5,43	1,08
	Plankton	s.e.	-18,77	3,04	9,38	1,37
		t.c.	-20,08	2,64	9,27	0,72
	POM	s.e.	-25,06	0,73	6,39	3,15
		t.c.	-26,46	2,25	5,57	2,50
	SOM	s.e.	-23,34	0,34	8,25	0,52
		t.c.	-24,05	0,65	9,20	0,69
BA	Macroalgae (*Ulva* sp.)	s.e.	-17,45	0,77	8,55	0,21
		t.c.	-17,36	1,25	7,29	0,23
	Halophytes (*Sarcocornia fructicosa*)	s.e.	-28,00	0,04	3,01	0,19
		t.c.	-28,00	0,04	3,01	0,19
	Seagrasses	s.e.	-9,26	0,12	8,03	0,41

		t.c.	-9,53	0,92	5,94	0,16
	Plankton	s.e.	-15,68	2,07	8,26	0,09
		t.c.	-14,60	2,62	7,62	0,28
	POM	s.e.	-20,15	0,48	4,23	0,51
		t.c.	-20,73	0,20	5,43	0,08
	SOM	s.e.	-20,96	0,09	2,52	0,90
		t.c.	-21,14	0,32	3,92	0,79

Results of the Bayesian mixing model, applied to estimate the contribution provided by the potential sources of organic matter to the isotopic pathway of *S. aurata* for each size class in each sampling position of station DE are showed in Figure 62. For *S. aurata* size class 1, the most likely contribution was given by particulate organic matter (POM), both in intertidal creek (mode = 0.62) and in saltmarsh edge (mode = 0.51), indicating no large difference between sampling position. As for the class 2, instead, the main source supporting the trophic pathway was *Ulva* sp. (mode = 0.59 in intertidal creek and 0.47 in saltmarsh edge). Lastly, among the two-different sampling positions, for both size classes and in both position, plankton, SOM and Halophyte sources provided a lower contribution in isotopic signature of *S. aurata*. Results of mixing models thus provided evidence that the contribution of the potential sources of organic matter to the trophic pathway of *S. aurata* clearly varied across size classes, particularly between post-larvae of size class 1 and size class 2, with no large differences across sampling positions (fig. 62). Overall, the general results summarized by size revealed a distinction between the trophic pathways of post-larval stage and juvenile (fig. 62).



A)



B)

Figure 62 – Boxplot of contribution of organic matter sources to the trophic pathway leading to S. aurata *(size class 1 and 2) in the two sampling positions of DE station (A = saltmarsh edge, B = tidal creek), with the relative credibility intervals. Boxes represent 25, 50 and 75 percent quantiles and whiskers represent 5 and 95 percent of credibility interval.*

3.3.3 Secondary production of *S. aurata*

To highlight the potential nursery value of saltmarsh habitat in the Venice lagoon, secondary production of *S. aurata* was calculated. All individuals collected in DE station, both in saltmarsh edge and in intertidal creek was cumulated to obtain more realistic results. *S. aurata* individuals, collected from February (first sampling campaign) to end of May (sixth sampling campaign) were used and all belonging to the 0-group cohort.

Firstly, the absolute growth rate (AGR, mm/day) was calculated using the mean of standard length of the individuals (tab. 37) to observe the growth of individuals during the different sampling campaign. Absolute growth rate (mm/day) had it maximum growth in March (0.52 mm/day) and during the first sampling campaign of April (0.38 mm/day). The secondary production was later calculated for each sampling interval between sampling campaign (tab. 37) and it showed the highest peaks in March and April, respectively 5.20 and 6.74 g w.w./m^2/month. Results show that *S. aurata* grow mainly in March and April, both in length and in biomass. Finally, the mean values of secondary production calculated cumulating all the individuals collected in DE station from February to May was 2,59 g w.w/m^2/month.

Table 37 – Density, Absolute growth rate and secondary production of S. aurata *collected in DE station during the sampling campaign.*

Sampling campaign	N° of *S. aurata*	Density (ind/100m^2)	AGR (mm/day)	Secondary Production (g w.w./m^2/month)
1 (February)	30	5.17	0.02	0.16
2 (March)	140	19.61	0.14	1.08
3 (April)	28	3.01	0.52	5.20
4 (April)	108	14.40	0.38	6.74
5 (May)	63	6.77	0.24	3.21
6 (May)	2	0.20		

3.4 Discussion

In the Venice lagoon, the saltmarsh habitats of the northern sub-basin are selected by many marine migrant fish at least during one period of their life cycle within the lagoon (see Chapter 1) and they are used for different purposes in relation to the ontogenetic growth of the individuals (see Chapter 2). In particular, *S. aurata* individuals massively concentrate in saltmarsh habitats of the northern sub-basin especially until they reach 35 mm standard length (see Chapter 2). Saltmarsh habitats can be different mainly in relation to their location within the sea-lagoon edge gradient, to the influence of salinity and freshwater inputs and to hydrodynamic conditions. Moreover, saltmarsh habitats are morphologically complex tidal habitats that are well known to contain high nutrient loads and thus attract a large number of organisms that act as primary prey for fish (Boesh and Turner, 1984; Laffaille et al., 2002). Inside the saltmarsh habitats on the flood tide, intertidal marsh creeks are colonized by fish periodically, instead they return to the adjacent mudflat or

subtidal channel on the ebb (Cattrijsse and Hampel, 2006). These movements made by fish are still largely unknown but are mail related to the presence and availability of prey and resources or shelter zone.

Using an integrated approach combining the stomach content, the stable isotope and the head shape morphology analyses, this study helps to assess the habitat preferences and the feeding ecology of a target marine migrant species (*S. aurata*) in saltmarsh habitats. In this study it was possible to detect differences among the saltmarsh edge and the tidal creek and observe ontogenetic diet changes during growth. The purpose was to understand how juvenile of *S. aurata* use the saltmarsh habitat and which are the trophic relationships that increase the nursery value of a transitional water ecosystem.

Stomach content

Among the different saltmarsh habitats sampled, the ones located near the sea inlet, characterized by a higher influence of salty sea water, are less colonized than the ones located near the lagoon edge. Small *S. aurata* (size class 1, less than 20 mm) are indeed present with high concentrations in the inner lagoon saltmarsh stations still from the first sampling campaign. This should indicate that *S. aurata* individuals, after entering in the lagoon from the sea inlet, especially from late February (Ferrari and Chieregato, 1981; Rossi, 1986), start immediately to move towards the inner part of the lagoon probably following a saline gradient (Cabral et al., 2007; Islam et al., 2006; Marshall and Elliott, 1998; Vasconcelos et al., 2011).

No differences among the two positions sampled in saltmarsh habitats (tidal creek and saltmarsh edge) were detected considering standard length, total weight and eviscerated total weight. Aforementioned, the entering and the first growth within the lagoon habitats is similar regardless the position: individuals probably move periodically among the two positions, exploiting resources of the tidal creek and the saltmarsh edge in a balanced way and thus growing in a similar way. However, differences between the sampling positions were observed both considering *S. aurata* stomach content and environmental prey availability. These differences probably indicate that each portion of the saltmarsh has a specific role and is colonized in different times and for different trophic reasons.

Generally, the number of individuals with a full stomach was higher in the tidal creek rather than in the saltmarsh edge, indicating that probably this portion of saltmarsh contains more prey and resources. Confirming the hypothesis of the presence of more resources inside the tidal creek, arrive from the observation of the number of ingested preys found inside the stomach of *S. aurata*. However, even if the number of prey eaten was greater in the tidal creek for both size class 1 and 2, in the saltmarsh edge preys had a bigger biovolume. This could be attributed to different reasons: i) in the two positions different prey taxa, with different biovolumes, are present or ii) the *S. aurata* individuals prefer or select different taxa as prey in relation to the position in the saltmarsh.

Analyzing in detail the taxa ingested, *S. aurata* ate mainly zooplanktonic and benthic preys, belonging to five taxa of Animalia: Amphipoda, Copepods (Calanoida, Cyclopoida, Harpacticoida), Decapoda, Mysidacea and Polychaeta. Each prey was selected by *S. aurata* with different quantity in relation with ontogenetic growth. No strong differences appeared between sampling positions, considering both dietary index and feeding strategy. Recognizing preferences in diet, small *S. aurata* (size class 1) ate mostly small prey as Harpacticoida, Cyclopoida and Calanoida, and in small part, Amphipoda. Calanoida were preferred only in the first sampling campaign especially in the tidal creek. However, in the saltmarsh edge, during the first sampling campaign some *S. aurata* size class 1 were extremely selective towards Calanoida. Conversely, Amphipoda and larger prey taxa were eaten and selected, considering both prey preferences and feeding strategy, especially in April, when individuals reach size class 2. These results suggest that during the first arrival of individuals within the lagoon, coming as larvae from the sea, probably *S. aurata* still eat planktonic prey. In relation to the growth of *S. aurata*, in April, some individuals started to change their diet towards larger prey, showing a shift in diet. Furthermore, *S. aurata* size class 2 still preferred Harpacticoida but also prefer large prey such as Amphipoda and Polychaeta, with no strong differences between tidal creek and saltmarsh edge. Finally, large prey such as Mysidacea and Decapoda were eaten by few individuals, mostly in April in saltmarsh edge, probably indicating that i) a new shift in diet is coming or ii) Mysidacea and Decapoda in saltmarsh edge are more abundantly present.

The analysis of dietary composition and feeding strategy using gut content unfortunately did not help comparing the ingested prey items with environmental availability of the possible prey (zoo-plankton, zoo-iperbenthos and zoo-benthos). The two positions (tidal creek and saltmarsh edge) indeed, were different in prey availability. In general, the possible prey was abundantly higher, both in number and biovolume, in May rather than in March, indicating that secondary production explodes and increases greatly with the arrival of spring and warm season. Calanoida and Cyclopoida, preferred by *S. aurata* during the first sampling campaigns (February and March), were present with high density and biovolumes in March in tidal creek. Harpacticoida, preferred prey of all size individuals, both in March and May, were present with high density in tidal creek. Later, during warm temperature, in May, when *S. aurata* size class 2 prefer larger preys, higher abundance and biovolume of Amphipoda and Polychaeta were present in tidal creek. To assess quantitatively the prey selection of *S. aurata* and the relationship between ingested prey and available prey, Relativized Selectivity Index (Vanderploeg and Scavia's index, E*) was used. Using this index appeared that Harpacticoida, which were preferred by individuals belonging both to size class 1 and 2, were selected positively only by size class 2 *S. aurata*. Conversely, Cyclopoida, which was present especially inside tidal creek in March, were strongly selected by *S. aurata* size class 1. Amphipoda were selected only by *S. aurata* size class 2 and avoided by other individuals and Polychaeta, present especially in tidal creek, were avoided by small *S. aurata* and selected by size class 2 individuals. These results could indicate that *S. aurata* enter in tidal creek due to the high abundance of preferred prey. These preferred prey present in the environment

are Cyclopoida and Calanoida, at the beginning of lagoon colonization, and Harpacticoida and Amphipoda during warmer months. This entry in tidal creek occur during the first arrival in the lagoon, when size class 1 individuals search and select Cyclopoida (present especially in tidal creek) and avoid large prey as Polychaeta (present especially in saltmarsh edge). Moreover, this entry in tidal creek occur during the period of growth within saltmarsh habitats, when the raising of the water temperature increases the secondary production of possible prey, and thus when size class 2 individuals search and select Harpacticoida, Amphipoda and Polychaeta (present especially in tidal creek).

Overall, *S. aurata* individuals belonging to the same size class, even if collected in the two different sampling positions, behave in a similar way, preferring the same preys. These results suggest that individuals move periodically between the saltmarsh edge and the tidal creek in order to find and exploit the preferred resources. The ontogenetic growth of *S. aurata* seems to be the strongest driver that moves the trophic ecology of this species and its habitat preferences. Indeed, as observed for the other species (Aarnio et al., 1996; Baldo and Drake, 2002; Ferrari and Chieregato, 1981), *S. aurata* individuals during growth, change their preferences in diet also in relation with their body shape changes (Russo et al., 2007), the development of the digestive tract (Elbal et al., 2004) and teeth-age adaption (Cataldi et al., 1987).

The *S. aurata* diet changes within saltmarsh habitats during the first ontogenetic growth, were also analyzed in detail dividing the individuals in smaller size classes (range 5 mm). The total ingested biovolume of *S. aurata* increased rapidly during the entire sampling period. In general, this could indicate that the presence of many and different preys in saltmarsh habitats helps individuals to accumulate a lot of energy right from the beginning of the lagoon colonization. Considering the average number of ingested prey, smaller *S. aurata* (size class A, 15<Standard Length<20mm) ate less than 10 prey for individuals while *S. aurata* size class B (20<SL<25mm) ate the largest number of prey. Finally, from 25 mm standard length the number of preys decreased and big *S. aurata* ate less preys. The decreasing number of ingested prey and the increasing number of the total ingested biovolume were observed also considering the most common taxa: Harpacticoida and Amphipoda. The decrease in number of ingested prey with the coinciding increase in the total ingested biovolume means that the average size of the ingested prey changes. As previously observed from the analysis of *S. aurata*'s preferences and feeding strategy, the taxa and the species eaten by *S. aurata* change with ontogeny, increasing in size.

The *S. aurata* diet changes, observed during growth, were compared with the development in the head shape morphology with the purpose to observe any common feature. The most marked changes were observed among the smaller individuals: between *S. aurata* size class A and B, thus from 15-20 to 20-25 mm. However, the increases in the head shape were observed between size class B and C and C and D, even if they were not statistically significant. In general, *S. aurata's* expansion in the head dimension, appears especially in the upper part above the eyes. The rounding of the head and the tapering of the south are the most evident

changes. These changes can be summarized in a downward displacement of the mouth and a general swelling of the head in the maxilla, bringing the head from a longer and tapered shape to a squatter and flattened one. The movement of the mouth downwards, could help individuals to change their diet, from planktivory to benthivore, while the swelling of the jaw, combined with the development of the musculature, could help to make stronger bites to increase the size of preys. Lastly, changes in diet seem to be strongly related to the changes in the head morphology.

Stable isotope

The stable isotope approach, which reflects the diet over a long period of time (weeks or months), was used to observe differences between sampling positions inside the saltmarsh habitats and between size classes of *S. aurata*. Results showed evidence of differences between sampling stations. However, the biggest differences were detected between size classes. Indeed, a significant ontogenetic trend in the isotopic signaturewas observed.

Analyzing the relationship between standard length and $\delta^{13}C$ or $\delta^{15}N$, the results confirmed the absence of strong differences between sampling positions while small differences were observed between sampling sites DE and BA. Considering $\delta^{15}N$, differences between size classes were observed in the saltmarsh station near the sea inlet (BA). In this station (BA), size class 1 individuals had similar isotopic values of both $\delta^{13}C$ and $\delta^{15}N$, indicating a low trophic position. These results suggest that small *S. aurata* found in BA station, since they showed similar isotopic values, had just entered the lagoon and probably ate similar resources. Even in DE tidal creek the small *S. aurata* (size class 1) had similar values of $\delta^{13}C$ and $\delta^{15}N$, indicating a similar utilization of resources. Conversely, small *S. aurata* (size class 1) collected in DE saltmarsh edge included a wide range of isotopic values, probably indicating that individuals had eaten a larger variety of food. Analyzing the isotopic signature of the individuals belonging to size 1 and 2, considering $\delta^{13}C$, no differences were observed between the sampling positions, implying that presumably the use of resources remains similar among the stations. However, strong differences in $\delta^{13}C$ were found between the small individuals (size class 1) and the bigger ones (size class 2 and 3), both in DE and BA station and both in the saltmarsh edge and tidal the creek. These results indicate that, as observed with stomach content analysis, *S. aurata* individuals of the same size class eat similar resources in both stations or positions, but these preferences in diet change with growth. Analyzing the isotopic signature considering nitrogen ($\delta^{15}N$), therefore the trophic position, differences were found between size classes and between sites (DE and BA). Again, as observed for $\delta^{13}C$, differences were detected between size class 1 and bigger individuals, indicating probably the occurrence of a strong shift in diet and thus in trophic position.

The differences between the saltmarsh edge and the tidal creek and between size classes were investigated analyzing the isotopic niches and the biplot $\delta^{13}C$-$\delta^{15}N$. The position of the isotopic niche represents the type of resources that are being used. Different positions of the isotopic niches could be explained by a shift in

the use of resources. The overlap of niches represents a similarity in the use of resources among individuals and the width of the isotopic niches and their metrics helps comparing the generalist (large niche width) or specialist (small niche width) behavior of individuals and the variety in resources consumed. In general, the isotopic niche of *S. aurata* sampled in this work varied in width, shapes and position especially between size classes, even within the same site or sampling position. The position of size class 1 *S. aurata* collected in BA station, located on the lower-left part of the biplot $\delta^{13}C$-$\delta^{15}N$, could indicate that they had a low trophic level, probably because they had just entered from the sea. As a general trend, as the size class increased from 1 to 2, the isotopic niche moved along both axes and differences in niches position appeard clear. This shift towards more enriched values of both $\delta^{13}C$ and $\delta^{15}N$ during early stages (from size class 1 to 2) could thus be explained with a shift in the use of resources, that is linked to the trophodynamic of *S. aurata* (Andolina, 2017). These changes generally correspond to the transition phase from pelagic-planktivorous, which characterize the post-larvae entering from the open sea (size class 1), to benthonic, which characterized the individuals inside the shallow habitats of the lagoons (Andolina, 2017). This transition phase is well observed with the stomach content analysis (Ferrari and Chieregato, 1981; Russo et al., 2007; Andolina, 2017). Considering the types of resources used, an overlap of isotopic niche was observed among individuals belonging to the same size classes, even if they were collected in two different positions (saltmarsh edge or tidal creek). No overlap of isotopic niches was observed between size class 1 and 2 individuals. Only the isotopic niches of the individuals belonging to size class 2 and 3 collected in BA station were strongly overlapped, indicating a similar use of resources. This probably indicates that *S. aurata*, growing from size 1 to size class 2, focuses on different resources but these preferences remain similar between sampling positions. The results confirm what previously observed: the strongest changes appeare at the beginning of lagoon colonization (from size class 1 to 2). The smallest and the biggest isotopic niche were recorded for size class 1 in respectively DE tidal creek and saltmarsh edge. Size class 1 individuals collected in BA station had a relatively small niche width while size class 2 individuals collected in DE had a proportionately large one. Analyzing the width of the niches, the results indicate that size class 1 individuals collected in BA station were quite selective. This selective behavior appears also in size class 1 individuals collected in the tidal creek. Furthermore, individuals belonging to size class 1 collected in DE saltmarsh edge, which probably travelled from the sea inlet till the lagoon, were entirely generalist. An interesting discover was that the niche of size class 1 individuals of DE tidal creek and DE saltmarsh edge were overlapped, indicating a similar resource utilization. Among size class 2 individuals, the ones collected in the tidal creek had a smaller niche width than the ones caught in the saltmarsh edge, indicating that probably inside the tidal creek *S. aurata* individuals manage to behave in a more selective and specialized way.

To analyze correctly the isotopic signature and the isotopic niche, to detect any differences between sampling positions, stations or size classes, environmental sources of organic matter were analyzed. Results of mixing models provided evidence that the contribution of potential sources of organic matter to the trophic pathway

of *S. aurata* clearly varied across size classes, with no strong differences across sampling positions. Generally, the strongest differences in basal sources occured between sampling site DE and BA. Indeed, differences between sampling positions occured only considering $\delta^{13}C$. Especially in DE station higher values of $\delta^{13}C$ were found in the saltmarsh edge compared to the tidal creek. Mixing the models elaborated for each sampling position of DE station, provided a quantitative description of the support of the basal sources to early development stages of *S. aurata*. In general, for *S. aurata* size class 1, the most likely contribution was given by particulate organic matter (POM) in both the intertidal creek and the saltmarsh edge, indicating a similar behavior in both sampling positions. As *S. aurata* growed (size class 2), the main source supporting the trophic pathway were *Ulva* sp., in both sampling positions. In accord with what observed by Andolina (2017) the importance of *Ulva* sp. increased in size class 2 individuals. This result may indicate that size class 2 *S. aurata* eat organisms that are closely associated with the *Ulva* sp. while small individuals are more associated with the water column (POM). Lastly, using mixing models, for both size classes and both sampling positions, plankton, SOM and Halophyte provided a low contribution.

The use of an integrated approach, combining stomach content, stable isotope and the head shape analysis helped to determine the feeding ecology of *S. aurata* in the saltmarsh habitats during growth. In this work it was possible to hypothesize and evaluate the reason of the different utilization of the tidal creek and the saltmarsh edge by *S. aurata* during the first period within the lagoon habitats. Also, it was possible to detect the changes in diet and the use of resources during growth. The most evident differences occured among the first developmental stage (less than 25 mm standard length) and main reason that drived the movement of *S. aurata* among the habitats could have been the presence of food and resources. Overall, the behavior of *S. aurata* changed mainly among size classes: small individuals are associated mainly with water column and small prey while bigger individuals, which feed large prey, probably are associated with macroalgae and substrate. These preferences in diet furthermore changed during growth both considering the stomach content and the stable isotope, influencing therefore the movement of individuals. In general, in this study it was possible to observe that the major differences in diet and feeding ecology of *S. aurata* occured depending on size classes and not on the sampling position. Therefore, individuals of the same size class search for the best resources moving from the saltmarsh edge to the tidal creek. Finally, the study and the evaluation of the secondary production helped to assess the potential nursery role of the saltmarsh habitat. No studies concerning secondary production of juveniles *S. aurata* had been conducted in Venice lagoon. For the management of the lagoon habitats and to evaluate the nursery role of transitional water ecosystems, particularly attention should be given to each position of the saltmarsh habitats, and especially the tidal creeks since they seem to contain an abundant quantity of food and resources preferred by *S. aurata*.

GENERAL CONCLUSION

Transitional water ecosystems provide to human a wide range of valuable ecosystem services and goods (Newton, 2018; Rova et al., 2015, 2019). Among the different ecosystem services that transitional water ecosystems provide, the maintenance of transitional and marine fisheries is extremely important (Barbier et al., 2011). Generally, the maintenance of fisheries in these ecosystems is governed by the provision of suitable reproductive habitats and nursery grounds, or shelter living space with high habitat quality, food sources and good hydrodynamic conditions (Barbier et al., 2011; Newton, 2018). Moreover, transitional water ecosystems represent essential habitats for juvenile marine migrant fish species, performing the function of elective nursery areas for their juvenile stages (Beck et al., 2001; Boesh and Turner, 1984; Cabral et al., 2007; Dahlgren et al., 2006; Deegan et al., 2000; Elliott and Hemingway, 2002; McLusky and Elliott, 2004; Mendes et al., 2014; Vasconcelos et al., 2007, 2008; Whitfield and Pattrick, 2015). Inside transitional water ecosystems, juvenile marine migrant fish find more suitable condition for metabolic growth, namely high food availability, favorable water temperature and low biotic stress (e.g. less predation) (Beck et al., 2001; Blaber and Blaber, 1980; Cabral et al., 2007; Dahlgren et al., 2006; Elliott and Hemingway, 2002; Gibson, 1994; Gillanders et al., 2003; McLusky and Elliott, 2004; Miller et al., 1985; Tournois et al., 2013; Vasconcelos et al., 2010, 2011; Whitfield and Pattrick, 2015).

It is well known that transitional water ecosystems, occupying highly prized locations, are some of the most heavily used and threatened natural systems in the planet (Barbier et al., 2011; Bassett et al., 2013; Lotze et al., 2006; Sheaves et al., 2015; Worm et al., 2006). Unfortunately, ecological needs and human demands can conflict sharply (Borde et al., 2003; Chittaro et al., 2009) and some habitats used by juvenile fish, (e.g. saltmarsh) are extremely vulnerable to degradation or loss (Brown, 2006) and can easily alter, reduce or disappear (Tagliapietra et al., 2011). The increasing difficulties associated to protection of an entire ecosystem, due to limited time and funds (Mohan et al., 2015), has led to the need of identification the conservation priority. The identification of nursery habitats is indeed an extremely important tool for the maintenance and conservation of the ecosystem services provided by transitional water ecosystems (Newton, 2018) and to generate strategies for the maintenance of fishery resources (Avigliano et al., 2017; Beck et al., 2001). Moreover, identify nursery habitats could help to prioritize the management actions towards specific and valuable habitats or portions of the lagoon (Sheaves et al., 2015). Due to the high complexity, many useful approaches to estimate the nursery value of a transitional water ecosystem, individually, are not able to provide a complete view of the problem (Sheaves et al., 2015). Therefore, to identify the true nursery value of a habitat it is essential to understand and consider all the complex dynamics that support nursery function (Sheaves et al., 2015), combining various approaches and techniques. Even if

density of individuals could help understand the role of different transitional water ecosystem habitats, many other factors must be considered to identify the true nursery habitats.

In this study, an integrated approach was used, considering as many factors as possible (e.g. the sea-lagoon connectivity, the use of lagoon habitats during ontogenetic growth, the response of individuals to abiotic conditions, the trophic relations within the habitats).

In the first part of the thesis the sea-lagoon connectivity and the entrance in the whole Venice lagoon of marine migrant fish species through all the three sea inlets were analyzed. For two years, standardized sampling of eggs, larvae and juveniles were conducted along three sea-lagoon edge gradients, both in marine and in lagoon stations, to identify any differences between sub-basins. It was observed that for most species, not all the life stages (eggs, larva and juveniles) were found within the lagoon. Furthermore, the entry phase in the lagoon changed according to different taxa. While *S. pilchardus* and *S. sprattus* seems to enter the lagoon already at eggs and larvae stage, other very common species such as *Chelon* spp. and *S. aurata* seems to complete the larval phase at sea and then enter the lagoon at a more advanced ontogenetic stage, the juvenile stage. This indicate that for these species the entrance in the lagoon does not seem to be attributable to a mere passive transport with tidal current. The reason of these differences in lagoon entry could be attributed to various reason: i) different species can spawn in different marine areas and the passive transport and the time of arrive near the coast could varied in relation to wind, currents and distance of spawning area from the lagoon, ii) some species, during juvenile phase, could prefer areas of canal than shallow water, making difficult to capture them with seine net.

The three sea inlets are different in structure, in direction and strong of forcing winds (*bora* from N-E and *scirocco* from S-E) (Bellafiore et al., 2008; Gacic et al., 2002; Massalin and Canestrelli, 2004) and in water exchange. However, results of ichthyoplankton component, which is strongly related to hydrodynamic characteristics (Bolle et al., 2009; Chiappa-Carrara et al., 2003; Perez-Ruzafa et al., 2004; Robins et al., 2012), does not show significant differences between sub-basins or between positions (sea-lagoon). These results indicate the presence of a strong sea-lagoon connectivity through all the three sea inlets. According to Ghezzo et al. (2010) models, the construction of mobile barrier of Mo.S.E., useful to protect Venice from high tide extreme events (Campostrini and Dabalà, 2017), should change flows through the sea inlets. These modifications could influence the entrance and the retention of organisms within the lagoon habitats.

The pattern resulting from the analysis of post-larval and juvenile component, due to their different swimming behavior in the water masses and to their different arrival in lagoon water, was more complex. *Chelon* spp. and *S. aurata* were the most represented taxa and were found in all three sub-basins with high abundance in both sampling years. Analyzing the spatial differences of juvenile densities and analyzing the colonization and the center of gravity index (I_C, COG), it was possible to observe that the areas under the

influence of the Malamocco and Chioggia inlets (central and south sub-basins) are characterized by the highest abundance of marine migrant fish. However, in these two sub-basin fish remains in the marine stations rather than accumulate within the lagoon. Conversely, in the north sub-basin higher density of marine migrant, especially *S. aurata* and *C. saliens*, was found in the lagoon stations, indicating a quite stable transport of individuals inside this sub-basin.

These results seem indicate that the north portion of the Venice lagoon attract the juvenile marine migrant fish. In north sub-basin fish seem to concentrate in lagoon station before the other sub-basins. An explanation for this great colonization could be attributed to hydrodynamic and meteorological favorable condition that drive individuals near the coast of Lido inlet rather than Malamocco and Chioggia. A second explanation could be attributed to the different morphology of the three sea inlets (Bellafiore et al., 2008; Gacic et al., 2004), which can facilitate or slow down the entry of individuals from the coast. Lastly, the three sub-basins could be colonized in a different way in relation to their environmental characteristics and to the habitats composition (Franco et al., 2006, 2009; Franzoi et al., 2010; Malavasi et al., 2004; Tagliapietra et al., 2009). The presence of different interconnected type of habitats such as seagrass beds, sand flats, mud flats, saltmarshes and intertidal creek, which can play different functional roles could influence the active entrance of juvenile marine migrant from the sea. Probably, the presence of suitable habitats (e.g. marsh creeks) located near the sea inlets facilitate the colonization of this sub-basin. In the central and south sub-basins, the absence of a high structured mosaic of habitat distributed linearly along the sea-lagoon edge gradients still near the sea inlets, could had negatively influenced the distribution of marine migrant juvenile fish in these sub-basins, favoring a greater colonization of the northern sub-basin. Moreover, the north sub-basin, is the one with the lowest salinity due to the presence of the main freshwater tributaries in the lagoon (Zonta et al., 2005). Freshwater and substance coming from the mainland through the rivers, probably helping to increase the production and thus the trophic resources, is very sought after by juveniles. Indeed, among the environmental parameters, salinity could play an important role attract individuals preferably in north sub-basin.

Overall, in the first chapter, results seem to highlight a strong connection between sea and lagoon and a passive transport of eggs and larvae within the lagoon through the sea inlets. No differences between sub-basins has been detected considering ichthyoplanktonic community. Conversely, analyzing the juvenile composition, which can move actively to found favorable morphological or environmental condition, differences between sub-basins appear. The north sub-basin seems to show the best conditions for the colonization of marine migrant fish, as for example the presence of a more complex mosaic of habitats, suitable environmental conditions and probably trophic resources or shelter zone.

In the second chapter of the thesis, the research was focused on the north sub-basin, which seemed abundantly colonized by juveniles of marine migrant fish species. In this chapter the distribution of juvenile

fish in different habitats during the period of growth within the lagoon has been analyzed. The presence of many different types of shallow water habitats in the Venice lagoon (Franco et al., 2006, 2010; Franzoi and Pellizzato, 2002; Franzoi et al., 2005) often found in proximity to each other - especially in the Northern sub-basin - allows species to occupy multiple habitats during their development, due to ontogenetic habitat shifts (e.g. Adams et al., 2006; Bostrom et al., 2011; Elliott and Hemingway, 2002). Accordingly, it is well known that, inside transitional water ecosystems, various factors such as environmental characteristics, spatial availability (Able and Fahay, 1998; Elliott and Hemingway, 2002; Herzka, 2005), changes in body morphology, swimming ability and development of digestive tract (Cataldi et al., 1987; Pita et al., 2002; Russo et al., 2007; Tancioni et al., 2003) can lead to changes in preference towards a certain habitat or environmental conditions (Adams et al., 2006; Beker and Sheaves, 2005; Minello et al., 2003). These changes can occur also during the first months of permanence of these species within the Venice lagoon. To represent different ontogenetic stages, the three species belonging to Genus *Chelon* and *Sparus aurata*, the most abundant and frequent marine migrant species in the north sub-basin of Venice lagoon during the sampling period, were divided in different size classes following the literature about diet habits (Baldo and Drake, 2002; Cataldi et al., 1987; Ferrari and Chieregato, 1981; Russo et al., 2007). In this work it has been showed that changes in habitats and environmental conditions can be effectively detected by considering two to three different size classes. Furthermore, results suggest that it is better to consider different size classes separately, because for each species, each size classes behave differently showing different association with habitat and environmental conditions

According to their spawning season, *C. auratus*, *C. ramada* and *S. aurata* start to enter the Venice lagoon during the colder months. Results indicate that generally the three size classes of *C. auratus*, *C. ramada* and especially *S. aurata* have a stable alternation in presence and abundance inside the habitats of the Venice lagoon. In contrast, *C. saliens* has more than one period of entrance inside the lagoon (Gandolfi et al., 1991) and a constant presence inside the lagoon (Franzoi et al., 2010). Finally, also the presence and the abundance of *S. pilchardus* and *P. flesus* seem to be constant during all the sampling period while *S. sprattus*, as previously pointed out by Solberg et al. (2015) and Dulcic (1998), prefer colder month as March.

Considering habitat typology, except for *C. saliens*, which prefers marsh creeks and concentrates in saltmarsh habitats located near the lagoon edge, results show that the other species changes their preferences towards habitat and environmental conditions with ontogeny, according also to Nagelkerken (2007), Whitfield and Pattrick (2015) and Ribeiro et al. (2012).

Considering habitat typology, the seagrass meadows are worldwide considered to have a fundamental role for fish fauna and in maintaining populations of commercially and recreationally exploited fisheries (Jackson et al., 2011; Vizzini et al., 2002). Indeed, seagrass meadows generally perform important functions as feeding, shelter and nursery areas (Heck et al., 1997, 2003; Jackston et al., 2001; Nagelkerken et al., 2008, 2015;

Whitfield, 2016), are a major primary producer, supporting detritus-based trophic webs (Nordlund et al., 2016; Scapin et al., 2018) and are preferred by many marine migrant species, as reported by other studies (e.g. Ford et al., 2010; Heck et al., 2003; Whitfield, 2016). However, Franco et al. (2006) observe that, considering the whole Venice lagoon, the seagrass bed habitats have the lower densities of juvenile marine migrant fish, suggesting a minor nursery role. The results of Franco et al. (2006) also suggest that even seagrass beds habitats less degradated, as the one located in the south sub-basin (Curiel et al., 2014; Sfriso and Facca, 2007), perform a minor nursery role. As observed also by Franco et al. (2006), overall, in this study, among the different habitats present in the northern sub-basin of Venice lagoon, seagrass beds do not seem to be preferred by many marine migrant species. Being colonized for a short time and by a few marine migrant species, this habitat does not seem to support massively the growth of the marine migrant individuals and thus it does not play any strong nursery role in the Venice lagoon. Conversely, saltmarsh habitats being colonized by more marine migrant species during at least one period of their life-cycle (e.g. *C. saliens*, *S. aurata* and *S. sprattus*), as observed also by other works both in the Venice lagoon (Franco et al., 2006) and in other areas (Cattrijsse and Hampel, 2006; Deegan et al., 2000; Rebeiro et al., 2012; Whitfield, 2016), seems to act an important role for juvenile marine migrant fish.

Among environmental parameters, salinity, turbidity and confinement affect the distribution of many species. Inner stations of the lagoon, characterized by low salinity values, low percentage of sand, long water residence time and high turbidity seem preferred by many marine migrant species (e.g. *S. pilchardus*, *S. sprattus*, *P. flesus*, *C. ramada*, *C. saliens*), as pointed out also by other authors (Bodinier et al., 2010; Harrison and Whitfield, 2006). This is probably due to their higher tolerance to salinity variations and to the higher abundance of trophic resources that characterize confined lagoon areas (Islam et al., 2006; Marshall and Elliott, 1998). Among the different considered habitats, the preferences of individuals of the different species toward a specific habitat (e.g. marshes) could be related to the lower predation risk. Moreover, saltmarshes provide a good food-rich place to forage (Boesh and Turner, 1984; Irlandi and Crawford, 1997) as well as protection from predation (Boesh and Turner, 1984; Irlandi and Crawford, 1997; Minello and Zimmerman, 1983). However, the preferences toward a specific habitat could be probably also related to the trophic and feeding role of these habitats. Indeed, the diet seems to be the factor that strongly affect the first developmental stages (Pita et al., 2002; Russo et al., 2007; Tancioni et al., 2003) and the possible preys distribute differently inside the transitional water ecosystems (De Biasi et al., 2003; Kneib, 1984; De Souza et al., 2013). *S. aurata* individuals, for example, once entering inside the lagoon, concentrates in marsh creek independently from their location in the sea-lagoon edge gradient, probably for trophic or shelter reasons. After *S. aurata* individuals start growing, they prefer saltmarshes located in the inner part of the lagoon, indicating a progressive entrance and colonization of the lagoon habitats. Probably, for some marine migrant species (e.g. *C. saliens* and *S. aurata*), the presence of suitable habitats (e.g. marsh creeks) located near the sea inlets could facilitate the colonization of individuals from the sea and toward the inner part of the lagoon.

Indeed, some species prefer a certain habitat typology still from their entrance into the lagoon and consequently individuals belonging to these species search for it along the sea-lagoon gradient and use it as stepping stone.

From the second chapter of the thesis it appears clear that not all the lagoon functions as nursery for the species and that individuals of the same species can use different lagoon habitats, often located in different portion of the sea-lagoon edge gradient, in relation with ontogenetic stage or can use the same habitat, as saltmarsh for example, as a stepping stone for the colonization of the inner part of the lagoon. Consequently, it appears extremely important to identify and protect the nursery habitats that provide the more species and the most recruits to adult populations, even if they change with growth of individuals (Mohan et al., 2015; Sheaves et al., 2015), because the identification of nursery areas is a very important tool to generate strategies for the maintenance of fishery resources (Avigliano et al., 2017; Beck et al., 2001). The results of the second chapter suggest therefore that saltmarsh habitats of the Venice lagoon, and especially those located near the lagoon edge, support, in general, greater density of marine migrant species. In addition, a particular attention should be paid to the presence of possible predator or to the trophic and feeding relationships that could explain why individuals concentrate in some particular areas and within some particular position of transitional water ecosystems, such as the inner saltmarsh habitats in the case of the Northern sub-basin of the Venice lagoon.

Using an integrated approach combining the stomach content, the stable isotope and the head shape morphology analyses, the third chapter of the thesis helps to assess the habitat preferences and the feeding ecology of a target marine migrant species (*S. aurata*) in saltmarsh habitats. The habitats selected for this study were saltmarshes located in the northern sub-basin of the Venice lagoon. In this part it was possible to detect differences among the saltmarsh edge and the tidal creek and observe ontogenetic diet changes during growth. The purpose was to understand how juvenile of *S. aurata* use the saltmarsh habitat and which are the trophic relationships that increase the nursery value of a lagoon.

Among the different saltmarsh habitats sampled, the ones located near the sea inlet, characterized by a higher influence of salty sea water, are less colonized than the ones located near the lagoon edge. This should indicate that *S. aurata* individuals, after entering in the lagoon from the sea inlet, especially from late February (Ferrari and Chieregato, 1981; Rossi, 1986), start immediately to move towards the inner part of the lagoon probably following a saline gradient (Cabral et al., 2007; Islam et al., 2006; Marshall and Elliott, 1998; Vasconcelos et al., 2011). No differences among the two positions sampled in saltmarsh habitats (tidal creek and saltmarsh edge) were detected considering standard length, total weight and eviscerated total weight. Individuals probably move periodically among the two positions, exploiting resources of the tidal creek and the saltmarsh edge in a balanced way and thus growing in a similar way. However, differences between the sampling positions were observed both considering *S. aurata* stomach content and

environmental prey availability. The number of individuals with a full stomach was higher in the tidal creek rather than in the saltmarsh edge, indicating that this portion of saltmarsh contains more prey and resources, as confirmed by environmental prey availability analysis. These differences probably indicate that each portion of the saltmarsh has a specific role and is colonized in different times and for different trophic reasons. Moreover, result of prey selectivity index suggest that *S. aurata* enter in tidal creek due to the high abundance of preferred prey.

The total ingested biovolume of *S. aurata* increase rapidly during all sampling period, indicating that the presence of many and different prey in saltmarsh habitats helps individuals to accumulate a lot of energy right from the beginning of lagoon colonization. Analyzing in detail the taxa ingested, *S. aurata* ate mainly zooplanktonic and benthic preys, belonging to five taxa of Animalia: Amphipoda, Copepods (Calanoida, Cyclopoida, Harpacticoida), Decapoda, Mysidacea and Polychaeta. Each prey was selected by *S. aurata* with different quantity in relation with ontogenetic growth. No strong differences appeared between sampling positions, considering both dietary index and feeding strategy. Instead, results suggest that the major changes occur during the first arrival of individuals within the lagoon. *S. aurata* individuals, coming as larvae from the sea and probably still eating planktonic prey (e.g. Cyclopoida and Calanoida), change diet preferences toward larger and benthic prey (e.g. Harpacticoida, Amphipoda and Polychaeta). A confirm of this hypothesis emerges analyzing the carbon and nitrogen stable isotope: in the saltmarsh near the sea inlet, size class 1 individuals have low and similar isotopic values of both $\delta^{13}C$ and $\delta^{15}N$, indicating generally a low trophic position and probably that they are still enter the lagoon. Overall, both using stomach content and stable isotope analysis, seems that *S. aurata* individuals belonging to the same size class, even if they were collected in the two different sampling positions, behave in a similar way, preferring the same preys. These results suggest that individuals move periodically between the saltmarsh edge and the tidal creek in order to find and exploit the preferred resources. The ontogenetic growth of *S. aurata* seems to be the strongest driver that moves the trophic ecology of this species and its habitat preferences.

Changes in diet observed during growth were compared with change in head shape morphology with the purpose to observe any common feature. The most marked changes were observed among the smaller individuals: between *S. aurata* size class A and B, thus from 15-20 to 20-25 mm. In general, *S. aurata's* expansion in the head dimension, appears especially in the upper part above the eyes. The rounding of the head and the tapering of the south are the most evident changes. These changes in head morphology can be summarized in a downward displacement of the mouth and a general swelling of the head in the maxilla, bringing the head from a longer and tapered shape to a squatter and flattened one. The movement of the mouth downwards, could help individuals to change their diet, from planktivory to benthivore, while the swelling of the jaw, combined with the development of the musculature, could help to make stronger bites to increase the size of preys. Moreover, these changes seem to be strongly related and agree with the

changes in diet: especially the increase in mean prey size and the diet shift from Cyclopoida towards Harpacticoida.

The use of an integrated approach, combining stomach content, stable isotope and the head shape analysis helped to determine the feeding ecology of *S. aurata* in the saltmarsh habitats during growth. In the third chapter it was possible to hypothesize and evaluate the reason of the different utilization of the tidal creek and the saltmarsh edge by *S. aurata* during the first period within the lagoon habitats. Also, it was possible to detect the changes in diet and the use of resources during growth. The most evident differences occurred among the first developmental stage (less than 25 mm standard length). The main reason that drive the movement of *S. aurata* among the habitats seems to be the presence of food and resources. Overall, the behavior of *S. aurata* changed mainly among size classes: small individuals are associated mainly with water column and small prey while bigger individuals, which feed large prey, probably are associated with macroalgae and substrate. These preferences in diet furthermore changed during growth both considering the stomach content and the stable isotope, influencing therefore the movement of individuals. In general, in this study it was possible to observe that the major differences in diet and feeding ecology of *S. aurata* occured depending on size classes and not on the sampling position. Therefore, individuals of the same size class search for the best resources moving from the saltmarsh edge to the tidal creek. For the management of the lagoon habitats and to evaluate the nursery role of a lagoon, particularly attention should be given to each position of the saltmarsh habitats, and especially the tidal creeks since they seem to contain an abundant quantity of food and resources preferred by *S. aurata*.

From the results of this thesis, the complexity in understanding and evaluating the nursery role of the transitional water ecosystems appears clear. Appear clear that some portion of the lagoon of Venice (the north sub-basin and the inner less salty water of this sub-basin) and in particular some habitats (saltmarshes) seem selected and preferred by juveniles. However, the preferences toward habitat or environmental conditions change depending on species and, within the same species, on ontogenetic stage. The hypothesis that the changes in habitat and environmental preferences are mainly related to dietary preferences and prey availability seems to be confirmed analyzing the habits of a commercially important marine migrant fish species, *S. aurata*. Therefore, to increase and manage the nursery values of a transitional water ecosystems, the human actions should consider all the different aspect that drive behavior of the fishes, namely for example the entrance in transitional water ecosystems, the colonization of different shallow water habitats, the abiotic characteristics and the position of the habitats within the transitional water ecosystems, the feeding preferences of the different species and the prey availability. Moreover, must be taken into account that all these aspects changes in relation with ontogeny.

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APPENDIX A

Table A1 - Environmental parameters collected in the stations sampled with bongo net during the eight samplings campaign of the two sampling cycles, divided for sea-lagoon transect.

	Temperature (° C)								
Sampling cycle	North sub-basin Campaign	MAn	LEn	LIn	BOn	SA	SF	BU	DE
I	I	14.50	13.06	12.98	13.10	12.89	13.08	8.72	5.99
I	II	7.95	7.65	7.94	8.11	7.58	7.72	7.11	5.1
I	III	9.96	9.73	9.43	9.59	9.40	9.70	9.45	8.03
I	IV	14.05	14.53	14.60	14.71	15.05	16.02	16.93	18.3
II	I	12.68	12.82	13.43	13.01	12.31	12.78	10.96	5.68
II	II	5.41	5.29	5.41	5.17	4.80	5.14	4.54	2.08
II	III	8.43	8.34	8.40	8.32	8.34	8.40	8.49	8.59
II	IV	12.68	12.31	12.43	12.65	12.20	13.19	14.10	13.8
Sampling cycle	Central sub-basin Campaign	MAc	LEc	LIc	BOc	FI	SL	CA	
I	I	12.07	12.48	12.57	11.72	11.72	10.55	9.62	
I	II	7.33	7.24	8.12	7.87	7.72	6.33	6.12	
I	III	10.12	9.95	10.00	10.10	10.08	9.81	9.71	
I	IV	13.44	14.16	13.83	12.73	12.97	14.58	15.36	
II	I	12.37	12.62	12.94	12.83	12.81	10.85	10.49	
II	II	6.65	5.93	5.62	5.82	5.70	4.32	4.05	
II	III	8.53	8.47	8.74	8.57	8.91	9.20	9.76	
II	IV	13.04	12.47	11.99	12.35	12.31	13.76	14.44	
Sampling cycle	South sub-basin Campaign	MAs	LEs	LIs	BOs	VA	CH	NO	
I	I	11.76	12.04	11.89	12.26	11.56	11.09	7.09	
I	II	6.98	7.31	8.38	8.50	7.68	8.16	5.02	
I	III	9.95	9.65	9.70	9.87	9.53	9.39	9.04	
I	IV	13.46	12.84	12.89	12.52	12.76	12.53	15.73	
II	I	12.74	13.24	13.15	14.06	13.35	13.54	8.73	
II	II	5.63	5.61	5.95	5.95	5.87	5.94	2.23	
II	III	8.72	8.75	8.64	8.60	8.47	8.65	9.26	
II	IV	12.12	12.16	12.17	11.90	11.90	12.02	13.21	

	Salinity (psu)								
Sampling cycle	North sub-basin Campaign	MAn	LEn	LIn	BOn	SA	SF	BU	DE
I	I	36.67	35.33	35.29	35.28	35.16	35.31	32.36	26.1
I	II	37.33	37.26	35.88	37.35	36.44	36.46	35.76	25.9
I	III	41.19	38.72	38.92	39.09	38.62	39.67	38.61	9.03
I	IV	40.10	37.26	37.87	38.28	37.96	37.28	33.86	14.0
II	I	39.94	39.53	40.79	39.90	39.39	39.33	36.66	25.1
II	II	39.50	41.00	41.00	40.00	40.00	40.50	39.50	30.0
II	III	37.54	37.88	37.45	37.18	36.52	37.26	35.49	28.8
II	IV	36.79	37.04	37.14	37.20	37.51	36.19	34.40	13.2

Sampling cycle	Central sub-basin Campaign	MAc	LEc	LIc	BOc	FI	SL	CA
I	I	35.50	35.53	35.56	33.81	35.38	34.84	33.94
I	II	35.95	37.23	36.74	37.75	37.67	35.88	34.98
I	III	40.86	40.67	41.10	41.60	41.45	38.53	36.59
I	IV	41.62	39.43	39.99	42.63	42.26	40.17	39.27
II	I	36.01	35.80	38.02	37.38	37.50	36.26	35.09
II	II	44.10	42.20	41.77	42.10	41.86	40.55	38.57
II	III	38.13	37.52	36.95	37.20	36.25	34.36	34.66
II	IV	36.54	36.30	37.53	36.75	36.96	35.73	34.93
Sampling cycle	South sub-basin Campaign	MAs	LEs	LIs	BOs	VA	CH	NO
I	I	35.76	35.57	35.54	35.78	35.44	34.72	32.45
I	II	35.92	35.75	37.09	37.90	36.66	37.23	33.46
I	III	39.67	39.82	40.33	39.67	39.11	37.70	10.19
I	IV	41.90	38.12	39.37	40.56	42.59	42.90	36.09
II	I	37.46	37.69	38.94	40.99	39.04	39.50	33.19
II	II	36.50	34.50	35.00	35.00	33.00	34.00	30.00
II	III	37.49	37.62	38.35	37.65	38.34	37.52	26.88
II	IV	35.97	35.64	35.66	36.07	36.19	35.95	30.40

	Dissolved oxygen (% saturation)								
Sampling cycle	North sub-basin Campaign	MAn	LEn	LIn	BOn	SA	SF	BU	DE
I	I	83.22	84.30	84.02	82.58	84.09	83.08	84.61	86.75
I	II	98.50	98.74	100.30	96.83	104.83	99.58	94.53	98.73
I	III	90.97	91.08	89.24	90.45	90.13	90.40	91.97	100.3
I	IV	102.47	102.53	102.32	97.26	103.06	97.54	106.94	111.6
II	I	89.26	88.26	86.66	90.74	84.67	89.89	92.21	113.5
II	II	78.51	79.85	77.57	77.80	80.68	77.30	78.66	94.4
II	III	98.26	92.41	96.06	95.53	95.40	95.73	98.28	123.1
II	IV	103.54	100.97	102.95	100.98	102.09	95.44	89.89	74.9
Sampling cycle	Central sub-basin Campaign	MAc	LEc	LIc	BOc	FI	SL	CA	
I	I	85.25	78.13	83.28	80.09	84.05	83.81	85.41	
I	II	108.69	97.27	98.64	97.69	97.65	91.63	104.94	
I	III	90.88	91.08	90.86	89.77	89.55	90.48	89.60	
I	IV	101.55	104.61	103.61	100.34	99.76	95.20	90.82	
II	I	87.83	89.54	86.68	91.70	93.91	89.52	89.74	
II	II	81.78	81.53	80.31	85	80.21	81.33	80.79	
II	III	99.79	99.58	97.08	96.96	94.63	97.01	95.10	
II	IV	118.33	119.81	111.38	114.32	110.39	109.94	107.60	
Sampling cycle	South sub-basin Campaign	MAs	LEs	LIs	BOs	VA	CH	NO	
I	I	88.24	86.55	85.11	84.38	84.63	81.79	87.98	
I	II	109.29	102.00	100.68	92.62	88.22	90.62	75.92	
I	III	90.91	90.47	89.99	89.65	90.07	89.63	77.52	
I	IV	102.36	99.49	99.35	97.72	98.33	99.19	88.57	
II	I	90.32	85.13	86.62	87.15	89.69	89.70	87.32	
II	II	85.09	80.10	80.12	80.88	78.58	80.04	79.2	
II	III	98.93	96.21	96.38	97.55	94.52	91.39	76.46	

II	IV	107.13	109.78	105.97	104.44	105.07	105.38	86.21	

	Turbidity (ftu)								
Sampling cycle	North sub-basin Campaign	MAn	LEn	LIn	BOn	SA	SF	BU	DE
I	I	3.21	4.18	5.29	5.36	8.58	6.78	27.73	10.5
I	II	1.72	1.30	1.88	1.81	3.39	2.99	3.02	2.39
I	III	4.25	7.67	5.93	13.15	17.90	16.84	13.04	11.0
I	IV	1.02	1.22	3.30	5.83	13.13	7.64	13.58	147
II	I	2.27	2.30	2.70	2.82	4.14	3.79	4.46	4.24
II	II	1.93	3.34	3.20	5.26	6.32	6.59	6.08	66.5
II	III	3.98	7.70	7.19	10.92	11.74	12.65	10.81	6.24
II	IV	1.18	0.67	1.68	1.36	2.92	6.90	10.76	28.4
Sampling cycle	Central sub-basin Campaign	MAc	LEc	LIc	BOc	FI	SL	CA	
I	I	4.69	4.65	4.79	4.52	4.86	4.79	18.75	
I	II	1.33	1.39	2.14	1.59	2.01	4.50	19.73	
I	III	3.18	3.42	5.00	4.22	4.64	5.25	7.35	
I	IV	0.84	0.77	1.30	0.78	1.78	5.03	7.22	
II	I	6.46	5.25	9.32	6.86	9.27	12.81	22.25	
II	II	1.37	6.74	8.30	7.93	12.09	20.27	13.98	
II	III	3.38	3.43	4.02	3.67	5.14	5.67	16.26	
II	IV	0.56	0.14	1.98	0.75	1.65	6.47	5.62	
Sampling cycle	South sub-basin Campaign	MAs	LEs	LIs	BOs	VA	CH	NO	
I	I	2.65	3.08	2.97	3.03	2.91	3.52	12.91	
I	II	1.26	0.96	1.28	1.32	2.25	1.41	1.42	
I	III	5.49	5.30	8.82	12.10	11.16	11.28	22.15	
I	IV	1.00	3.12	1.50	1.72	2.07	2.18	4.37	
II	I	2.48	4.78	4.56	3.53	4.26	4.97	5.20	
II	II	2.20	3.14	3.08	3.20	3.51	3.65	3.8	
II	III	2.47	3.30	6.58	4.79	6.37	6.32	8.43	
II	IV	0.29	1.22	3.70	4.67	1.09	3.94	5.15	

	Chlorophyll in water (μg L^{-1})								
Sampling cycle	North sub-basin Campaign	MAn	LEn	LIn	BOn	SA	SF	BU	DE
I	I	1.24	0.88	0.95	0.96	1.01	1.05	1.58	1.07
I	II	0.71	0.60	0.82	0.62	0.56	0.60	0.56	1.03
I	III	0.75	0.98	0.55	1.00	0.96	0.81	0.83	12.03
I	IV	1.12	0.63	0.53	0.60	0.95	0.54	1.00	3.21
II	I	1.02	0.89	0.80	0.92	0.80	0.96	0.57	0.62
II	II	0.43	0.44	0.43	0.46	0.47	0.45	0.32	0.59
II	III	1.15	1.47	1.20	1.16	1.45	1.24	1.25	1.66
II	IV	1,80	1,63	1,37	1,08	1,77	1,49	1,61	4.03
Sampling cycle	Central sub-basin Campaign	MAc	LEc	LIc	BOc	FI	SL	CA	
I	I	0.85	0.69	0.78	0.51	0.50	0.57	0.83	
I	II	0.85	0.89	0.64	0.95	0.70	0.74	0.88	
I	III	0.99	1.03	0.81	0.71	0.99	0.80	0.73	

I	IV	1.86	2.59	1.72	1.22	1.30	0.87	1.01
II	I	1.51	1.49	1.38	1.08	1.40	1.22	1.67
II	II	0.49	0.69	0.50	0.46	0.39	1.00	0.79
II	III	0.76	1.03	0.60	1.03	0.92	1.19	1.55
II	IV	2,37	2,32	1,70	1,74	1,97	1,50	1,39
Sampling cycle	South sub-basin Campaign	MAs	LEs	LIs	BOs	VA	CH	NO
I	I	0.43	0.43	0.45	0.51	0.43	0.48	0.79
I	II	0.52	0.71	0.57	0.59	0.55	0.75	0.60
I	III	0.96	0.66	0.84	1.21	0.83	0.66	2.04
I	IV	2.25	1.72	1.20	1.24	0.98	1.47	1.92
II	I	0.84	0.78	0.96	0.76	0.55	0.98	1.10
II	II	0.18	0.24	0.17	0.19	0.25	0.24	0.33
II	III	1.15	1.07	1.33	0.96	1.23	1.02	1.27
II	IV	2,62	2,35	2,50	2,79	2,38	2,60	2,60

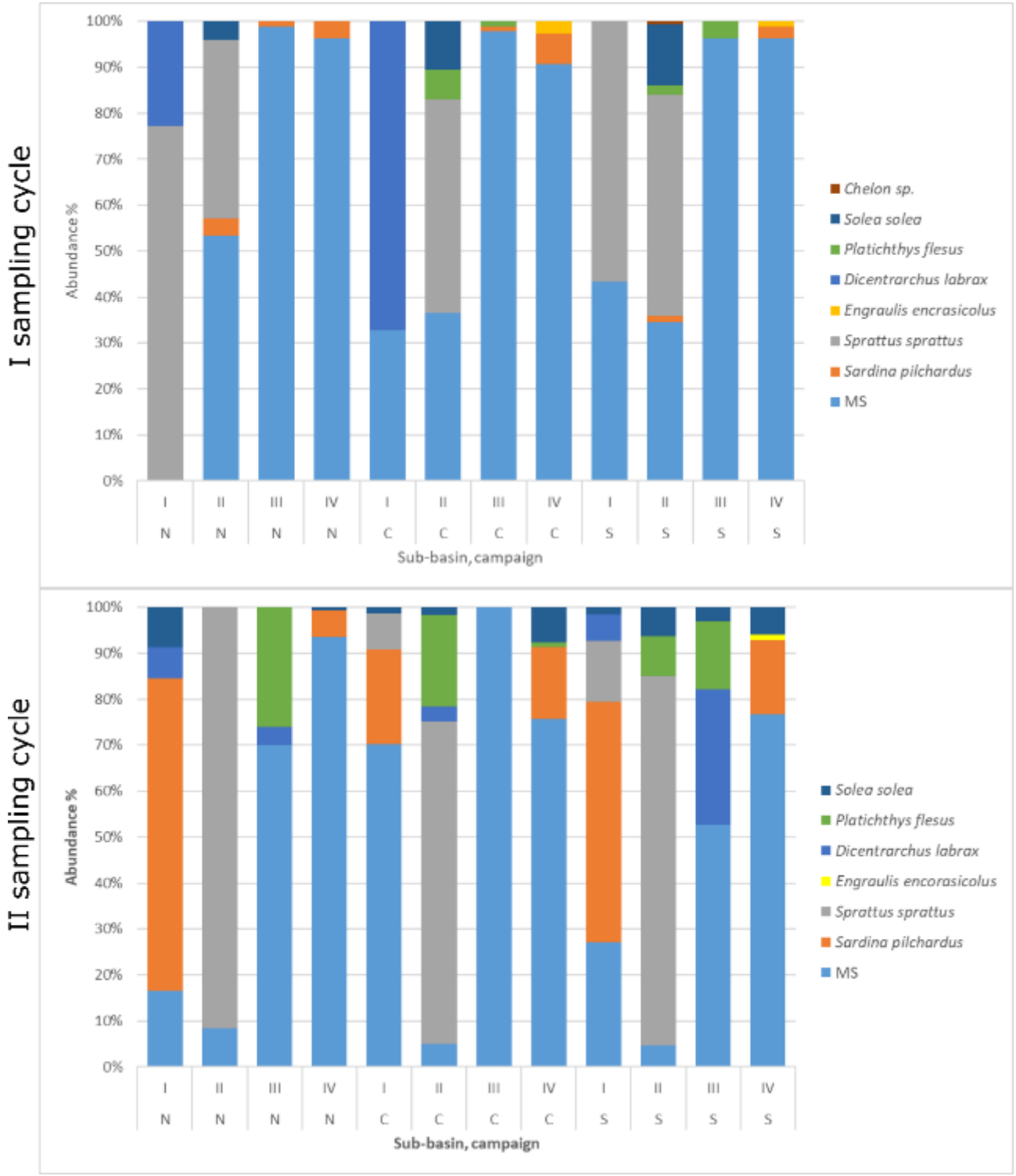


Figure A1 – Ichthyoplankton, eggs only. Abundance % of each species/taxa calculated on the mean density by sub-basin and by campaign, separately for sampling cycle. N = north sub-basin, C = central sub-basin, S = south sub-basin. I, II, III, IV = sampling campaigns. MS = Marine Straggler.

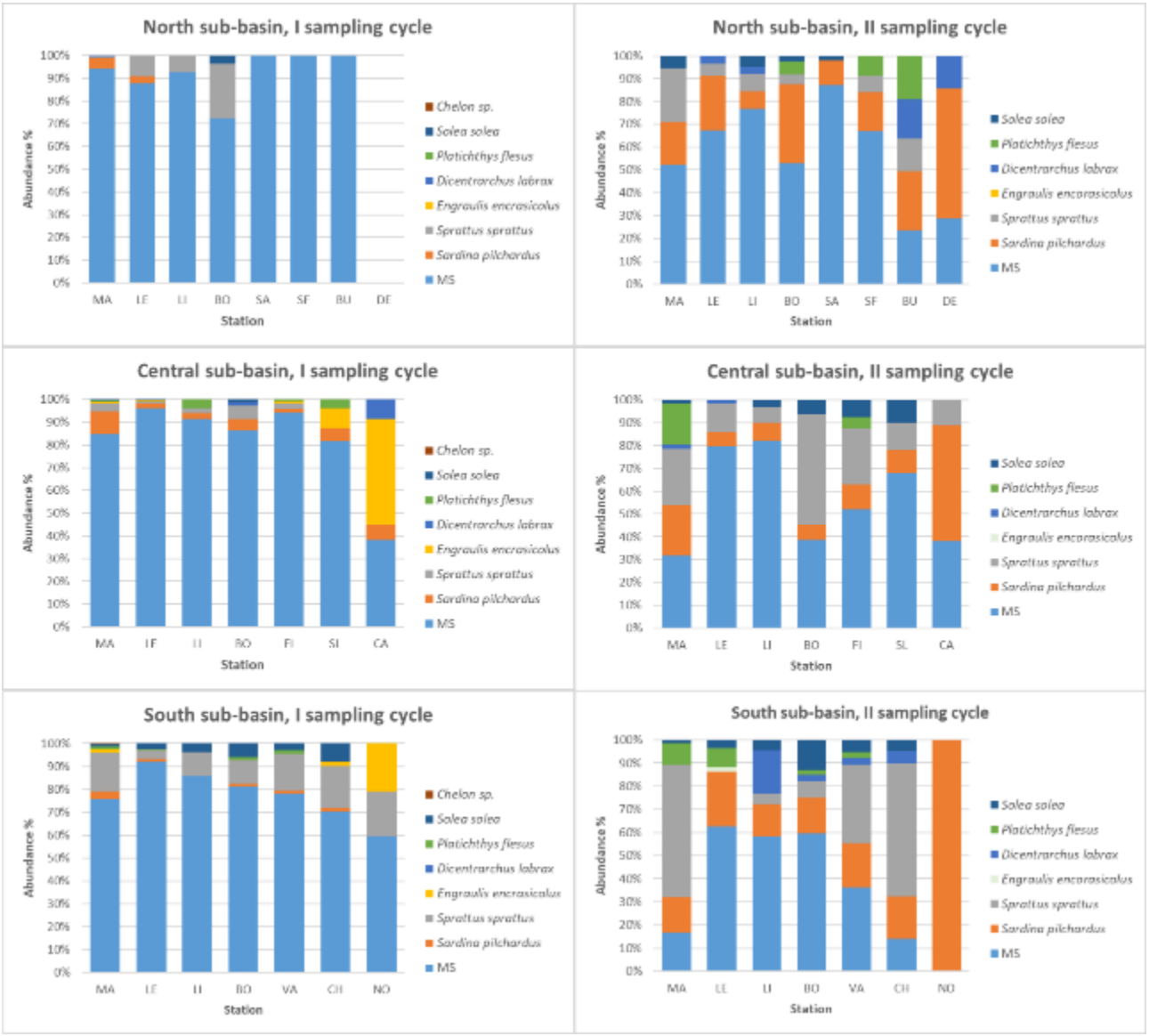


Figure A2 – Ichthyoplankton, eggs only. Abundance % of each species/taxa calculated on the mean density by station, separately for sampling cycle and sub-basin. MS = Marine Straggler.

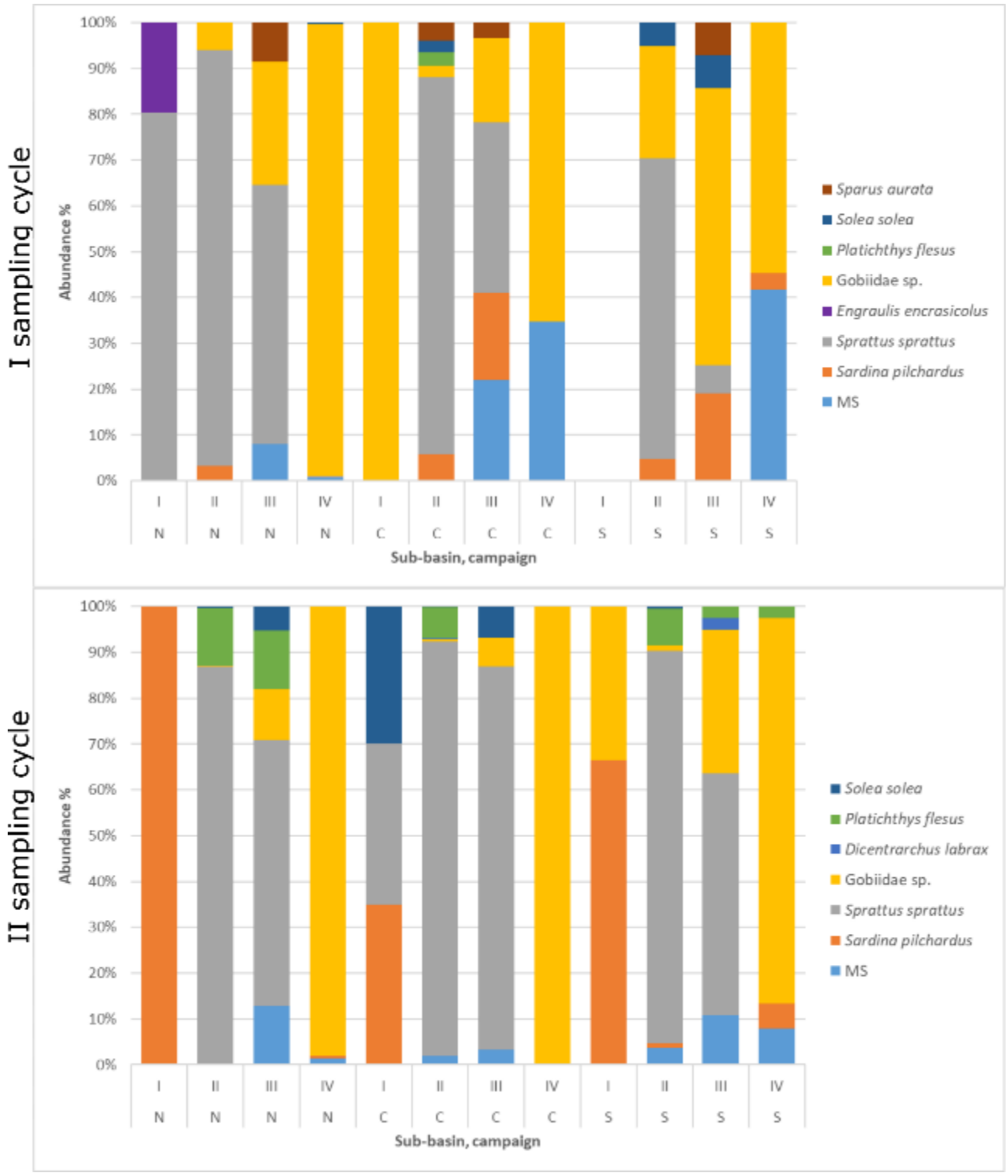


Figure A3 – Ichthyoplankton, larvae only. Abundance % of each species/taxa calculated on the mean density by sub-basin and by campaign, separately for sampling cycle. N = north sub-basin, C = central sub-basin, S = south sub-basin. I, II, III, IV = sampling campaigns. MS = Marine Straggler.

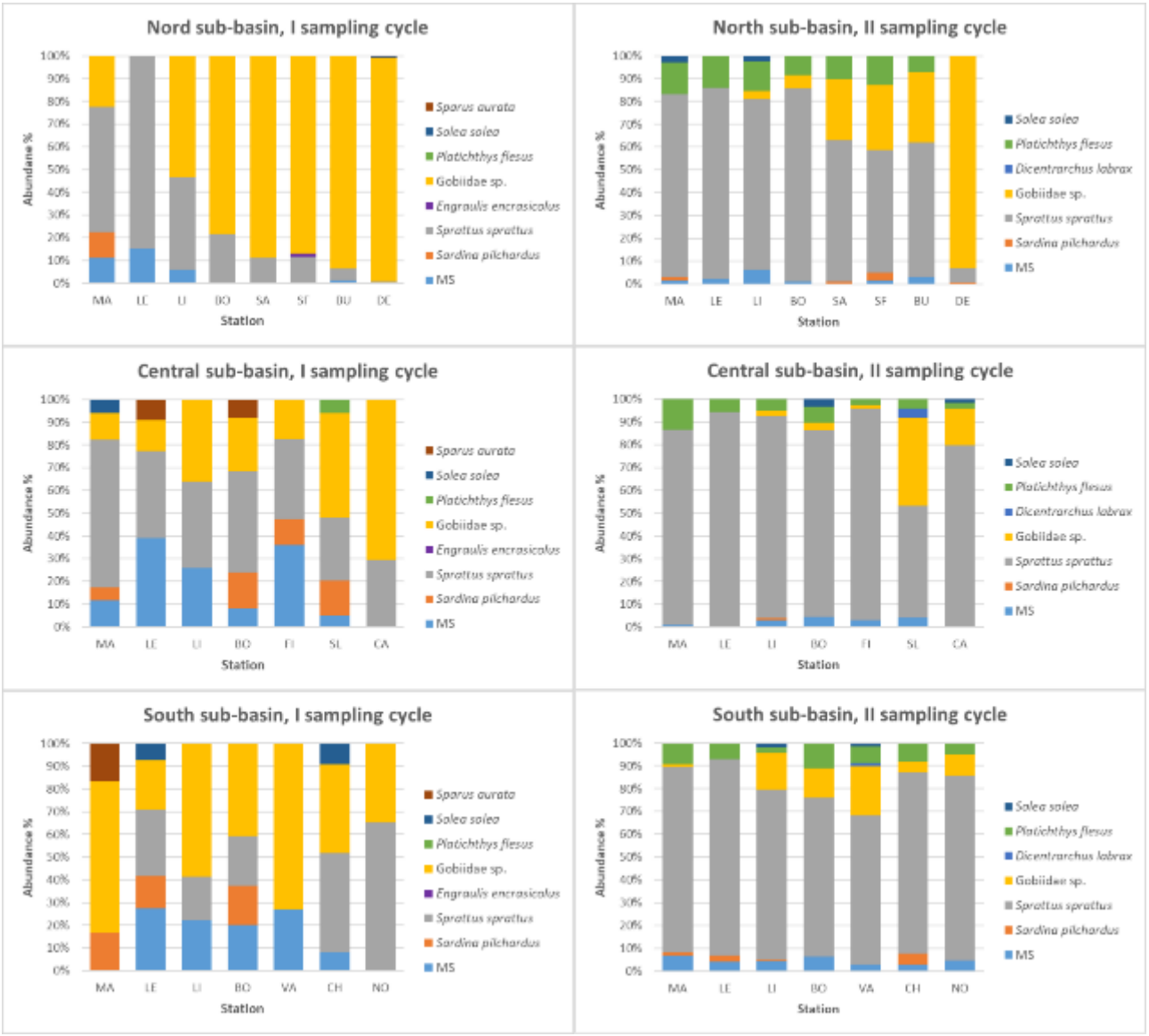


Figure A4 – Ichthyoplankton, eggs only. Abundance % of each species/taxa calculated on the mean density by station, separately for sampling cycle and sub-basin. MS = Marine Straggler.

Table A2. Environmental parameters collected in the stations sampled with seine net during the eight samplings campaign of the two sampling cycles, divided for sea-lagoon transect.

Temperature (° C)

Sampling cycle	North sub-basin Campaign	PS	SN	BA	CR	SC	DE
I	I	10.91	11.50	10.71	9.56	8.82	8.54
I	II	12.47	12.93	11.23	12.43	13.69	14.98
I	III	16.23	16.23	18.94	20.72	22.40	23.53
II	I	12.31	13.17	9.79	11.71	11.87	12.98
II	II	13.37	13.48	12.16	12.95	13.38	14.16
II	III	17.69	17	17.93	22.36	16.36	21.15
Sampling cycle	Central sub-basin Campaign	AL	MU	OT	RA	LT	
I	I	9.95	10.31	9.22	9.27	8.40	
I	II	10.27	11.58	10.51	10.04	12.84	
I	III	15.36	16.25	18.20	17.25	20.88	
II	I	10.89	10.18	11.65	11.08	12.70	
II	II	13.18	12.49	14.28	15.17	14.64	
II	III	18.03	19.11	19.69	21.05	20.07	
Sampling cycle	South sub-basin Campaign	CA	SM	PC	TR	VD	
I	I	8.89	9.53	9.86	11.56	10.65	
I	II	11.82	11.72	11.31	14.36	12.75	
I	III	16.00	16.00	19.22	22.08	22.26	
II	I	10.78	10.38	10.54	8.63	9.38	
II	II	13.74	13.53	14.96	15.41	15.67	
II	III	15.45	16.35	17.58	20.61	23.58	

Salinity (psu)

Sampling cycle	North sub-basin Campaign	PS	SN	BA	CR	SC	DE
I	I	42.35	40.60	39.13	31.84	34.78	30.35
I	II	28.67	33.48	38.18	36.06	37.51	23.61
I	III	36.80	39.32	38.93	35.66	33.74	19.62
II	I	34.00	34.38	35.40	33.51	34.84	24.72
II	II	23.93	28.51	32.90	32.97	33.61	20.22
II	III	28.86	30.46	30.75	30.48	31.17	12.20
Sampling cycle	Central sub-basin Campaign	AL	MU	OT	RA	LT	
I	I	39.36	39.52	33.75	32.74	23.77	
I	II	40.10	40.71	34.87	32.32	31.72	
I	III	37.11	35.86	39.96	39.65	33.71	

II	I	36.60	36.06	35.07	31.66	31.65
II	II	31.29	31.05	34.76	28.62	27.69
II	III	33.64	34.24	34.11	26.88	23.3

Sampling cycle	South sub-basin Campaign	CA	SM	PC	TR	VD
I	I	35.16	38.35	39.60	25.73	23.60
I	II	40.08	38.01	37.72	33.26	32.23
I	III	41.02	41.02	39.20	29.91	29.06
II	I	36.36	35.48	36.83	23.18	28.45
II	II	34.60	32.78	34.88	26.83	18.45
II	III	34.06	35.76	34.3	26.22	22.92

	Dissolved Oxygen (% saturation)						
Sampling cycle	North sub-basin Campaign	PS	SN	BA	CR	SC	DE
I	I	90.2	91.7	98.16	99.06	94.78	98.98
I	II	98.7	98.9	83.47	101.80	119.51	113.13
I	III	98.99	97.79	173.58	124.74	135.35	129.85
II	I	97.56	112.16	88.67	99.97	100.23	122.16
II	II	102.47	105.71	80.58	69.55	90.43	88.90
II	III	93.36	86.56	90	107.73	82.93	135.87
Sampling cycle	Central sub-basin Campaign	AL	MU	OT	RA	LT	
I	I	95.7	95.5	95.5	96.27	84.96	
I	II	90.0	88.6	98.3	94.2	89.30	
I	III	112.7	112.0	121.3	113.76	123.36	
II	I	87.59	100.30	128.40	101.06	90.16	
II	II	101.14	103.41	122.38	111.19	83.52	
II	III	87.78	91.22	107.86	102.72	96.26	
Sampling cycle	South sub-basin Campaign	CA	SM	PC	TR	VD	
I	I	91.1	90.9	87.87	120.37	93.41	
I	II	92.1	93.4	91.75	118.9	112.41	
I	III	102.8	102.77	117.56	106.51	90.93	
II	I	100.18	111.00	100.35	85.64	85.67	
II	II	98.69	106.26	109.97	95.96	96.45	
II	III	87.04	93.88	91.61	113.64	77.91	

	Turbidity (ftu)						
Sampling cycle	North sub-basin Campaign	PS	SN	BA	CR	SC	DE
I	I	24.3	107	1.44	5.79	5.72	7.06
I	II	4.4	12.3	2.6	5.73	4.41	14.05
I	III	3.81	10.09	3.14	6.88	10.90	119.63

II	I	1.19	3.14	2.54	2.94	3.92	9.31
II	II	1.91	1.50	1.65	8.50	3.49	10.14
II	III	10.12	10.04	7.47	26.63	10.68	53.78
Sampling cycle	Central sub-basin Campaign	AL	MU	OT	RA	LT	
I	I	5.8	22.3	10.6	12.24	13.37	
I	II	5.5	20.4	3.0	4.09	14.62	
I	III	0.9	1.3	5.9	19.62	12.54	
II	I	5.98	6.39	2.23	3.95	11.86	
II	II	1.73	0.54	0.02	7.72	13.31	
II	III	1.41	13.59	5.07	13.63	27.19	
Sampling cycle	South sub-basin Campaign	CA	SM	PC	TR	VD	
I	I	2.5	5.8	17.06	9.57	4.12	
I	II	14.5	19.7	10.06	27.89	16.92	
I	III	2.00	1.96	5.56	10.60	18.68	
II	I	3.38	7.03	4.38	5.66	8.74	
II	II	0.71	2.84	3.11	16.18	26.26	
II	III	2.86	9.34	3.89	21.07	25.49	

	Chlorophyll in water (μg L^{-1})						
Sampling cycle	North sub-basin Campaign	PS	SN	BA	CR	SC	DE
I	I	0.69	2.87	0.78	0.77	0.89	1.60
I	II	1.41	1.36	0.77	0.73	0.47	3.43
I	III	1.36	0.95	0.46	1.40	1.43	4.87
II	I	0.90	1.53	1.19	1.40	0.84	3.25
II	II	2.68	2.28	10.79	2.45	2.60	3.00
II	III	0.67	1.32	0.52	2.14	0.85	5.28
Sampling cycle	Central sub-basin Campaign	AL	MU	OT	RA	LT	
I	I	1.18	2.37	1.02	1.33	1.66	
I	II	0.31	0.35	0.29	0.57	1.18	
I	III	0.8	1.37	0.79	1.09	1.37	
II	I	0.90	0.51	0.64	0.95	1.02	
II	II	5.64	3.15	0.52	1.79	1.36	
II	III	0.34	0.40	0.23	1.10	1.59	
Sampling cycle	South sub-basin Campaign	CA	SM	PC	TR	VD	
I	I	0.93	1.16	0.92	1.14	1.61	
I	II	0.88	1.15	1.84	1.52	3.17	
I	III	0.8	0.52	1.25	2.03	2.59	
II	I	1.41	0.93	2.20	2.30	1.85	
II	II	2.81	3.35	2.98	11.15	18.21	
II	III	0.74	1.17	0.81	3.87	4.30	

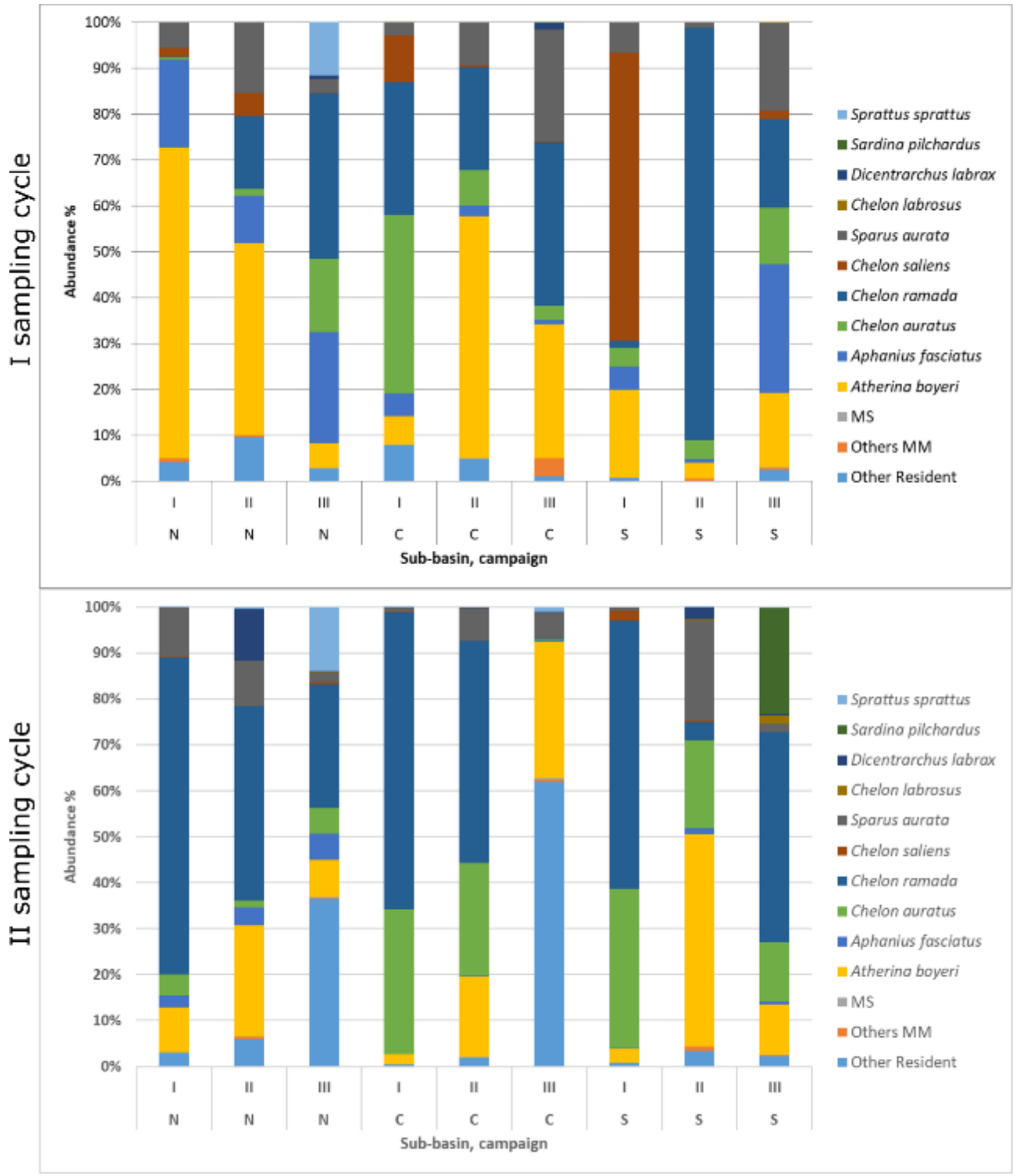
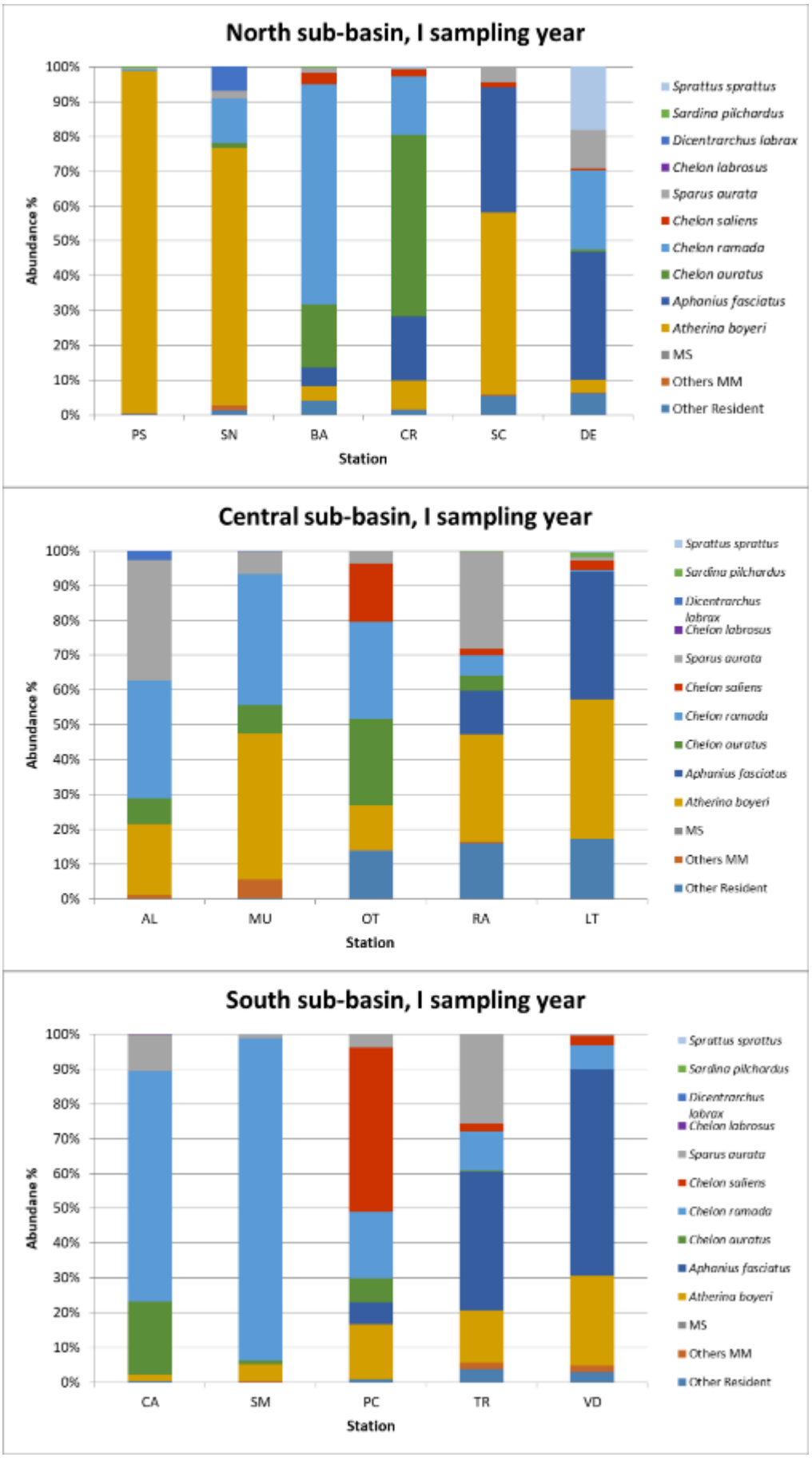


Figure A5 – Juvenile only. Abundance % of each species/taxa calculated on the mean density by sub-basin and by campaign, separately for sampling cycle. N = north sub-basin, C = central sub-basin, S = south sub-basin. I, II, III = sampling campaigns. MS = Marine Straggler, MM = Marine Migrant

A)


North sub-basin, I sampling year
Abundance %
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
PS
SN
BA
CR
SC
DE
Station
Sprattus sprattus
Sardina pilchardus
Dicentrarchus labrax
Chelon labrosus
Sparus aurata
Chelon saliens
Chelon ramada
Chelon auratus
Aphanius fasciatus
Atherina boyeri
MS
Others MM
Other Resident
Central sub-basin, I sampling year
Abundance %
AL
MU
OT
RA
LT
Station
Sprattus sprattus
Sardina pilchardus
Dicentrarchus labrax
Chelon labrosus
Sparus aurata
Chelon saliens
Chelon ramada
Chelon auratus
Aphanius fasciatus
Atherina boyeri
MS
Others MM
Other Resident
South sub-basin, I sampling year
Abundane %
CA
SM
PC
TR
VD
Station
Sprattus sprattus
Sardina pilchardus
Dicentrarchus labrax
Chelon labrosus
Sparus aurata
Chelon saliens
Chelon ramada
Chelon auratus
Aphanius fasciatus
Atherina boyeri
MS
Others MM
Other Resident

B)

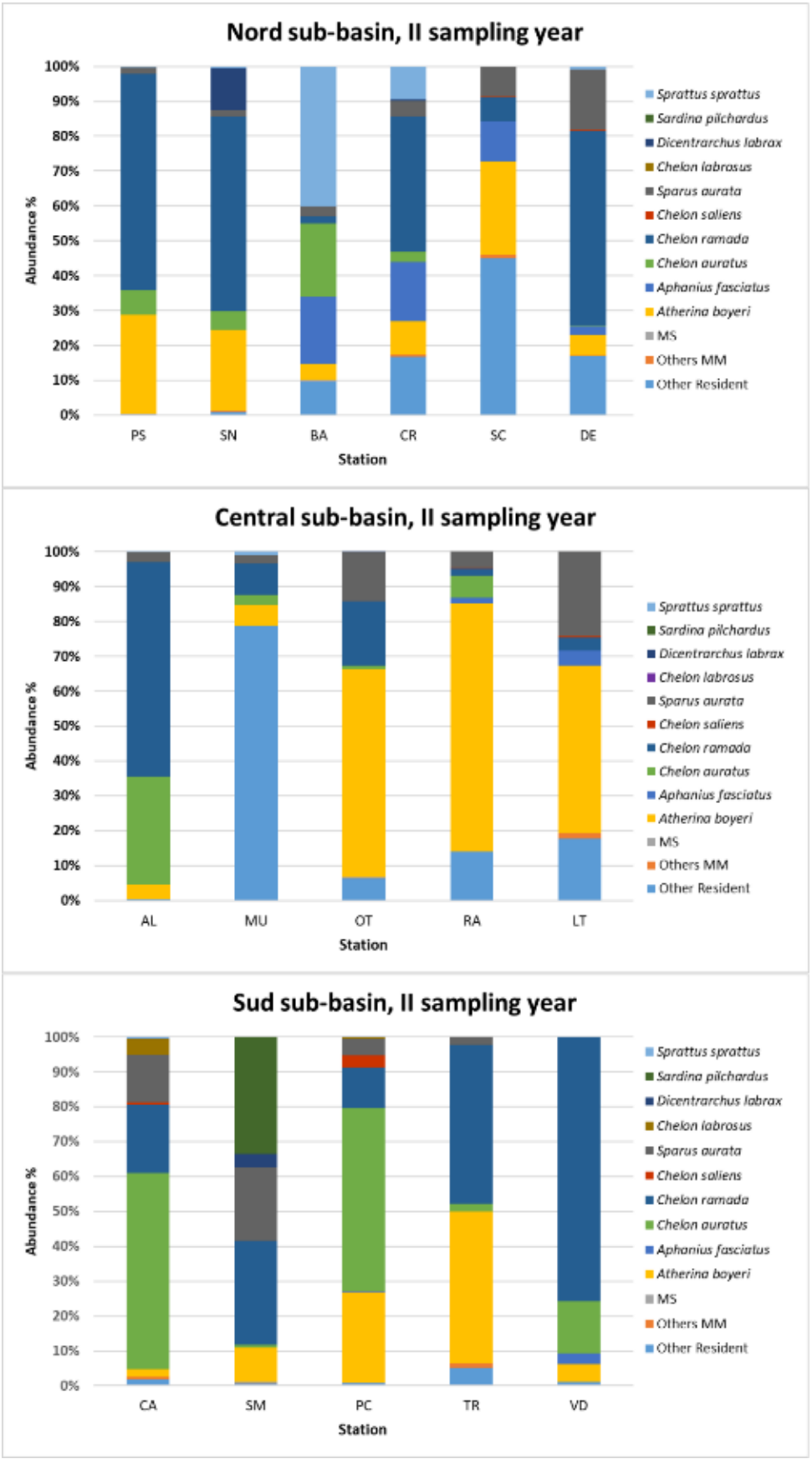


Figure A6 – Juvenile only. Abundance % of each species/taxa calculated on the mean density by station, separately for sampling cycle and sub-basin. MS = Marine Straggler, MM = Marine Migrant. A = I sampling cycle, B = II sampling cycle

APPENDIX B

Table B1 – Dietary indices (%N, %V, %F) of S. aurata for each prey category, calculated for each date, station, position and size class. (s.e. = saltmarsh edge, t.c. = tidal creek).

data	**19/02**						**21/03**								
stations	**DE i.c.**			**DESE s.e.**			**DE i.c.**			**DESE s.e.**			**DESE s.e**		
size class	**CL 1**			**CL 1**			**CL 1**			**CL 1**			**CL 2**		
n° of *S. aurata*	22			8			32			30			3		
Indices/Taxon	%N	%V	%F	%N	%V	%F	%N	%V	%F	%N	%V	%F	%N	%V	%F
Harpacticoida	70,1	40,3	95,5	65,2	44,3	87,5	68,9	29,7	90,6	62,6	4,2	84,0	90,9	24,0	66,7
Amphipoda	0,0	0,0	0,0	4,3	14,1	12,5	0,0	0,0	0,0	0,8	5,7	4,0	0,0	0,0	0,0
Polychaeta	0,0	0,0	0,0	0,0	0,0	0,0	0,6	52,9	6,3	4,1	88,5	20,0	3,0	75,5	33,3
Calanoida	20,7	52,1	50,0	4,3	22,0	12,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Cyclopoida	8,0	6,1	13,6	26,1	19,5	37,5	30,4	17,3	59,4	32,5	1,5	44,0	6,1	0,6	33,3
Mysidacea	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Decapoda	1,1	0,7	4,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

data	**07/04**											
stations	**DE i.c.**			**DESE s.e.**			**DE i.c.**			**DESE s.e.**		
size class	**CL 1**			**CL 1**			**CL 2**			**CL 2**		
n° of *S. aurata*	3			4			3			6		
Indices/Taxon	%N	%V	%F	%N	%V	%F	%N	%V	%F	%N	%V	%F
Harpacticoida	60,0	2,1	66,7	69,4	8,9	75,0	77,8	2,3	66,7	90,5	6,1	66,7
Amphipoda	20,0	96,1	33,3	2,8	28,2	25,0	0,0	0,0	0,0	1,6	2,0	16,7
Polychaeta	0,0	0,0	0,0	2,8	60,7	25,0	3,7	86,0	33,3	4,8	26,6	50,0
Calanoida	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Cyclopoida	20,0	1,8	33,3	25,0	2,1	25,0	14,8	0,6	66,7	0,0	0,0	0,0
Mysidacea	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	38,2	16,7
Decapoda	0,0	0,0	0,0	0,0	0,0	0,0	3,7	11,0	33,3	1,6	27,1	16,7

data	**15/04**						**04/05**						**26/05**		
stations	**DE i.c.**			**DESE s.e.**			**DE i.c.**			**DE i.c.**			**DESE s.e.**		
size class	**CL 2**			**CL 2**			**CL 2**			**CL 3**			**CL 3**		
n° of *S. aurata*	46			4			24			2			2		
Indices/Taxon	%N	%V	%F	%N	%V	%F	%N	%V	%F	%N	%V	%F	%N	%V	%F
Harpacticoida	97,8	40,7	100	35,7	0,2	75,0	78,6	8,6	66,7	0,0	0,0	0,0	0,0	0,0	0,0
Amphipoda	0,7	43,6	39,1	14,3	4,2	50,0	16,8	62,8	83,3	0,0	0,0	0,0	100	100	100
Polychaeta	0,2	8,2	13,0	0,0	0,0	0,0	2,5	24,7	37,5	100	100	100	0,0	0,0	0,0
Calanoida	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Cyclopoida	1,0	0,4	28,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Mysidacea	0,0	0,0	0,0	28,6	64,9	25,0	0,3	0,1	4,2	0,0	0,0	0,0	0,0	0,0	0,0
Decapoda	0,2	7,2	8,7	21,4	30,7	50,0	1,9	3,8	8,3	0,0	0,0	0,0	0,0	0,0	0,0

data	**21/06**			**04/05**					
stations	**DE i.c.**			**BA s.e.**			**BA s.e.**		
size class	**CL 3**			**CL 2**			**CL 3**		
n° of *S. aurata*	1			24			6		
Indices/Taxon	%N	%V	%F	%N	%V	%F	%N	%V	%F
Harpacticoida	0,0	0,0	0,0	67,9	1,1	45,8	6,3	0,0	16,7
Amphipoda	33,3	17,7	100	24,8	98,3	66,7	75,0	90,6	83,3
Polychaeta	33,3	76,8	100	0,7	0,4	4,2	0,0	0,0	0,0
Calanoida	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Cyclopoida	0,0	0,0	0,0	6,6	0,2	4,2	0,0	0,0	0,0
Mysidacea	33,3	5,5	100	0,0	0,0	0,0	0,0	0,0	0,0
Decapoda	0,0	0,0	0,0	0,0	0,0	0,0	18,8	9,4	16,7