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ELABORATO DI LAUREA

Preference of Venice Lagoon sediment by the invasive blue crab *Callinectes sapidus* Rathbun, 1896 and the native green crab *Carcinus aestuarii* Nardo, 1847 in relation to its grain size

Tutor: Prof. Alberto Barausse
Dipartimento di Biologia

Co-tutor: Dott. Filippo Piccardi
Dipartimento di Biologia

Laureando: Luca Brunoni

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I. Introduction

1.1 Alien and Invasive Species

An organism that may survive and reproduce outside of its known range is the definition of an alien species according to the terminology proposed by Occhipinti-Ambrogi & Galil (2004). This definition may then be further specified depending on the species population expansion and its impact on the new environment or by its mode of introduction. With the development of transportation, by sea, land or air, more and more species have been displaced from their native range into other environment, thus being identified as alien species (Hulme, 2009). But not all alien species are a danger to their new environment, the real danger comes from species that are able to exponentially grow their populations and expand their territories. These potentially dangerous species are defined as invasive alien species (Occhipinti-Ambrogi & Galil, 2004); an example of an invasive species is the Eastern gray squirrel *Sciurus carolinensis*, native to North America, that is invading countries like Italy, the United Kingdom, Ireland and many others, where it is outcompeting and causing the decline of native species like the Eurasian red squirrel *Sciurus vulgaris* (Bertolino et al., 2013). Another useful tool for defining alien and invasive species is proposed by Colautti and MacIsaac (2004) as a framework for defining different terminologies relative to biological invasions. This framework is divided into different stages corresponding to different moments that are usually referred using different terms: for example, the term alien species can refer to stages I through V in the framework, and the term invasive species can refer to stages IVa, IVb and V. These stages are heavily conceptualized on the propagule pressure factor that influences biological invasions (Colautti & MacIsaac, 2004).

The process for an alien species to become invasive is called invasion, and it is influenced by three main factors: propagule pressure, environmental factors and biotic factors (Catford et al., 2008). Propagule pressure is defined as the amount and frequency that individuals from alien species arrive in a new environment (Simberloff, 2009). These individuals must then face the hurdles that are the new environmental and biological factors, also defined as resistance to the invasion process. In an aquatic environment some of the main abiotic factors that can limit the spread of invasive species are water temperature, salinity, and inorganic resources availability, as they can directly influence various biological processes like metabolism or osmoregulation, therefore limiting population growth (Catford et al. 2008). Some species are more tolerant to variation of certain environmental factors, such as euryhaline and eurytherm species that tolerate wider ranges of salinities and ambient temperatures respectively. These generalist species have a higher probability of being successful in the invasion process (Keller et al., 2011).

The main biotic factors that influence an invasion are the resistance of the local community to invasions due to the presence of strong competitors in the native species and the biological traits of the invading alien species that promote population growth (Catford et al. 2008). An example of biotic resistance of the local community to an invasion is in the interaction between the invading crab *Carcinus maenas* Linnaeus, 1758, with the native *Callinectes sapidus* in the eastern coast of the United States. A study by DeRivera et al. (2005) found that predation by *C. sapidus* on the invasive *C. maenas* prevented its spread to the south.

1.2 Biological invasions in the Mediterranean Sea

The Mediterranean Sea has been the stage of biological invasions for many years. This has been an ever-increasing trend since the opening of the Suez Canal, connecting the Mediterranean with the Red Sea, and the establishment of interoceanic trade routes (Corriero et al., 2015). The opening of the Suez Canal in 1869 and the surge in shipping routes through the Mediterranean pre-date most modern marine species studies, but the sizeable number of biological surveys and studies that have been conducted since the beginning of the 20th century allow, with a reasonable amount of certainty, to distinguish between allochthonous species and alien species (Galil, 2008).

Alien species are and have been introduced to the Mediterranean Sea by different anthropogenic pathways. Of the currently established alien species in the Mediterranean, 54% of them were introduced to it through the pathway that is the Suez Canal (Galil, 2008) in a process called “Lessepsian migration” from Ferdinand de Lesseps (Bianchi, 2007), either by natural dispersion of larval or adult forms or through transport by shipping vessels (Galil, 2008). An example of alien species that dispersed through the Suez Canal is that of the pearl oyster *Pinctada radiata*, whose rapid spread is attributed to shipping vessel transport or as epibionts on marine turtles (Galil, 2008). Shipping vessels can promote the dispersion of alien species by attachment of fouling, boring or adhering species to the vessel’s hull or through ballast water transport of pelagic species or larval forms (Galil, 2008). Ballast water is fresh- or salt-water loaded from the source port and held in the ballast tanks of ships. It is used to provide stability during the ships voyage and is discharged at the destination port after arrival and loading of new cargo. This practice has been widely used since its development in the mid-1850s, but since the beginning it has been an involuntary pathway of transporting alien species and is currently one of the leading causes for the spread of invasive species (Davidson et al., 2011).

Another way that alien species can be introduced to the Mediterranean is as commodities, either by stocking or aquaculture, which accounts for 11% of the currently established alien species. Stocking is a practice that involves the intentional release of animals to create populations for hunting and fishing or to act as biological control agents. Aquaculture involves the cultivation of aquatic organisms for a commercial purpose; these organisms can then become alien species by escaping from farms or just reproducing outside of their bounds (Keller et al., 2011). An example of this is that of the North American signal crayfish, *Pacifastacus leniusculus*, initially introduced to Europe for aquaculture, have now spread outside of aquaculture facilities and have become a big threat to native crayfish species, also causing the spread of alien diseases (Keller et al., 2011).

Biological invasions from alien species are also promoted by human pressures such as climate change, fishing and pollution that weaken the less tolerant native species reducing the biotic resistance of the local community. Additionally, traits that help alien species become invasive, such as adaptability to varying environmental condition, rapid growth, and reproductive rates, can often bring them advantages in dealing with climate change in comparison to native species, thus giving them an advantage in the invasion process (Mainka & Howard, 2010). The Mediterranean for the last decades has been undergoing a process of tropicalization in which sea water temperature keeps rising, probably caused by the combined action of climate change, Atlantic waters influx, and Lessepsian migration. This process is bringing great changes to the native biota of the Mediterranean by promoting the spread of thermophilic native or invasive species (Bianchi, 2007). All these factors that exert an influence on the Mediterranean ecosystem and its high invasion risk are well studied on their own, but the multiple interactions between them need further exploration in order to preserve the biodiversity of the Mediterranean Sea (Coll et al., 2010).

1.3 Invasive species in the Venetian Lagoon

The Venetian (or Venice) Lagoon is the largest Mediterranean lagoon, situated along the northern Adriatic Sea coast and is connected to the sea by the three inlets of Lido, Malamocco and Chioggia. It has an average depth of 1,5 m and a tidal regime delayed by the three inlets compared to the rest of the Adriatic (Ravera, 2000). About a tenth of the lagoon is occupied by saltmarshes which can be naturally occurring or artificially made and are home to plant and animal species adapted to varying high to low salinity and wetness (D'Alpaos, 2010).

The Venetian Lagoon has been recognized as an environment with a high risk of invasion (Marchini et al., 2015). This is because it is a very important hub of anthropogenic activities that are often associated with invasion risks, such as shipping and aquaculture. These activities generate a high propagule pressure that, together with the local environmental conditions, put the lagoon at high invasion risk. Other factors that influence this risk are the pollution of the lagoon caused by human activities like the petrochemical industry and agricultural and domestic discharge, that cause eutrophication and have severe consequences on the native species (Marchini et al., 2015), as well as the loss of tidal forms due to the ongoing erosion (Sarretta et al., 2010). A survey conducted in 2015 by Marchini et al. showed that there were 71 alien species documented in the Venetian Lagoon, and as of today they could be even more. These alien species include many different animal and plant taxa of mollusks, crustaceans, macroalgae, etc.

1.4 Invasive Blue Crabs and Native Green Crabs

One of the most relevant invasive species in the Venetian Lagoon in current times is the blue crab *Callinectes sapidus* Rathbun,1896 (Decapoda: Portunidae). It is a euryhaline and eurythermal species, native to the western coast of the North Atlantic Ocean, whose range has since expanded in Asia and Europe probably because of human activity (Galil, 2000). The first documented record of a blue crab in European waters is from the beginning of the 20th century, in the Biscay Gulf, France (Bouvier, 1901) while its first appearances in Mediterranean waters is dated to the mid 20th century (Banoub, 1963; Holthuis & Gottlieb, 1955). In Italy its first appearance dates to 1949 from the Grado Lagoon (Giordani Soika, 1951) where it was erroneously identified as *Neptunus pelagicus* until 1993, with the identification of two male individuals in the Venetian Lagoon (Mizzan, 1993). Due to its tolerance to wide ranges in environmental conditions the leading theory on the mode of introduction of *C. sapidus* in the Mediterranean is through ballast water transport (Manfrin et al., 2016). The blue crab is a commercially important species being commonly fished and sold in most American fish markets as seafood, and more recently even in the Mediterranean.

Blue crabs are organisms with evident sexual dimorphism, with immature females having a triangle shaped abdomen that becomes round with the onset of sexual maturity and males having an elongated abdomen (Van Engel, 1958). Mating of the blue crab begins in early May continuing through the summer in the low salinity waters of upper estuaries (Van Engel, 1958), in a complex process consisting of courtship, copulation, sperm storage and internal fertilization of eggs. After mating, male blue crabs remain dispersed in upper estuaries for their adult life and will mate more than once per season, while female blue crabs mate only once then migrate to lower estuaries and coastal areas where the fertilized eggs are brooded externally in high salinity waters until hatching after 14-17 days as planktonic zoea larvae (Millikin and Williams, 1984). Newly hatched larvae then develop in the open ocean through 7 stages of planktonic zoeal larvae in about 4 weeks with favorable conditions, to then metamorphose in a single megalopae stage. During this stage, thanks to their remarkable swimming ability, megalopae migrate upstream to estuaries where they are stimulated to metamorphose in juvenile individuals, with a carapace width of a few millimeters, by chemical cues associated with nursery habitats where they then remain for several months until reaching a width of 20-30 mm (Epifanio, 2019).

Early juvenile stage blue crabs settle in nursery habitats like seagrass beds and saltmarshes, where they can find protection and nutrition (Hines, 2007). After reaching a sufficient size they then move out of the nurseries to find a suitable habitat with plenty of organisms to feed on, like mollusks, small fish, algae, and other crustaceans, at times even practicing cannibalism (Prado et al., 2024). Growth rates during the early stages of juvenile blue crab development, up to 7 cm carapace width, do not vary significantly between sexes. After the early stages variation in growth per molt can occur, probably caused by diet quality and temperature differences (Millikin and Williams, 1984).

Male and female blue crabs reach sexual maturity respectively after 18-19 and 17-19 post larval molts, with an average carapace width of 120 mm (Marchessaux et al., 2023). Males can keep molting after sexual maturity, reaching sizes up to around 241 mm (Millikin and Williams, 1984), while for females this is the final molt, also referred to as pubertal molt, and it causes morphological changes in the now adult crab, most notably it changes the shape of the abdomen from the immature triangular shape to a more rounded semicircular shape, more apt for brooding eggs (Epifanio, 2019). These characteristics and the generalist diet of *C. sapidus* make it exert a strong influence on native benthic communities, while the intraguild predation on native species with similar trophic role could also have a negative effect on the local biodiversity (Finke & Denno, 2005). These attributes of the blue crab could have negative effects on the fishing and aquaculture industries, while its inherent commercial value could instead bring positive effects (Prado et al., 2024).

The Mediterranean Green Crab *Carcinus aestuarii* Nardo, 1847 (Decapoda: Portunidae) is the most widespread crab species native to the Mediterranean Sea (Mori et al., 1990). It is commonly found in the Venetian Lagoon and is fished commercially mainly while it is still soft after molting, in a stage locally referred to as “moeca”, primarily during spring and autumn (Matozzo et al., 2013). *C. aestuarii* also plays an important role in lagoons as a component in the diet of many lagoon feeding fish or fish that migrate towards lagoons for trophic reasons (Mori et al., 1990). Green crabs have two breeding seasons, with a primary winter cycle for larger crabs and a secondary summer cycle for smaller crabs that cannot produce eggs during the primary cycle (Glamuzina et al., 2017). After mating female crabs migrate to lower littoral levels where they will remain to develop the eggs through fall and winter; according to Mori et al. (1990) oogenesis lasts about three months. After hatching a ~90 days larval stage follows, with four planktonic zoeal stages and one benthic megalopae stage, which then metamorphose to the first juvenile crab stage (Leignel et al., 2014). Green crabs then grow in the juvenile stage until reaching sexual maturity with an average carapace width of 29,1 mm for females and 31,4 mm for males (Mori et al., 1990). Green crabs also show clear sexual dimorphism with males being larger than females (Matozzo et al., 2013).

C. aestuarii is an euryhaline and eurythermal species, also known for its tolerance to hypoxia, air exposure and food deprivation during molting. These characteristics are what allow green crabs to live in varying habitats like estuaries and lagoons and what makes them potentially a highly invasive species, which is the case for its relative *Carcinus maenas*, the European Green Crab, one of the world's worst invasive species (Leignel et al., 2014).

C. maenas has invaded many different regions, ranging from North American coasts to Australian and South African coasts. One case study was conducted on its invasion of the Atlantic coasts of North America, where it became established in the 1800s, specifically on its interaction with the native Blue Crab species *Callinectes sapidus*. The invasive *C. maenas* current range expands from Nova Scotia to Maryland, limited in the north by the cold-water temperature, and in the south by the interaction with *C. sapidus*. Both crab species show similar adaptability to temperature and salinity variations, being euryhaline and eurythermal species, and inhabit estuaries and bays with overlapping diet and habitat use. While the two species share many similarities, blue crabs grow up to twice the size of an adult European green crab and often predate smaller crabs including green crabs. This predation interaction between the two species limits the invasive species spread to the south in a remarkable show of biotic resistance to a biological invasion (DeRivera et al., 2005).

Similar interactions could be happening in the Venetian Lagoon, with the more recent invasion of *Callinectes sapidus* and the native *Carcinus aestuarii*. Both species are euryhaline and eurythermal species that migrate towards the sea during their reproductive cycle. Further study of this interaction could be useful in determining the effect and outcome of the blue crab invasion in the Venetian Lagoon. One important factor in these crab species life cycles is the use they make of habitats, specifically of the sediment substrates they inhabit, being benthic organisms. Sediment substrate is often used by crab species that exhibit burrowing behaviors, which allow them to find protection from predators or to prey on the organisms living inside it (Barshaw & Able, 1990) or to overwinter (Thomas et al., 1990). Both of the crab species under examination in this study exhibit burrowing behavior. Understanding the preferences and behaviors of sediment substrates by *C. aestuarii* and *C. sapidus* could be useful in determining the extent and type of interaction these two species might have with each other.

II. Objectives

Interactions between native and invasive species are one of the focal points in the resistance to biological invasions. With the ongoing invasion of *Callinectes sapidus* in the Venetian Lagoon, it becomes more and more important to study its interaction with the native species, even more for species that share similarities in habitat use and trophic level, which is the case for the native *Carcinus aestuarii*. To understand what interactions may happen between two such species, we first need to know where these interactions may take place and, crabs being benthic organisms, we can start by looking at the substrates they live on. The Venetian Lagoon is a diverse environment and the sedimentation of materials in the water column can be influenced by many factors like seawater input from the three inlets, water circulation inside the lagoon, the presence of saltmarshes, etc. Saltmarshes are an important habitat of the lagoon, being formed from the accumulation of sediment transported by the river tributaries or removed from the seafloor by tidal action, that can be inhabited by a variety of species (Ravera, 2000).

Another factor influencing sediment budget in the Lagoon is human activity, be it with negative impact on the environment like the dredging of canals or with positive impact, like environmental restoration efforts. One notable such case of restoration involved the creation of artificial saltmarshes with sediment from drainage sites of canals in the Venetian Lagoon, to restore the degradation of the ecosystem by erosion (Tagliapietra et al., 2018). Our study aims to analyze and compare the preference and behavior of juvenile *Callinectes sapidus* organisms and adult *Carcinus aestuarii* organisms for three types of Venetian Lagoon sediment, particularly in relation to grain size which is changing due to the ongoing process of erosion in the Venetian Lagoon (Molinaroli et al., 2009).

III. Materials and Methods

1. Sediments samples and grain size

In this study we took into consideration three different bottom substrates present in the Venice Lagoon: sediment taken from the lagoon floor in front of a natural saltmarsh, in front of an artificial saltmarsh, and sand from a sandy beach. These three sediments were sampled from the southern Venice lagoon at the following coordinates: 45° 13.470' Nord e 12° 13.174' Est for the natural saltmarsh, 45° 13.515' Nord e 12° 13.421' Est for the artificial saltmarsh, and 45° 14.3942' Nord e 12° 17.2845' for the sand.

The sediments were prepared for the grain size analysis with the removal of organic matter and saline content present in the samples (Roner et al., 2016), as they tend to cause aggregation of the sediment particles, thereby altering the grain size analysis results. Following this method, we prepared three 1000 ml beakers, each containing a subsample of about 20-30 g of one of the sampled substrates. Each subsample was treated with hydrogen peroxide for 24 h, after which they were treated with an NaClO solution for 24 more hours. The remaining solution was then put in the oven to dry at 70°C for about 48/72 hrs.

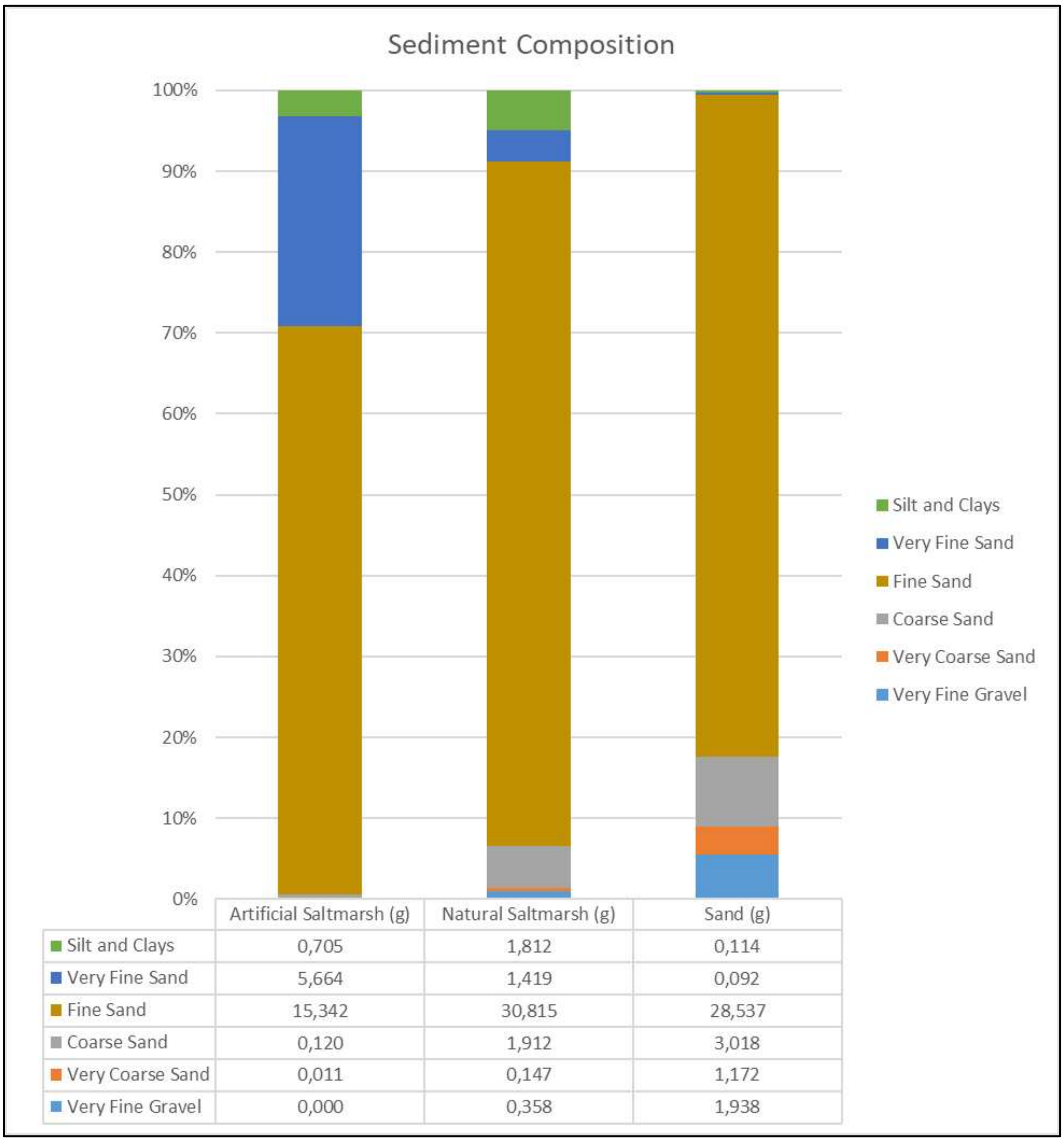
We then proceeded to sift the dried sample using a series of sieves with different mesh sizes corresponding to the main grain size classes of the Udden-Wentworth scale (Wentworth, 1922) and weighed each fraction using a precision scale (error ± 0.001 g). With this analysis we were able to identify 6 main grain size diameter categories, corresponding to certain classes in the Udden-Wentworth scale:

- With a grain diameter of less than 63 μm we have silt and clays;
- Between 125 μm and 63 μm is very fine sand;
- Between 500 μm and 125 μm is fine sand;
- Between 1 mm and 500 μm is coarse sand;
- Between 2 mm and 1 mm is very coarse sand;
- With a grain diameter above 2 mm we have gravel;

In the following table (Table 1) and graph (Fig. 1) respectively the composition of the sediment samples in grams over the total weight sample, and in percentages are reported.

Table 1: This chart shows the different sediment fractions categorized using the Udden-Wentworth scale and the weight in grams of each fraction for all three sediment types.

Wentworth Scale	**Size Range (metric)**	**Artificial [g]**	**Natural [g]**	**Sand [g]**
Very Fine Gravel	2-4 mm	0,000	0,358	1,938
Very Coarse Sand	1-2 mm	0,011	0,147	1,172
Coarse Sand	0,5-1 mm	0,120	1,912	3,018
Fine Sand	125 μm - 0,5 mm	15,342	30,815	28,537
Very Fine Sand	62,5 - 125 μm	5,664	1,419	0,092
Silt and Clays	< 62,5 μm	0,705	1,812	0,114
	Total Sample Weight:	21,842 g	36,463 g	34,871 g



	Artificial Saltmarsh (g)	Natural Saltmarsh (g)	Sand (g)
Silt and Clays	0,705	1,812	0,114
Very Fine Sand	5,664	1,419	0,092
Fine Sand	15,342	30,815	28,537
Coarse Sand	0,120	1,912	3,018
Very Coarse Sand	0,011	0,147	1,172
Very Fine Gravel	0,000	0,358	1,938

Fig. 1: This graph shows the composition in percentages of each sediment sample fraction categorized in classes using the Udden-Wentworth scale.

As shown in the graph, all three sediments vary in the concentration of fine or coarse dimensional classes, but the highest concentration of particles is in the fine sand class for all sediment samples. The sand sample is composed of 81,84% fine sand, while the rest of its composition is on the coarser side, showing the highest concentration of coarse sand, very coarse sand and gravel between the three sediments (respectively 8,65%, 3,36%, 5,56%).

The artificial saltmarsh has a lower percentage of fine sand (70,24%) also having the lowest percentage of coarser particles (0,55% of coarse sand and 0,05% of very coarse sand) and the highest amount of very fine sand (25,93%). It also shows higher concentration of silt and clays compared to the sand sample (3,23%). The natural saltmarsh instead has the highest percentage of fine sand (84,51%) but is at a middle ground compared to the other two sediments concerning the finer and coarser classes. It has a higher percentage of coarser sediments than the artificial saltmarsh but lower than sand (coarse sand 5,24%, very coarse sand 0,40%, gravel 0,98%) and a lower amount of very fine sand than artificial sediment compared to sand (3,89%). It also has the highest amount of silt and clays between the three sediments (4,97%).

Knowing the main grain size classes of our sediment samples is useful to our analysis as it allows us to distinguish more clearly which types of sediment could influence the crab behavior. To differentiate the substrates, we will take into consideration the average concentration of finer and coarser sediments, resulting in a scale ranging from the coarser sand to the natural saltmarsh and to the finer artificial sediment.

2. Specimens and their treatment

The crabs used in this analysis were gathered by professional fishermen in the southern Venice Lagoon, during the months of April and May 2024. The specimens gathered were adult individuals of *Carcinus aestuarii* and juveniles of *Callinectes sapidus*: this choice was made because they are similar in size and therefore trophic level during these life stages. Once caught, the two crab species received the same treatment: they were brought to the “Stazione Idrobiologica Umberto D’Ancona di Chioggia (University of Padova, Chioggia)” where they were kept in stabulation tanks (different tanks for each species) with flowing lagoon water for a period of 24-72 h to acclimatize after the stress of their transport to the station. After the initial period of acclimatization each crab was subjected to the behavioral testing. Following the test each crab was accurately measured: carapace width and length using calipers (error ±0.05 mm); its weight using a precision scale (error ±0.001 g), and its sex determined by the shape of the abdomen, being rounder for females and long and slender for males (Van Engel, 1958). After testing the green crabs’ specimens were released back into the Venice Lagoon while the blue crabs, being an invasive species, were disposed of.

3. Experimental setup

The experimental setup (Fig. 2) consisted of a glass tank (35 x 35 x 35 cm) with a square Plexiglass container at the bottom (35 x 35 x 5 cm) divided in 4 equal parts. Each of the quadrants was filled to about 5 cm with one of the 3 sediment samples chosen randomly, while one remains empty and was used as the control quadrant (also chosen randomly). At the center of the container a 10 cm white cylindrical PVC enclosure was placed, with 4 slits that enabled the Plexiglass dividers to slot into it, where the crab was placed at the beginning of each test. The cylinder was without a solid base, allowing the crab inside it to feel the different substrates before being allowed to roam; a white PVC support was placed in the empty quadrant fraction of the cylinder base to stop the crab from escaping from the enclosure before the test start. The glass tank was then partially filled with lagoon water, up to the top of the cylindrical enclosure, taking care not to disturb the sediment and murky up the water, after which followed a 5–10-minute wait to allow any suspended particles to settle.

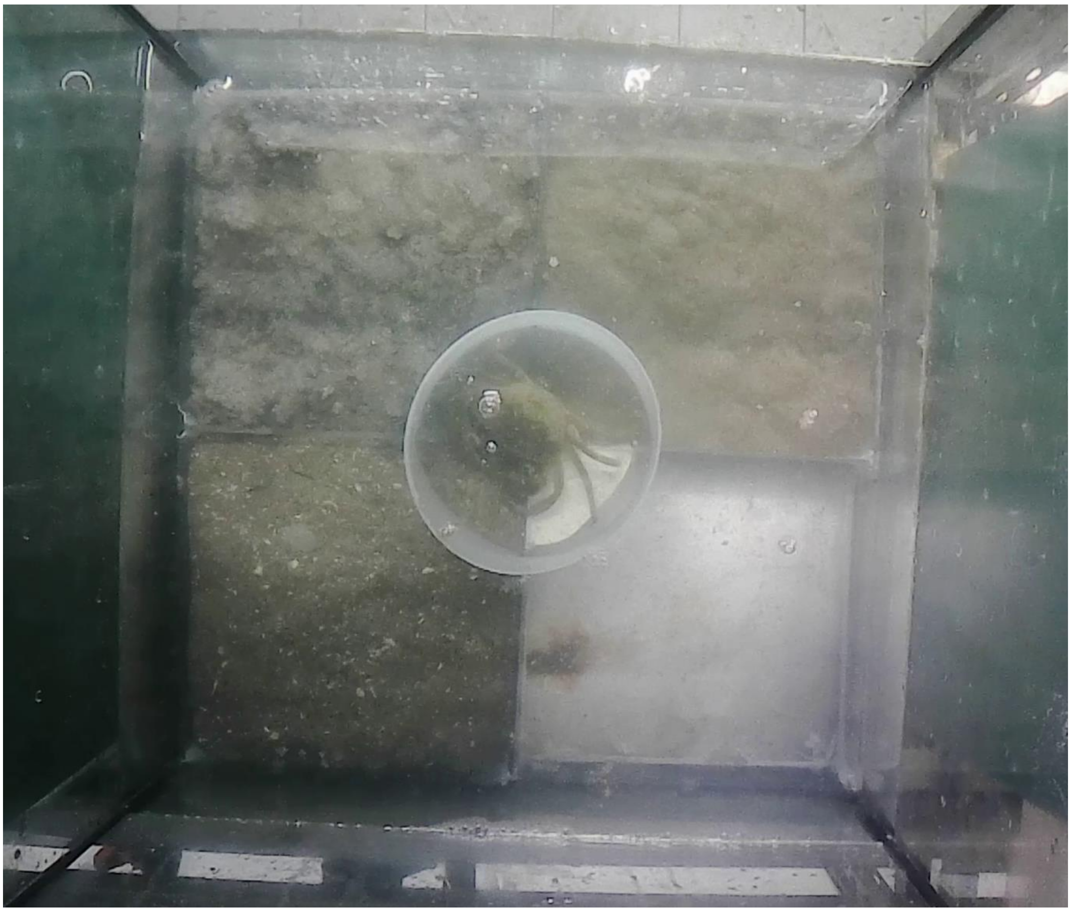


Fig. 2: Picture of the experimental setup complete with substrates randomly arranged in the 4 corners and crab inside the holding cell in the center. In this picture the substrates are, from top left to bottom right, natural saltmarsh, artificial saltmarsh, sand and control substrate. The crab at the center of the setup is a specimen of *Carcinus aestuarii*.

Above the center of the tank a Nilox Mini Wifi 3 digital camera was positioned to record the experiment with a resolution of 2k at 30 fps. After preparing the setup, the recording was started, and the crab put into the cylindrical enclosure at the center of the tank, where it is left to acclimatize for 3 minutes. After the acclimatization time the crab was released from the enclosure, free to move on the sediment for 10 minutes. Once the 10 minutes were up the recording is stopped, the crab is removed from the tank to be measured, weighed and have its sex determined and the tank is emptied, rinsed and prepared for the next test.

4. Software for data and statistical analysis

For the random choice of sediments in the experiment setup Google Random Number Generator was used by assigning a number between 1-4 to each quadrant and to each sediment plus the control. For data analysis, chart and graph creation we used Microsoft® Excel® for Microsoft Office 365 and RStudio: Integrated Development for R (*http://www.rstudio.com/*).

For the recordings analysis and the behavioral mapping we used the software BORIS (Behavioral Observation Research Interactive Software), version 8.27.1 (http://www.boris.unito.it/). The observed behaviors are movement, burrowing, standing still, as well as the crab position in relation to each sediment (natural saltmarsh, artificial saltmarsh, sand and control quadrant).

IV. Results

A total of 35 crabs was analyzed, of which 17 specimens were of *C. aestuarii*, all males without any visible defects, and 18 were of *C. sapidus*, 13 males and 5 females, also without any visible defects.

The blue crab size, measured in width of the carapace, ranged from a maximum of 10,7 cm to a minimum of 4,4 cm, with an average value of 8,44 cm. The green crabs size instead, ranged from a maximum of 4,9 cm to a minimum of 3,7, with an average value of 4,33 cm (Fig. 4). All of the *C. sapidus* specimens were juveniles, as male specimens reach sexual maturity at around 11,75 cm of carapace width, while female specimens reach it at around 12 cm of carapace width (Marchessaux et al., 2023). All the *C. aestuarii* specimens were adults, with the males of this species reaching their sexual maturity at 3,14 cm on average (Mori et al, 1990).

The blue crabs weight ranged from a maximum of 79,88 g to a minimum of 5,43 g, with an average of 47,17 g. The green crabs weight ranged from a maximum of 42,60 g to a minimum of 15,24 g with an average value of 27,72 g (Fig. 3).

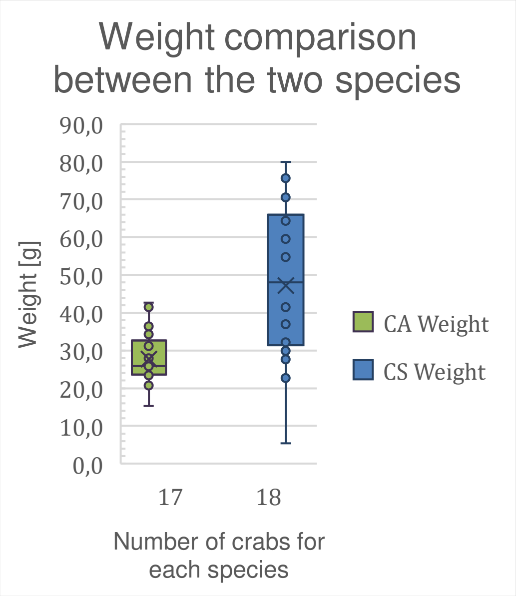


Fig. 3: This graph compares the weight in grams of *C. sapidus* (CS, in blue) and *C. aestuarii* (CA, in green). The two boxes in the graph represent the range of weight values between the first and third quartiles, also called the interquartile range, of the two species, while the whiskers that extend outside of the boxes indicate the variability outside of the interquartile range. Each dot represents an individual crab weight. From the graph we can tell that the weight range of *C. sapidus* is quite larger than the range of *C. aestuarii*, with a highest value that is almost double the green crabs max weight, and a lowest value lower than the green crabs.

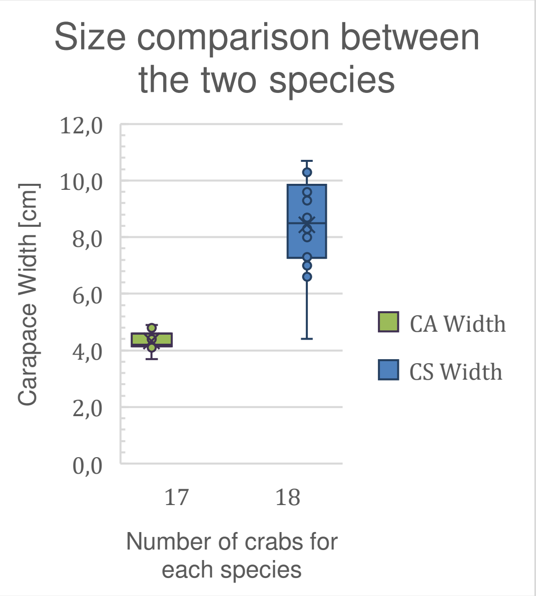


Fig. 4: This graph compares the carapace width of the two species, using it as a standard to compare their size. The two boxes in the graph represent the range of size values between the first and third quartiles of size, also called the interquartile range, of the two species, while the whiskers that extend outside of the boxes indicate the variability outside of the interquartile range. Each dot represents an individual crab size. As we can tell, *C. sapidus* (CS, in blue) is quite larger in size compared to *C. aestuarii* (CA, in green), with its lowest carapace width being on the higher side of the green crabs, while also having a higher variation in size.

From the above graphs we can infer that, even at the juvenile stage, most *C. sapidus* individuals are heavier and larger than adult *C. aestuarii* individuals.

The behavioral analysis using the software BORIS produced results that were divided into two categories: data concerning the crabs position on the different substrates (Artificial saltmarsh, Natural saltmarsh, Sand substrate, Control substrate) and data concerning the crabs behavior (Movement, Burrowing). These data were analyzed using the software RStudio to give us more insight on the average time spent on each substrate and on what behavior was prevalent between the two species.

1. Analysis of position data

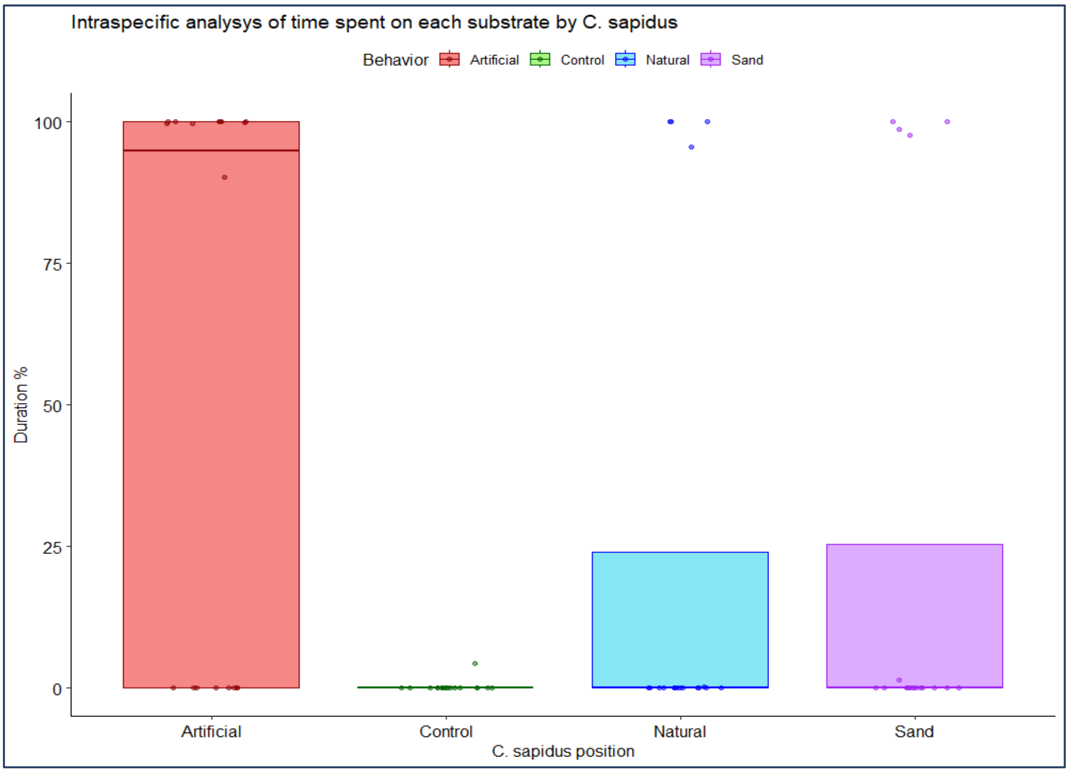


Fig. 5: This graph represents the time spent on each substrate by all 18 tested specimens of *C. sapidus*. The data input in the graph is the percentage of the total duration (around 600 seconds) of time spent on a given substrate by each crab, and each colored dot is one individual; the dots with a value of 0 represent the crabs that did not spend time on that specific substrate. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As is shown in the graph, the number of crabs that spent at least some time on each substrate is: 10 crabs on artificial saltmarsh, 5 crabs on natural saltmarsh, 5 crabs on sand and 1 crab on control.

As shown in the above Fig. 5, most of the blue crabs spent all or most time in the artificial saltmarsh sediment (10 crabs), while sand and natural saltmarsh came second place (5 crabs each); only one crab spent a short time on the control substrate. In general, individual crabs did not visit more than one substrate. An analysis of variance (ANOVA) conducted using the software RStudio detected statistically significant differences across substrates (ANOVA, F Value = 3,176, P Value = 0,0501); the post-hoc Tukey test showed that, when comparing the substrate permanence times for the three sampled sediments, the Artificial saltmarsh has a tendency to be preferred over the others (Table 2).

Post-hoc Tukey results	**Diff**	**Lwr**	**Upr**	**P Adj.**
Natural – Artificial	-32.9848	-69.4076	3.4380	0.083
Sand – Artificial	-32.8764	-69.2992	3.5464	0.085
Sand - Natural	0.1084	-36.3144	36.5312	0.99

Table 2: Table with the results of the post-hoc Tukey test done with RStudio on the position data for *C. sapidus.* In this table Diff is the difference between means of the two groups, Lwr and Upr are the lower and upper endpoint of the confidence interval at 95% and P Adj. is the adjusted P Value after multiple comparisons using Tukey's Honest Significant Differences method.

To summarize, other than for the control substrate that almost no crabs visited, we can confirm a preference for the artificial saltmarsh substrate by the blue crabs from these results.

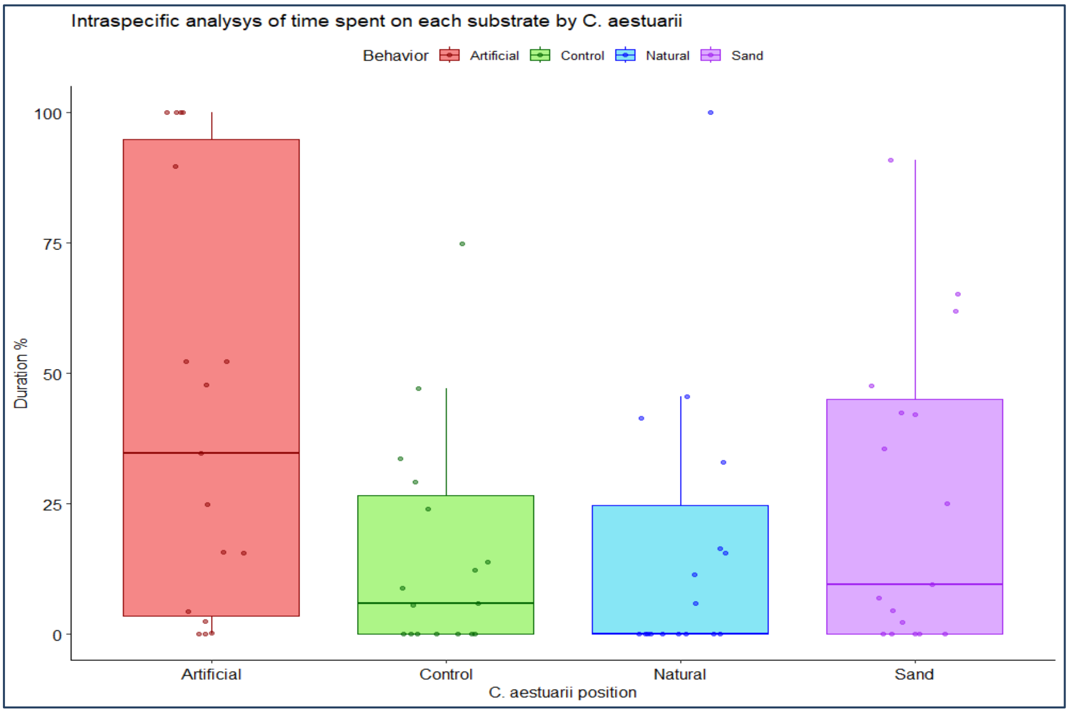


Fig. 6: This graph represents the time spent on each substrate by all 17 tested specimens of *C. aestuarii*. The data input in the graph is the percentage of the total duration (about 600 seconds) of time spent on the substrate by each crab, and each colored dot is one individual; the dots with a value of 0 represent the crabs that did not spend time on that specific substrate. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. From this graph we can see that 12 crabs visited the sand, 10 the control substrate, 15 the artificial sediment and 8 the natural sediment.

From the above Fig. 6, we can see that most green crabs spent at least some time on the artificial saltmarsh sediment (15 crabs) while a slightly lower number of crabs visited the other sediments though there is not an evident dislike over any of them. To test the significance of these results an ANOVA analysis was conducted, and the results showed that that there are differences significant enough to be considered valid (ANOVA, F Value = 3,071, P Value = 0,034). A post-hoc Tukey test further confirmed this statement when comparing between Control substrate and Artificial saltmarsh (Post-hoc Tukey test, Adj. P Value = 0,046) and more marginally when comparing between Natural and Artificial saltmarsh substrates (Post-hoc Tukey test, Adj. P Value = 0,057); all the other differences were not found to be significant at the 0.05 level by the post-hot Tukey test (Table 3 & Fig 7).

Post-hot Tukey Results	**Diff**	**Lwr**	**Upr**	**P Adj.**
Control – Artificial	-181.7558	-361.4876	-2.023954	0.046
Natural – Artificial	-175.9630	-355.6948	3.768811	0.057
Sand – Artificial	-111.9831	-291.7149	67.748693	0.36
Natural – Control	5.7928	-173.9390	185.524575	0.999
Sand – Control	69.773	-109.9592	249.504458	0.74
Sand - Natural	63.9799	-115.7519	243.711693	0.78

Table 3: Table with the results of the post-hoc Tukey test done with RStudio on the position data for *C. aestuarii*. In this table Diff is the difference between means of the two groups, Lwr and Upr are the lower and upper endpoint of the confidence interval at 95% and P Adj. is the adjusted P Value after multiple comparisons using Tukey's Honest Significant Differences method.

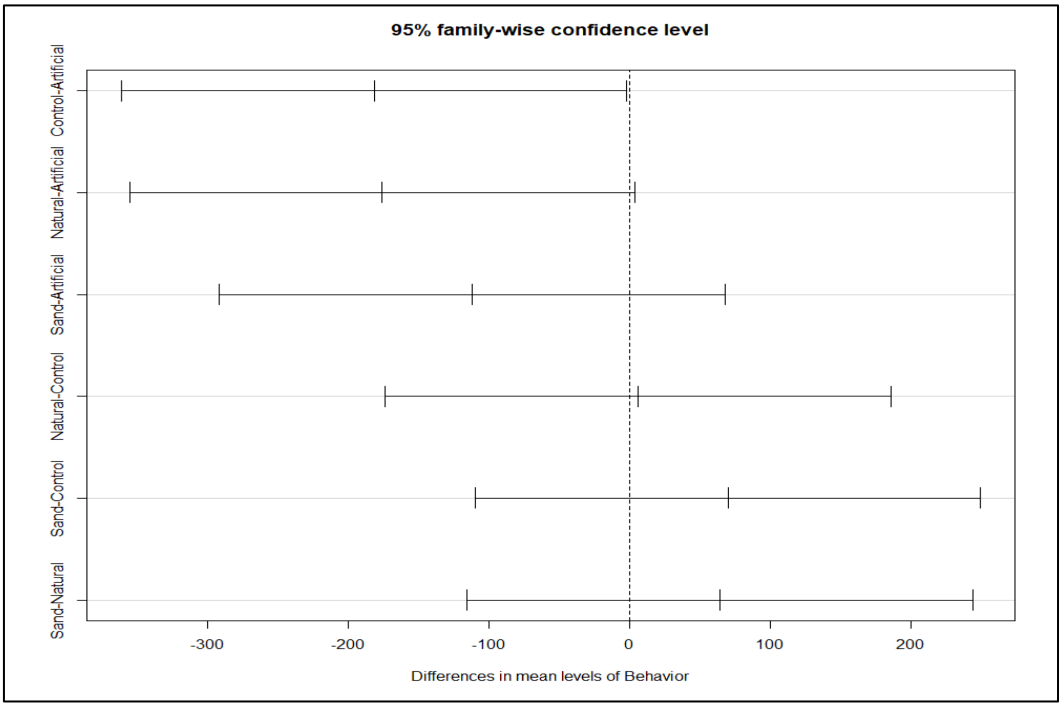


Fig. 7: Graph showing post-hoc Tukey test results on the ANOVA analysis of the position data for *C. aestuarii*. The bars represent the differences in means and 95% confidence intervals shown in Table 2.

In Fig. 8 we can see the comparison between the average time spent by the individuals of the two species on each substrate. Looking at this graph we can once again confirm that blue crabs spent the least time on the control substrate and that they spent more time on the artificial saltmarsh substrate, a tendency also seen in the green crab but more faintly.

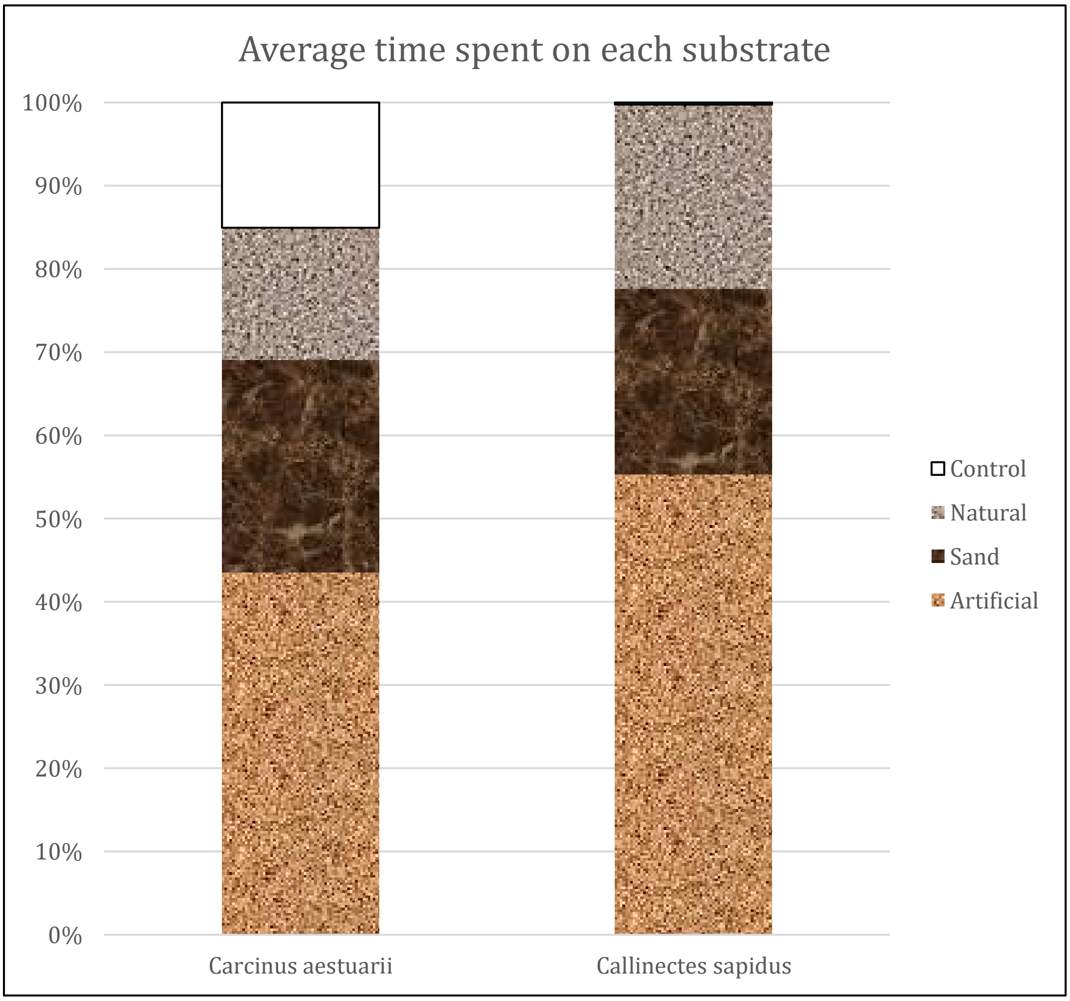


Fig. 8: In this graph are represented in percentage, the average times spent on each substrate by the two crab species.

Figures 9, 10, 11 and 12 show the interspecific comparison of time spent on each tested substrate between *C. aestuarii* and *C. sapidus*.

Fig. 9, 10 and 11 show respectively the comparison of the time spent on the Artificial saltmarsh substrate, Sand substrate and Natural saltmarsh by the two species under examination. The ANOVA variance analysis does not confirm the significance of the difference in time spent by the two species on neither the artificial saltmarsh substrate (ANOVA, F Value = 0,543, P Value = 0,467), the sand substrate (ANOVA, F Value = 0,079, P Value = 0,78) or the natural saltmarsh (ANOVA, F Value = 0,261, P Value = 0,613). Yet, the figures clearly show the differences in the number of substrates visited by the two species, which is lower in the blue crab, whose individuals tends to visit only one substrate each.

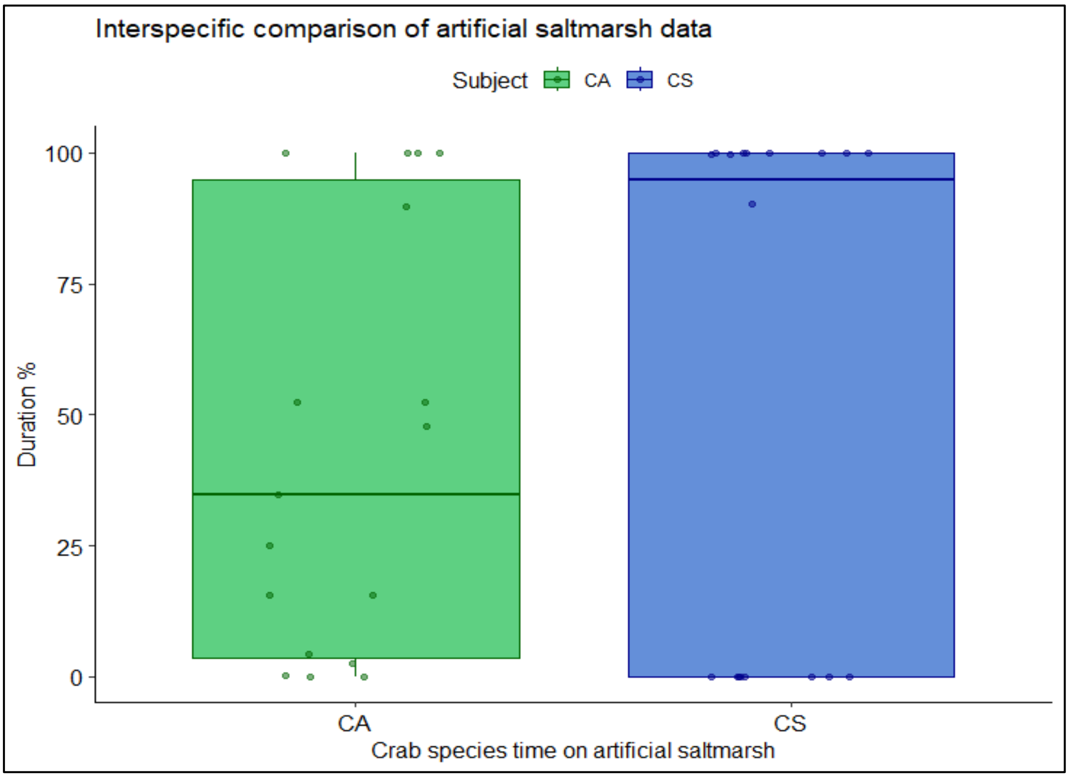


Fig. 9: This graph puts in relation the time spent on the artificial saltmarsh substrate by the two crab species. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not spend any time on this specific substrate. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As is shown in the graph, a total of 15 green crabs (green) and 10 blue crabs (blue) frequented this substrate, but the differences in time are not statistically significant.

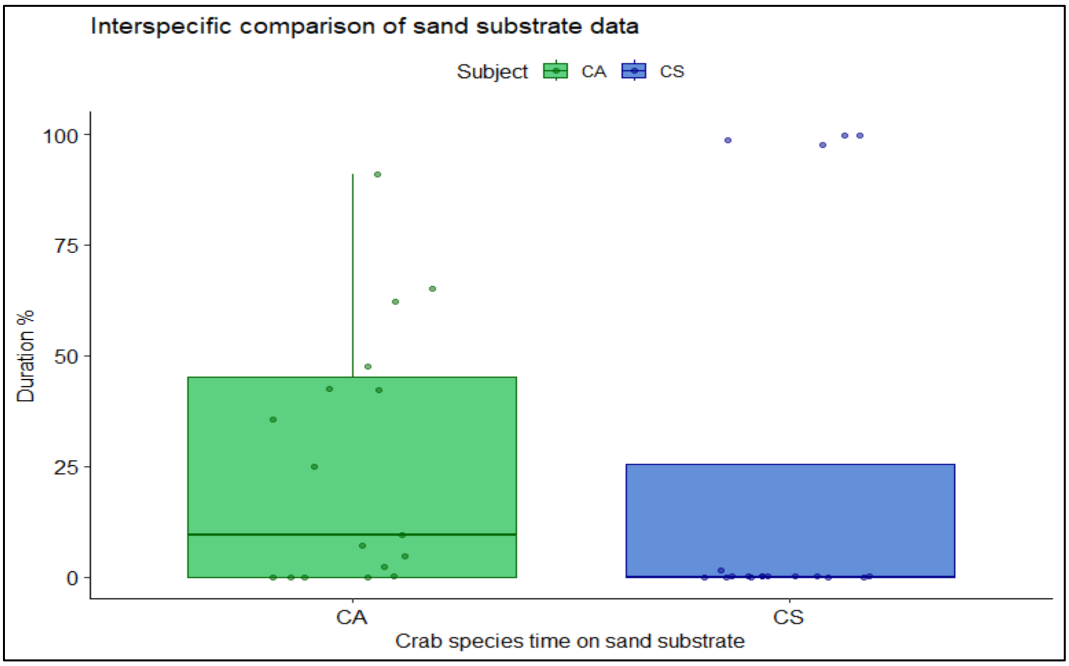


Fig. 10: This graph puts in relation the time spent on the sand substrate by the two crab species. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not spend any time on this specific substrate. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As is shown in the graph, a total of 12 green crabs (red) and 5 blue crabs (blue) frequented this substrate, but the differences in time are not statistically significant.

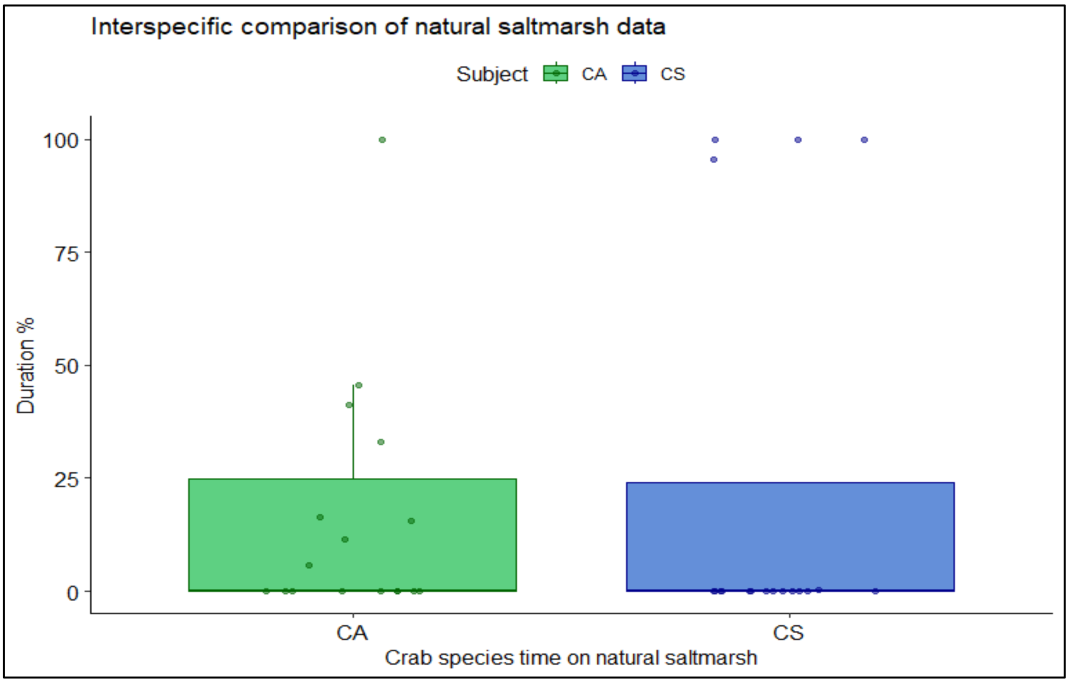


Fig. 11: This graph shows the correlation between the time spent by each species on the natural saltmarsh substrate. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not spend any time on this specific substrate. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As shown in the graph, 8 green crabs (red) and 5 blue crabs (blue) frequented this substrate. The differences in time are not statistically significant.

Fig. 12 shows the comparison of the time spent on the control substrate by the two species. Only one specimen of blue crab spent a small amount of time on the control substrate while it was more commonly frequented by the green crabs.

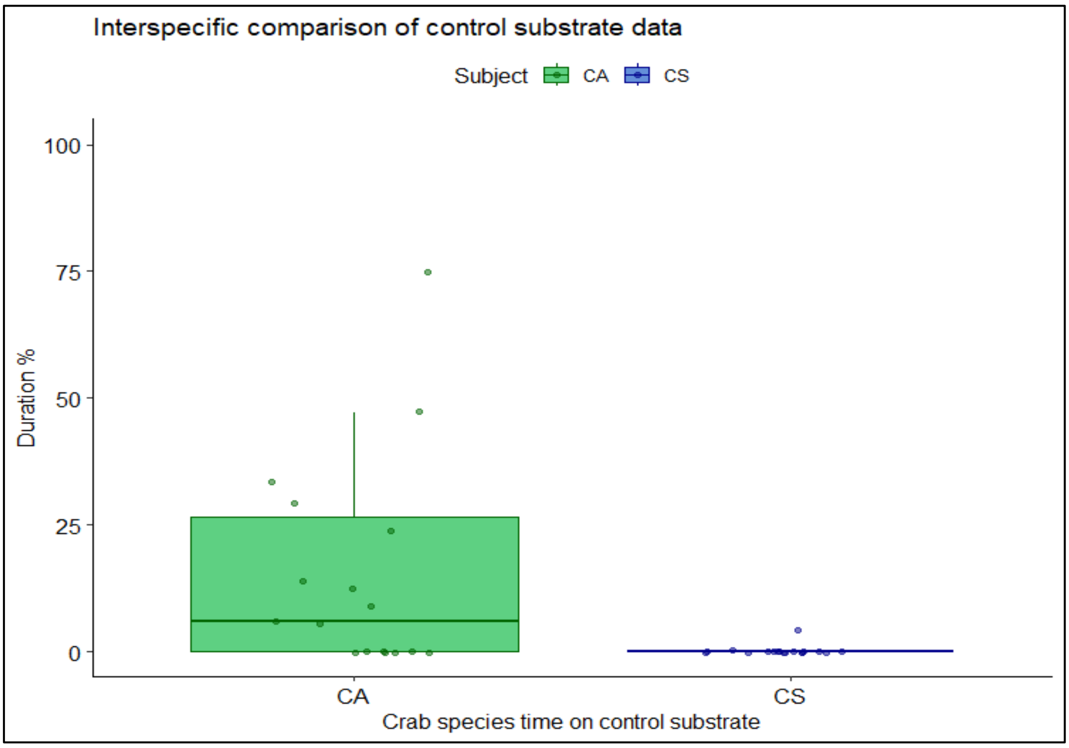


Fig. 12: This graph shows the correlation between the time spent by each species on the control substrate. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not spend any time on this specific substrate. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As shown in the graph, 10 green crabs (red) and 1 blue crabs (blue) frequented this substrate.

2. Analysis on behavior data

Fig. 13 shows the intraspecific analysis on behavior data by *C. sapidus*. The observed behaviors are movement atop the substrates and burrowing in one of the three sediment samples. From the graph we can see that the tested blue crabs spent most time burrowed rather than moving, and in fact only 10 crabs moved on the substrates, while all 18 crabs burrowed. The ANOVA variance analysis confirmed that the difference between time spent moving and burrowing is statistically very significant (ANOVA, F Value = 2829, P Value = <2e-16). Further confirming this, the average time spent moving by the blue crabs is 16,60 s, a very short time compared to the average time spent burrowing, 626,85 s.

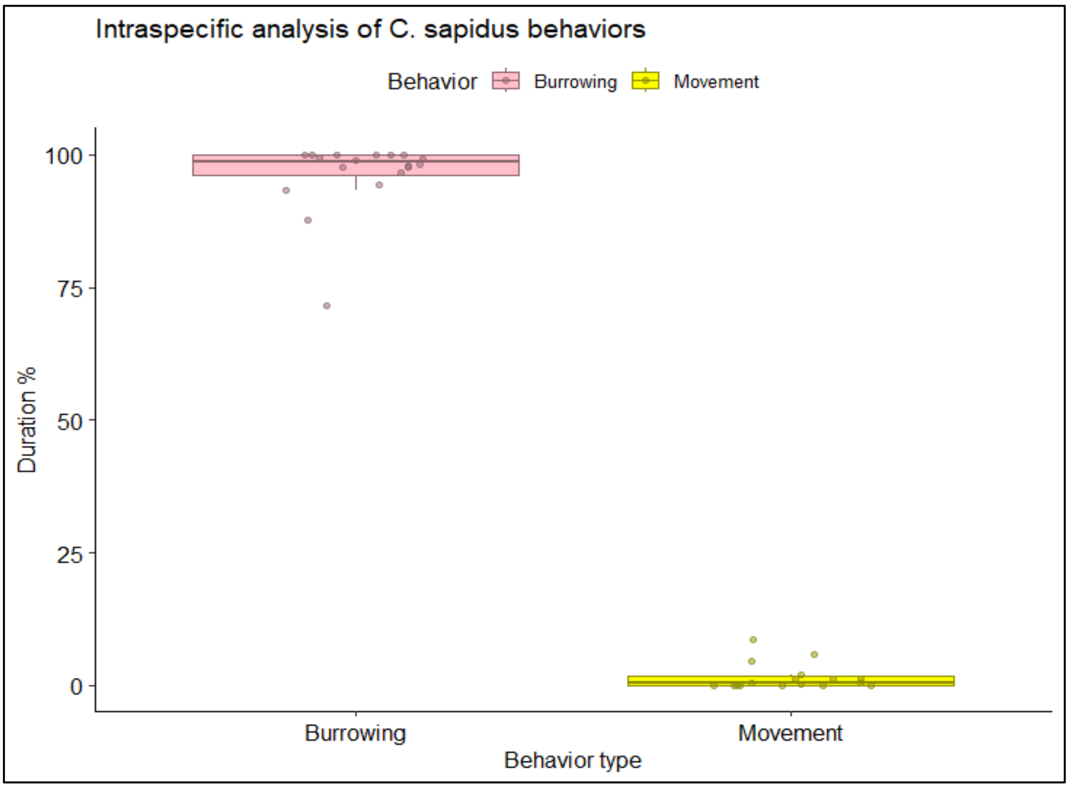


Fig. 13: This graph represents the time spent by specimens of *Callinectes sapidus* on the two observed behavior types: movement and burrowing. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not exhibit that specific behavior. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 – /+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. In this comparison the standing still behavior is not included for the sake of simplicity. As shown, all blue crabs spent most of their time burrowing, with only 10 of them moving for a short time, while the others burrowed as soon as they were freed from the holding cell. Evidently, blue crabs spend most of their time burrowed in the sediment.

Fig. 14 shows instead the intraspecific analysis on behavior data by *C. aestuarii*. The observed behaviors look the same from a qualitative but not quantitative point of view, and indeed in this case the difference is not statistically significant, as shown by the ANOVA analysis (ANOVA, F Value = 2,351, P Value = 0,135). The average time spent by the green crab while moving and burrowing are respectively 208,706 s and 413,759 s, values not far apart enough to be statistically relevant.

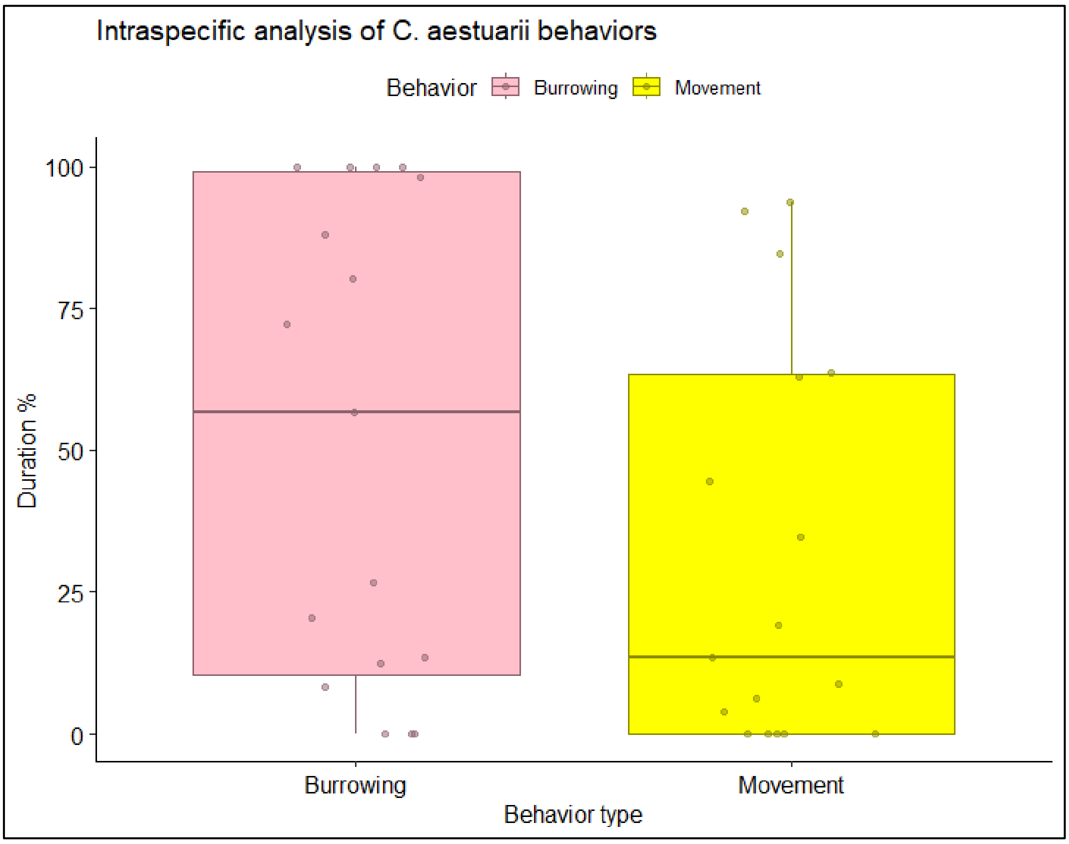


Fig. 14: This graph represents the time spent by specimens of *Carcinus aestuarii* on the two observed behavior types: movement and burrowing. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not exhibit that specific behavior. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As shown, 12 green crabs spent a good amount of time moving in the tank, while 14 of them spent time burrowed in the sediment. The differences in time are not statistically significant.

In Fig. 15 and 16 the interspecific analysis on the behavior data of the *C. aestuarii* and *C. sapidus* are represented.

Fig. 15 shows the relation between movement behavior duration in both species. It clearly shows a high amount of movement in the green crabs behavior, as opposite to the blue crabs. The variance analysis shows that the difference between the two species behavior is statistically significant (ANOVA, F Value = 11,86, P Value = 0,00162), meaning we can confirm that green crabs exhibit more movement behavior compared to blue crabs. On the other hand, Fig. 16 sets in comparison the two species burrowing behavior duration, and, opposite to the movement behavior graph, it shows that blue crabs burrow more and for longer periods of time than green crabs. This difference is proven significant by the ANOVA analysis (ANOVA, F Value = 19,64, P Value = 9,75e-05). On average green crabs spent 31,39% of their time moving and 62,59% of it burrowed in the sediment, the remaining ~6% was spent standing still. Blue crabs instead spent most of their time burrowed (96,39%), using only a short percentage of time to move around (2,6%).

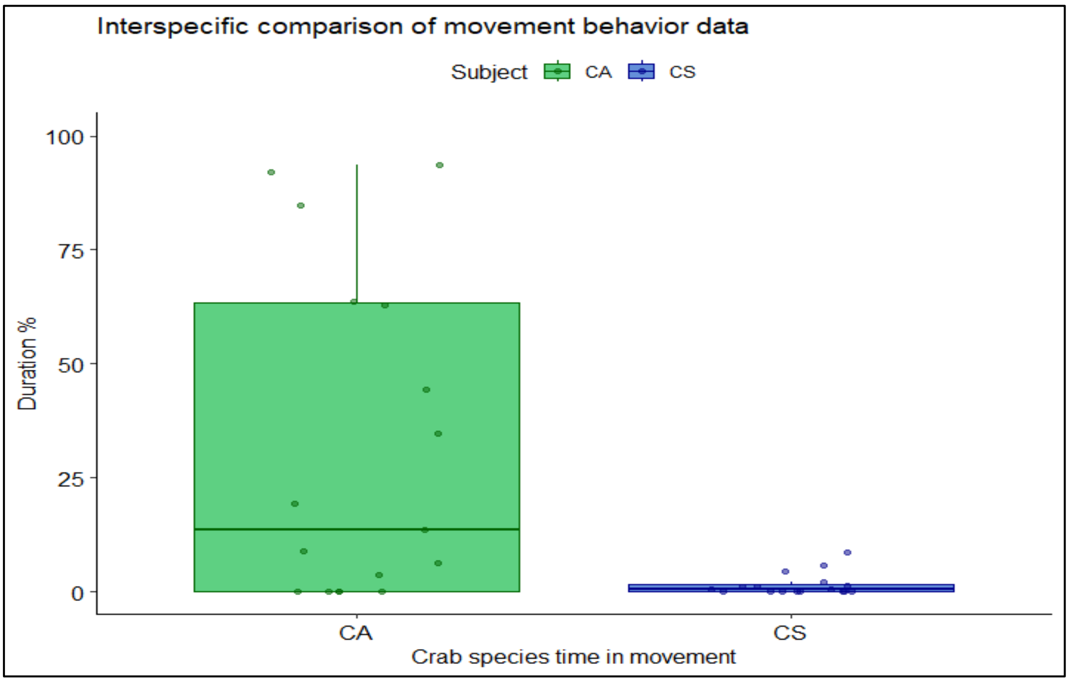


Fig. 15: This graph shows the correlation between the time spent by each species while moving. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not exhibit that specific behavior. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As shown in the graph, the average time spent moving by blue crabs is quite lower than the average time spent moving by the green crabs.

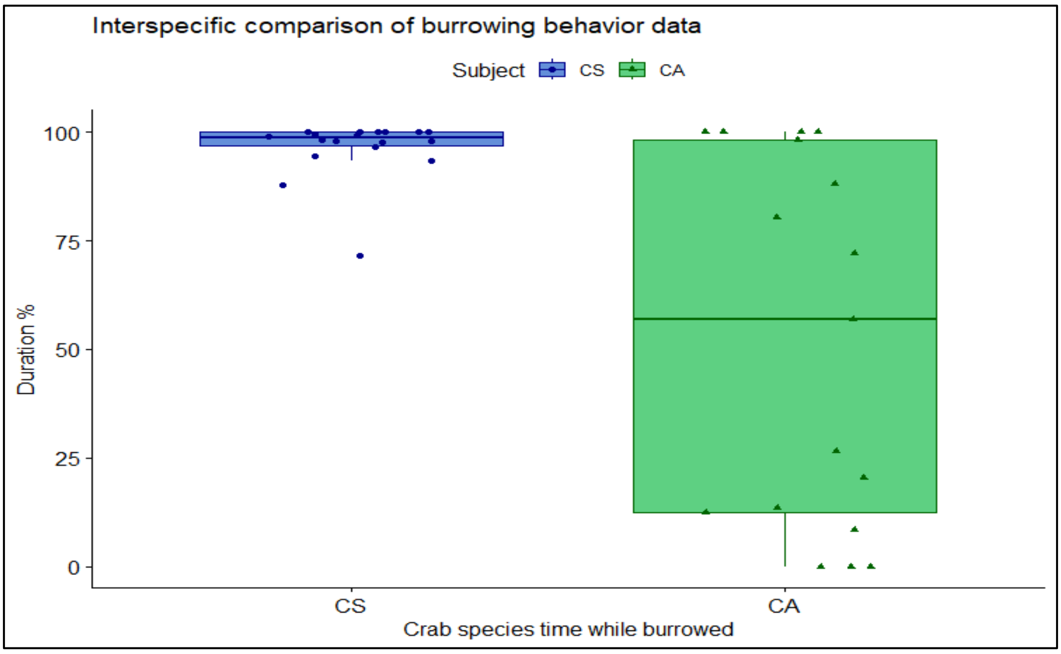
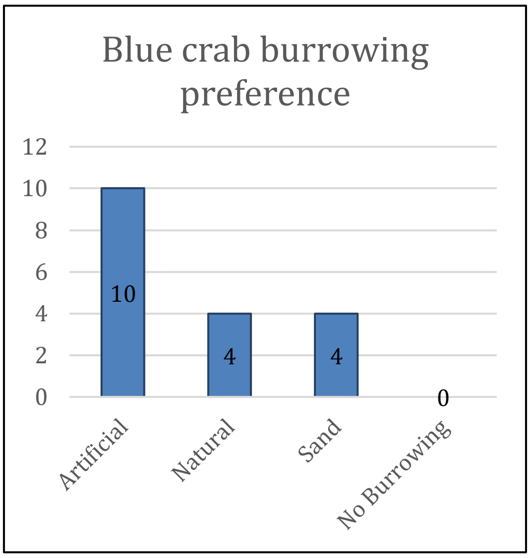


Fig. 16: This graph shows the correlation between the time spent by each species while burrowed. Each dot in the graph represents one individual, and the dots with a value of 0 represent the crabs that did not exhibit that specific behavior. The horizontal continuous line represents the median of the recorded durations, the light-shaded rectangles represent the values contained in the first to third quartile, also known as the interquartile range (IQR), and the whiskers that extend outwards from the boxes represent the variability outside of the IQR with a lower limit and an upper limit calculated from the IQR (Q1/Q3 –/+ 1,5 x IQR). Any data outside of these limits is represented as an outlier. As shown in the graph, the average time spent borrowed by blue crabs is quite higher than the average time spent burrowed by the green crabs.

Another aspect of the burrowing behavior is the choice of sediment to burrow into. Fig. 17 and 18 exhibit the choice of sediment the two species of crabs burrowed into during the experiment.



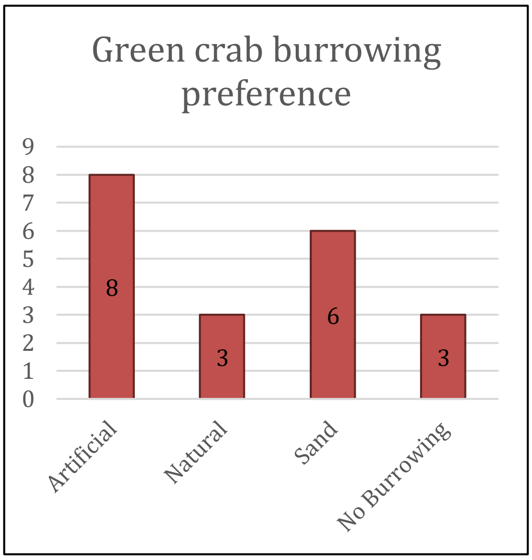


Fig 17: This graph shows the number of *C. sapidus* specimens that exhibited burrowing behavior and the sediments they chose to burrow into. As can be seen, all blue crabs exhibited burrowing behavior, with most of them burrowing in the artificial saltmarsh sediment.

Fig. 18: This graph shows the number of *C. aestuarii* specimens that exhibited burrowing behavior and what sediments they chose to burrow into. As shown, most green crabs exhibited burrowing behavior, with only 3 crabs not burrowing; of the burrowing crabs, most chose the artificial saltmarsh sediment, with the sand substrate coming close second.

V. Discussion

This analysis compares different behaviors, such as substrate preference, movement and burrowing behaviors, of the native *C. aestuarii* and the invasive *C. sapidus*. These results have shown that there are differences for the two species in these preferences and behaviors, both intra- and interspecifically. More specifically, the intraspecific analysis results on substrate permanence of the blue crabs have suggested a preference for the Artificial saltmarsh substrate (Fig. 5). This contrasts with the results from the study conducted by Bortot, (2022) which showed a clear preference of the blue crabs for sand substrate with a tendency to prefer sediments in relation to their fine sand content. Of note is that only one blue crab visited the control substrate, which could be an indicator of an aversion to artificial surfaces like glass and Plexiglass, or it could be attributed to them being juvenile crabs and therefore more skittish and quicker to seek refuge from potential threats, a hypothesis corroborated by the observation that, basically, each blue crab visits one substrate type only to immediately borrow in it (see below). For the green crabs the story is a little different: the results indicate a preference for the artificial saltmarsh compared to the natural saltmarsh and control substrates, but no avoidance of the control, and in general a tendency of each individual to visit more than one substrate type (Fig. 6). The interspecific confrontation of the substrate permanence data did not reveal any indication that either crab species had significant differences in substrate preferences than the other one (Fig. 9, 10, 11, 12).

The other part of this study involved the observation and analysis of the behaviors exhibited by the crabs: movement, standing still and burrowing. Intraspecifically, blue crabs exhibit a clear preference towards burrowing than movement, as more than 90% in average of their time was spent burrowed under the sediment (Fig. 13). For green crabs it is a less clear situation, with them spending a decent amount of time doing either behavior such that the statistical analysis found the differences in time spent not significant enough to be confirmed (Fig. 14). In the interspecific comparison, indeed, the statistical analysis clearly shows that blue crabs burrow more and for longer periods of time than green crabs, and, vice versa, that green crabs move more than blue crabs (Fig 15 & 16), coherently with the observations that each green crab individual visits more than one substrate. This difference in the burrowing behavior could be because adult green crabs are more bold and less prone to hiding in the sediment by burrowing as compared to juvenile blue crabs who have yet to finish growing into adults. Burrowing is a behavior commonly exhibited by blue crabs as it can be used to feed on prey up to 10-15 cm of depth and for juvenile individuals to hide from predators (Hines, 2007) and our study results confirm this with all tested blue crabs burrowing for most of the experiment duration. Green crabs also exhibit burrowing behaviors as means to escape predation in their juvenile stage, to overwinter, or to prey on burrowed organisms (Smallegange et al., 2009).

These results can be compared with the results from the similar study conducted by Bortot (2022), whose results showed a significant preference in substrate by the blue crabs in relation to its fine sand content, with a higher preference for the artificial saltmarsh sediment and sand substrate and no clear preference shown by the green crabs, hypothesized to be because of them recognizing their habitat quicker (Matozzo et al., 2013). Our studies yielded different results, with a preference shown by blue crabs towards the artificial saltmarsh but nothing in relation to the fine sand content; a possible dislike towards the control substrate was also highlighted. The green crabs showed a preference for the Artificial saltmarsh compared to the control and natural saltmarsh substrate. So, both species showed a preference for the Artificial saltmarsh substrate, which could be an indication of the invasive blue crabs adapting to the local environment of the Venice Lagoon in a similar way to the green crabs, or of the presence of traces of relevant resources, such as food, in the restored substrate.

VI. Conclusions

This study aimed to clarify the preference for sediment substrate by the invasive blue crabs and the native green crab, specifically looking for correlation with sediment type and grain size in hopes of finding results that could help in identifying possible colonization habitats for juvenile blue crabs in the Venetian Lagoon and to evaluate whether interactions between the two species could be happening.

Our results highlighted two significant preferences from the blue crabs regarding sediment permanence: a possible dislike towards the control substrate, a hard surface with no burrowing possibility leaving the crabs exposed, and a preference towards the Artificial saltmarsh. In relation to the behavior analysis blue crabs showed a clear preference to burrowing compared to moving on the substrate, possibly resulting from a more skittish nature of their juvenile stage. On the other hand, the green crabs sediment permanence analysis showed a preference for the Artificial saltmarsh compared to the control and natural saltmarsh substrate, but each individual typically visited more than one substrate unlike the blue crab. The behavioral analysis also did not shed light on a preference between moving or burrowing behavior. These results could further confirm the hypothesis of the adult green crabs leading a generalist lifestyle in the Venetian Lagoon.

From these results, competition between the two species is possible, with overlapping substrate permanence duration data, except for the control. A grain-size correlation to substrate preference is unlikely from these results, but grain size characterization may prove tricky when dealing with substrates rich in organic substance such as in natural environments of the Venice Lagoon and it may be improved using alternative laboratory methodologies. Also, as this experiment was conducted in laboratory conditions, results in the natural habitat and with different life stages of these crabs (i.e., also including adult blue crabs) could be different. A follow-up study on the possible interactions between the two crab species in a lab-controlled environment or also in their natural habitats could be useful to further confirm what kind of interactions can take place between them and whether their behavior in regard to substrate preferences changes when interacting with one another.

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