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Comparing the carbon and nitrogen fluxes in *Ostrea edulis* and *Crassostrea gigas* in the Venice lagoon

Comparison of fluxes undergoing different types of incubations, taking into consideration biofouling on the external part of the shells

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Degree: Environmental Sciences, Global Change Approach
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Project: MAREA (MAtchmaking Restoration Ecology and Aquaculture)
A restoration project aiming to bring back O. edulis in the North-West Adriatic addressing the feasibility of its cultivation.

Master's thesis

Assessing the carbon and nitrogen fluxes through respiration, calcification, and metabolism processes in *Ostrea edulis* compared to the ones of *Crassostrea gigas* in the Venice lagoon

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Environmental Sciences – Global Change and Sustainability Curriculum

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(*Ostrea Edulis* (MAREA, 2021))

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ABSTRACT

This master thesis focuses on the European oyster *Ostrea edulis*. The species is considered to be 'threatened and in decline', in several areas of European waters. In the last period of time, different countries, have embraced and approved strategies for its conservation and restoration (Rodrigues-Perez, et al., 2019), but also smaller realities are fighting for the same goal. The main goal was to find out the differences in the fluxes of O_2, NH_4^+, CO_2 of the two species, in order to provide an overview of said differences. For this analysis, the researcher performed incubations to find out more information, through measurements of various parameters (namely O_2, NH_4^+, CO_2, pH, temperature, and alkalinity) on the fluxes.

To measure N and C fluxes, the researcher used chambers and measured the parameters with special kits, in order to get an idea of the amounts of each. The values measured were then used to calculate the fluxes.

The experimental phase included three main types of incubations in the chambers: not clean shell with animal inside (NCWA), clean shell from microorganisms (epibionts) with animal inside (CWA), and clean shell without animal inside (CWOA). Said three different types of incubations were performed with the same individual in different days in order to have the comparison of the measured values with and without animal, and with and without epibionts.

The above-mentioned incubations were done in AMBIENT and AMBIENT+ few degrees. This means that the temperature in one tank (AMBIENT), in which the oysters were kept throughout the time of experiments, was not controlled; and in the other one (AMBIENT+) the temperature was kept constant with a heater. The reason behind controlling the temperature in one tank was to understand if temperature had an influence on the fluxes measured or not.

The results provided few insights on the differences in the fluxes of the two species, but unfortunately no real conclusion was drawn. This was due to errors in the measurements and calculations, and in the number of incubations, that should have been more in order to provide a more complete profile.

CHAPTER 1 – Introduction and theoretical framework

It has been stated repeatedly through scientific research, papers, thesis, books, media, and every other mean of communication between people, that habitats all around the world, both terrestrial and marine have been degraded, damaged, or ever lost in some cases. In the specific case of marine habitats, human pressures are causing huge and unprecedented damages throughout the global oceans. In fact, during the years very large areas of marine habitats with their biodiversity have been drastically changed, causing rapid declines of the biodiversity, and changing organism behaviour and in their biological and ecological roles in the ecosystems (OurSharedSeas, 2022). It is well known that humans rely on marine ecosystems for a large variety of purposes, including goods and services, which, have been limited or modified, and, in some cases, were lost (Lee, 2020). These environmental tragedies and degradation started rising a lot of interest in scientists and scientific communities in general, for ecological restoration. In fact, the number of restoration projects is increasing very rapidly in the last decades, across a range of both marine and terrestrial ecosystems, which, of course, requires and creates much more research on a multidisciplinary level (Lee, 2020). Multidisciplinary research is fundamental in order for supporting successful restoration projects. Studies and analysis stated that bivalve habitats, and specifically oyster beds, are among the most endangered habitats in the world (Beck, et al., 2011). In this thesis the comparison of various fluxes of two species of oysters (*Ostrea edulis* and *Crassostrea gigas*) will be analysed. The research that will be described in the following sub-chapters aims to provide useful insights for a specific restorative project, namely MAREA – MAtchmaking Restoration Ecology & Aquaculture (https://pric.unive.it/projects/marea/home).

1.1 Background information of Ostrea edulis in the world

(Fig. 1: *Ostrea edulis*, (Pouvreau, 2017))

Ostrea edulis (fig. 1), also known with the name of European flat oyster, in the past was very abundant in European waters and could be found from the Norwegian Sea to the Atlantic coast of Morocco (Laing, Walker , & Areal , 2006). It was formerly much cultivated before the stocks were reduced drastically by overharvesting and disease (CABI, Ostrea edulis (European oyster), 2022). It was reintroduced in many areas for aquaculture or fisheries, and, in some cases, established wild populations in some of these regions (CABI, *Ostrea edulis* (European oyster), 2022). It is usually found from the shallow waters of lagoons for example, to around 50/60 m in depth in certain specific cases (Olsen , 1883) (Thurstan, 2013). The areas in which densities of populations and oyster beds are higher in size, are found mostly in the areas of Sweden and Danish Limfjord, which, compared with other areas, are less affected by anthropogenic impacts (Pogoda, 2019) (Thorngren, 2019). Indeed, also the nutrient composition in the water, the water temperature, the right level of salinity and currents play a huge role in the ability of *Ostrea edulis* to thrive in certain waters compared to others. In fact, *Ostrea edulis*, being a filter feeder, feeding on suspended organic particles, is associated with highly productive estuarine and shallow coastal water habitats, where nutrients are abundant, because of mud, rocks, muddy sand, muddy gravel with shells and hard silt are present (MarLIN, 2017). As fig. 2 shows, its distribution is mostly in west Europe and at a lower degree in central America.

(Fig. 2: Distribution of *Ostrea edulis* in the world ((OBIS), 2022))

Its shell is rounded and has external grooves and ridges radiating from the hinge; the left valve is saucer-shaped with pink or purple markings, and the right valve is flat and usually brown/yellow in colour (Yonge, 1926). The left valve is less marked than in cupped oysters of the genus *Crassostrea*, which will be described in the following sub-chapter. It grows up to 11 cm long, rarely larger. The inner surfaces are pearly, white, or bluish grey, often with darker blue areas (MarLIN, 2017). Moreover, a lifespan of 5-10 years is typical in most individuals, however they can reach even 15 years of age.

1.1.1 MAREA project – new frontiers

During the last decade, several projects, both local and global, concerning restoration of oyster beds have been implemented all around the world. Through conferences and networks such as NORA (https://noraeurope.eu/), communities of researchers, and activists form in order to undergo projects that aim to restore oyster beds and share knowledge on the importance of them for our environment. The MAREA (Matchmaking

Restoration Ecology & Aquaculture) project (grant agreement: 886037, June 2021-December 2023), was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie). Masterminded by Camilla Bertolini and supervised by prof. Roberto Pastres from Ca Foscari University of Venice.

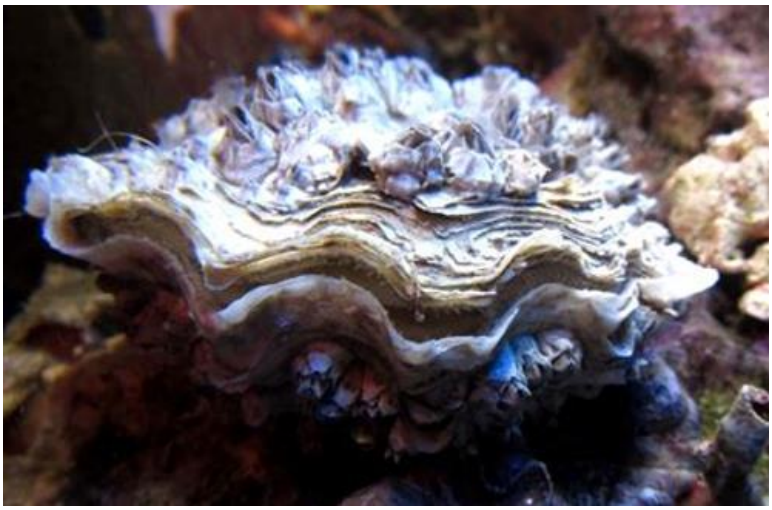
The main objective of this project is to demonstrate how ecosystem restoration theory can be incorporated within current aquaculture, to increase revenues while minimizing impacts on ecosystems. In the specific case of MAREA, the specific objective is to incorporate oyster reefs restoration, of *Ostrea edulis*, with mussel aquaculture (Bertolini & Pastres, 2021). The researchers behind the MAREA project are working on models to make future projections in order to understand how the population which has been reintroduced will behave and if there would be ecosystem services involved in its reintroduction.

Ostrea edulis reefs were destroyed and overexploited due to intensive trawling and dredging activities, and the populations suffered very bad consequences bringing some reefs to even disappear. The idea behind MAREA is to protect these restored oyster reefs from the damaging activities just mentioned, because trawling or dredging are not allowed within the mussel farm where the project takes place. The reef that is being restored and that will be protected, will act as a "mother reef", providing a source of new seeds, both to replenish natural populations in the Venice lagoon, but also to be collected to start a new chain of oyster farming based on local production (Bertolini & Pastres, 2021).

One of MAREA main objective is the development of mathematical models to make projections for future developments of oyster beds.

The experiments carried out in this master thesis were designed with the aim of supporting the parameterization of these models. This is not one of the objectives of the whole research, but it is one of the positive consequences that it might have. The results, will be useful to understand the fluxes of the two species of oysters analysed and to understand whether *O. edulis* would be more beneficial to the lagoonal environment of Venice, compared to *C. gigas.*

1.2 Background information of Crassostrea gigas in the world

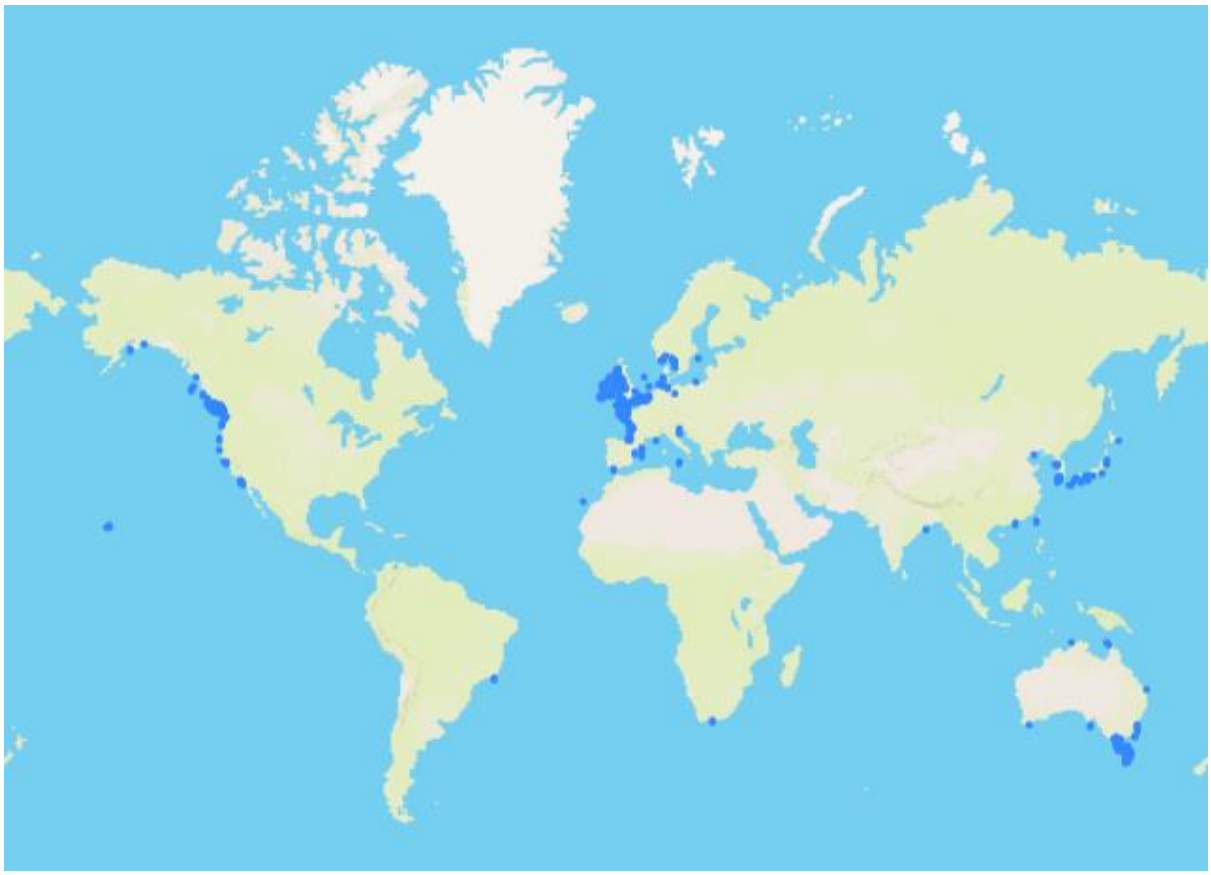


(Fig. 3: *Crassostrea gigas*, (stringfixer, 2022))

Crassostrea gigas (Fig. 3), also called Pacific oyster or *Magallana gigas* originally comes from north-eastern Asia (Japan) but at present is farmed in many countries worldwide. In fact, it is considered as one of the most "globalised" marine invertebrates. It is characterized by a high level of invasiveness, and therefore is considered as a pest or a noxious species in some cases (Ashton, 2001) (Blake, 2001). In some of these regions of the world, transfer restrictions are being applied, and in some other areas instead the species poses no problem because is considered to have an economic interest (Escapa, et al., 2004).

It can resist to freezing air temperature (-17°C) as well as a 20°C difference between low and high tide in winter. On the other hand, in summer conditions, it can resist up to 45°C on a muddy bottom. It has been translocated in many areas of the world with very different water conditions and climate because it is highly tolerant to a wide range of seawater temperature and salinity. Moreover, it has the capacity to grow in highly variable environments, from estuarine areas to brackish waters, to offshore areas in oceanic waters (CABI, Magallana gigas, 2022). It is very adaptable also in its nutrition. In fact, it is a plankton feeder, filtering phytoplanktonic species for food of a large gamma, but also ingesting detrital particulate organic matter. As Fig. 4 shows, its distribution is wider compared to *Ostrea edulis*, in fact it can be found again in western Europe and in

part of central America, but also in south-east Asia, Australia, and in smaller quantities in South Africa and South America.



(Fig. 4: Distribution of *Crassostrea gigas* in the world ((OBIS), 2022))

Crassostrea gigas has an elongated rough shell which can reach 20-30 cm in size. The two valves are usually very solid but unequal in size and shape. The valve itself is slightly convex and the right valve is quite deep, and cup shaped. Usually, like *Ostrea edulis*, they show purple steaks and spots, while the inner part is white (CABI, Magallana gigas, 2022).

1.2.1 Crassostrea gigas in the lagoon of Venice

At present, *Crassostrea gigas* it is widespread in the Mediterranean Sea. It was deliberately introduced in the Lagoon of Venice in 1966, where it was already present spontaneously since few years (Ligabue, 2022). At present, it can be found at high densities almost everywhere, even in the city canals, as it can tolerate marked fluctuation in pH and concentration of dissolved oxygen. Its presence is causing ecological problems and was probably one of the causes of the alleged decline of the autochthonous species *Ostrea*

edulis (Ligabue, 2022). Moreover, it is also found in internal areas where *Ostrea edulis* was never found before (Ligabue M. d., 2022).

1.6 Impact of anthropogenic activities on the two species of oysters in the lagoon of Venice

Lagoons, and in general coastal systems are among the most vulnerable ecosystems in the world and at the same time economically most important ones (Madricardo, et al., 2019). Humans, since these ecosystems are of much importance for goods and services of any kind, exploit them usually way too much, creating challenges for both flora and fauna to live. The lagoon of Venice is no exception. Negative human actions have huge effects on living organisms in the lagoon, which makes it difficult for them to thrive.

Examples of negative anthropogenic activities resulting in challenges for oysters in the lagoon include dredging activities, fast growing scours around anthropogenic structures built to protect the historical city of Venice from flooding, ships traffic (both private boats and ferry/taxi boats), diffuse littering that dissolves and/or sink to the bottom (Madricardo, et al., 2019). In changing the balance of the lagoonal waters, also nutrient availability, water conditions and everything related to those is affected. In fact, many organisms, such as oysters, must adapt their habits in order to stay alive and to keep thriving in these changing waters. Rises of water temperatures, changing in the current's patterns, sea-level rise, acidification, and nutrients availability. Anyhow, as can be seen from basic observations, *Crassostrea gigas* manages to thrive in the Venice lagoon seemingly without any problems.

Concerning nutrients availability, it is important to say that excessive nutrients input from anthropogenic activities can alter the balance between inputs and outputs, as has been said before. This change in balance results in a variety of negative consequences for the overall ecosystem. (Valiela, et al., 1992). For example, Nitrogen (N) is of concern for lagoons and estuaries because it often limits primary production regulating the base of food webs (Howarth, 1988). For example, excessive N presence stimulates algal growth, leading most of the times to harmful algal blooms, which is the beginning of a death cycle of a certain system, because air and light start getting blocked in the water column, which makes water plants and algae die, which as a consequence lead other species of organisms to starve to death and as a consequence they start to decompose which leads to extended

eutrophication (Nixon, 2012). Anyhow, oysters and other suspension filter feeders may be of help to sink some of that excessive N in the water (Kellogg, Cornwell, Owens, & Paynter, 2013). This whole concept of anthropogenic activities resulting in challenges for oysters in the Venice lagoon is related to the objectives of this research that will be described in the following sub-chapters, in the sense that understanding the fluxes of dissolved O_2, NH_4^+, and CO_2, may be of help for future research on topics related to anthropogenic activities in Venice lagoon and how to contrast them with the help of oysters perhaps.

1.7 Natural challenges for oysters – biofouling and epibionts

Epibionts are marine micro and macro-organisms, including algae, cyanobacteria, plankton, small vertebrates, and bacteria. The large amount of in situ infrastructure in mariculture production, and also in cities like Venice, causes settlement by numerous species of epibionts, which as a consequence causes biofouling on the oyster reefs and cultivations (Guenther, 2006) (Mallet, 2009). In fact, biofouling is a recurrent problem for aquaculture especially worldwide, that can cause huge economic losses on the long-term (Adams, 2011) (Willemsen, 2005). It can cause economic losses in the sense that machinery with biofouling needs more energy to sustain itself, or simply can cause irreversible damages to the mechanics involved. In another sense, biofouling can also cause biological challenges to the bivalve involved. Theoretically, since epibionts are living organisms, with a metabolism and need to feed themselves, they can subtract nutrients from the bivalves and so also changing their fluxes of nitrates and carbonates. Moreover, they could, again theoretically, consume more oxygen in the water again causing problems for other organisms. For example, biofouling by algae or other encrusting species can be so damaging and extensive that could block the or obstruct the water flows and change the morphology of the water bodies, preventing bivalves from opening and closing their valves. In these cases, of course, biofouling drastically reduces the amounts of food, oxygen and nutrients reaching the bivalves, which will create short and long-term negative impacts on their respiration, feeding rate, and ecosystem services (Lacoste & Gaertner-Mazouni, 2015). In these cases, epibionts, can be true competitors for the bivalve in the area, because they subtract nutrients from them and increase the oxygen demand of oyster reefs. Natural challenges, like anthropogenic activities resulting

in challenges for oysters in the Venice lagoon, are both topics that are part of this research, and that hopefully will be of help in future research concerning the same area.

1.3 Objectives of the research

The two main objectives of the thesis are:

1) To compare the respiration and ammonia excretion rates, under different temperature and irradiation conditions of the two oyster species (*Ostrea edulis* and *Crassostrea gigas*).
2) To assess the potential role of epibionts (biofouling) on the overall consumption and production of O_2, NH_4^+, and CO_2 of individual oysters from both species (*Ostrea edulis* and *Crassostrea gigas*).

1.4 Research questions

The objectives formulate the following research questions:

1) "Which species consume more O_2, excrete more NH4 and emits more CO2, at the same temperature and light intensity level?"

2) "Do epibionts (biofouling) affect the consumption and production rates of O_2, NH_4^+, and CO_2?"

1.5 Hypothesis

As the literature states, temperature and light will have an impact on the consumption and production of the variables measured.

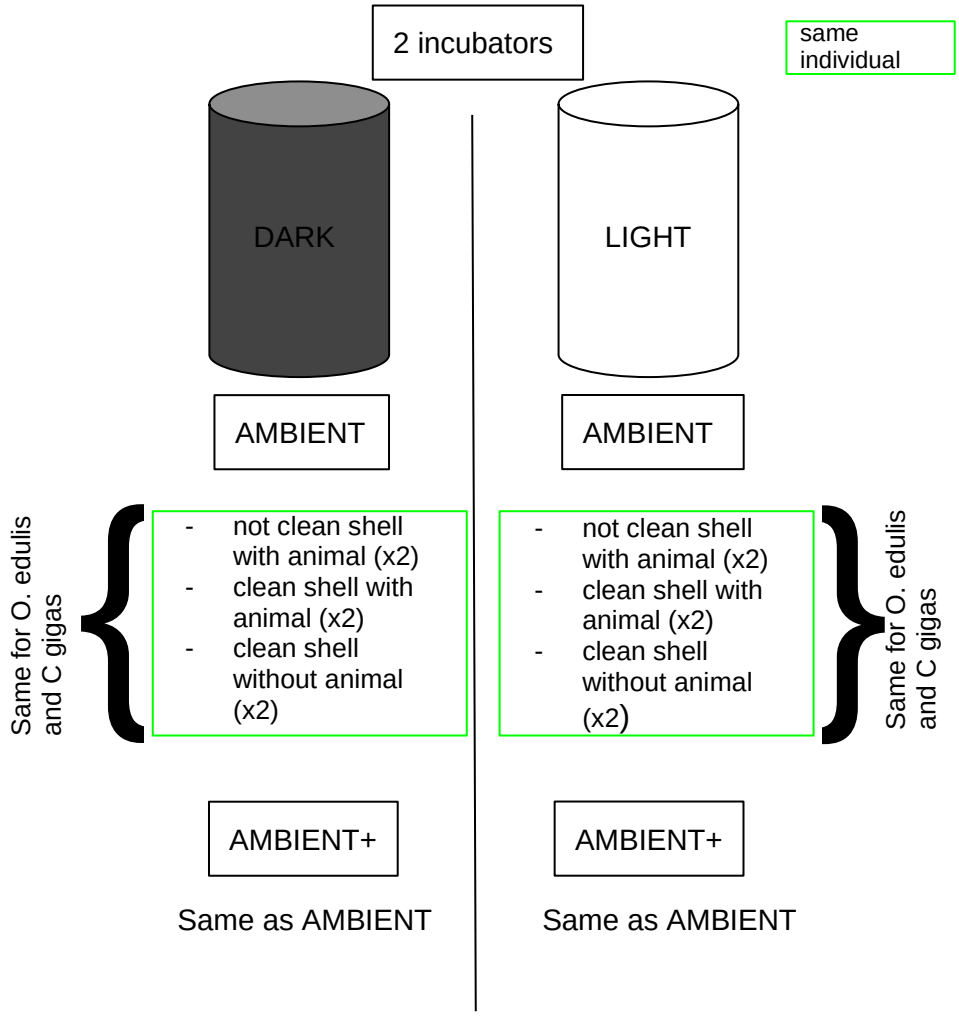
Moreover, it is hypothesized that epibionts, which are microorganisms which needs nutrients and light to survive, will influence the consumption and production of O_2, NH_4^+, and CO_2, during the incubation period of 5 hours.

CHAPTER 2 – Materials and methods

2.1 Description of experimental methods and sampling scheme (incubations and measurements)

The objectives stated in the previous chapter were achieved by designing and implementing a set of short-term incubation experiments in order to measure various parameters in order to be able to calculate the fluxes of O_2, NH_4^+, and CO_2:

- Shell with animal alive (not cleaned shell) – **NCWA** (not clean with animal)
- Shell with animal alive scrubbed from macro and microorganisms (epibionts, microalgae) – **CWA** (clean with animal)
- Shell scrubbed from macro and microorganisms (epibionts, microalgae) – **CWOA** (clean without animal)



(Fig. 5: incubations)

As can be seen from Fig. 5 the same individuals were used for all three situations (NCWA, CWA, and CWOA) in order to have the same amount of microorganisms and the same size of the area on the shell. This means that in total there were 24 incubations for each species, calculating 2 incubations for each situation multiplied by the two temperature situations (Fig. 5), which means that at least 24 individuals of *O. edulis* and 24 individuals of *C. gigas* were needed for the experiments. A total of 30 oysters for each species were collected, which means a total of 60 individuals (30 from *O. edulis* and 30 from *C. gigas*). This means that more oysters than needed were collected in order to be ready for any problem or inconvenience.

Each incubation consisted of one single oyster at a time in order to provide a profile for each situation (NCWA, CWA, and CWOA), and each incubation lasted 5h, because after several trials, more than 5 hours would have resulted in levels of dissolved O_2 that would have been too dangerous for the examined oyster. During the incubation, there will be a magnetic stirrer below the incubator which will operate a magnetic bar inside the chamber, in order to have a constant movement of the water.

In order to carry out the experimental design, 2 incubations (1 dark and 1 light) per daywere performed. The oysters were placed inside the incubator with the magnetic stirrer in the morning and stayed inside the chamber completely sealed for 5 hours; after that period, the measurements were taken and written down on a notebook and subsequently on an excel sheet.

On Tuesday the 29th of March the oysters were placed inside the basins and let them acclimate until Monday the 11th of April (2 weeks). The incubations started on the 11th of April as well. The incubations/measurements period, then, lasted until Friday the 20th of May.

As was said before, on top of the oysters' shells, a variety of microorganisms live and thrive (epibionts and microalgae). They are part of the analysis and of the incubations, so it was important to take them into consideration during the experimental phase. In order to do this properly, it was important to use the same individual for all the 3 incubations.

In order to represent as close as possible the conditions in the lagoon, all the above-mentioned incubation were done in ambient temperature (*AMBIENT*) (around 15°C) and

ambient temperature plus few degrees (*AMBIENT+*) (around 25°C) (Seatemperature, 2021) conditions, manually controlled in the basins in which the oysters will be kept.

The experimental design is summarized in tables 1 and 2, concerning, respectively, *O.edulis* and *C. gigas* . The oysters were given codes (see appendices for pictures of every oyster with the corresponding code) in order to insert the numbers correctly in the data sheets in excel, and in order to calculate the various parameters in connection to their weight. The codes were given with the letter O for *Ostrea edulis*, and with the letter C for *Crassostrea gigas*. The numbers close to the O or the C are simply in order of incubation. In table 1 for instance, all the incubations that were undertaken for *Ostrea edulis* are inserted in the right place in order to have a visualisation of the work that has been done. The same applies for table 2 which instead are all the incubations that has been undertaken for *Crassostrea gigas*. Moreover, in both tables all the parameters that have been measured are inserted. More specifically, as follows, all the parameters with are listed, with the instrumentation used to determine the variables:

- Dissolved O_2 was measured with HI—9146 Handheld Dissolved Oxygen Meter from HANNA instruments. The values were taken in the form of percentages, but then converted into mg/l of dissolved O_2 with the online tool Loligo online oxygen converter (https://www.loligosystems.com/convert-oxygen-units).
- NH_4^+ measurements were taken using a specific kit (Ammonium 3 from Nanocolor, 985003).
- Alkalinity was measured again with a kit (Alkalinity test from MColortest, 1.11109.0001)
- Temperature was measured and monitored with the same instrument used for dissolved O_2.
- CO_2 was calculated using the SeaCarb package in R-studio, inserting the alkalinity and pH measurements.
- pH was measured with a portable pH meter.

Thanks to these tables a good overview of the whole experiment procedure is shown, providing a full report of what has been done and what not.

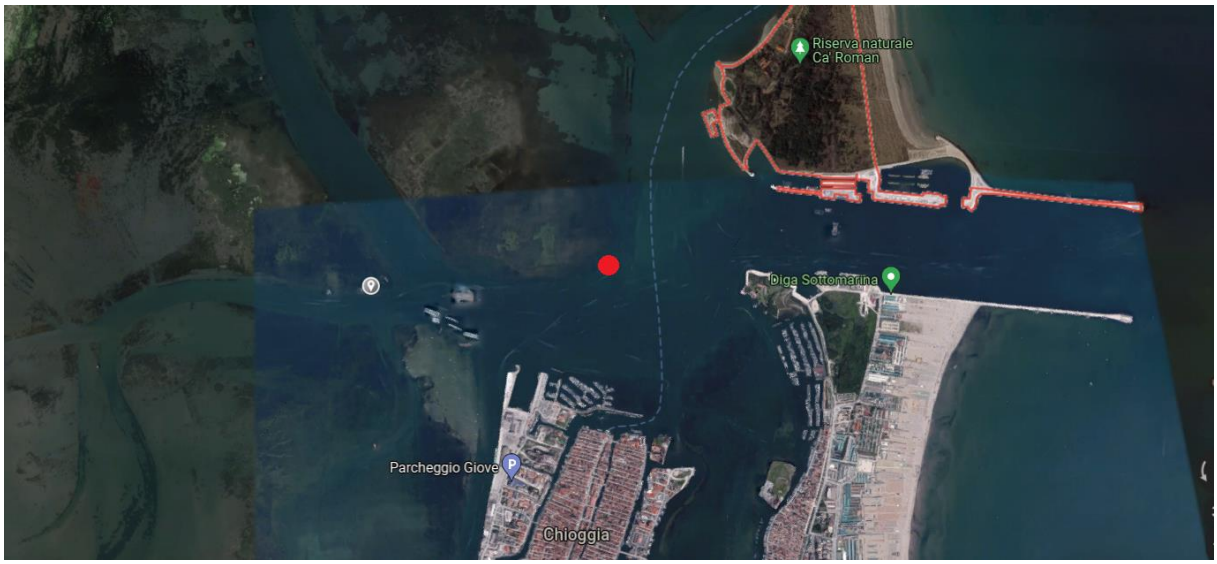
CASSOSTREA GIGAS								
			Dissolved O2	NH4+	Alkalinity	Temperature	CO2	pH
DARK INCUBATOR	AMBIENT+	NCWA	C6, C7	C6, C7	C6, C7	C6, C7	C6, C7	C6, C7
		CWA	C6, C7	C6, C7	C6, C7	C6, C7	C6, C7	C6, C7
		CWOA	C6, C7	C6, C7	C6, C7	C6, C7	C6, C7	C6, C7
	AMBIENT	NCWA	C1, C2	C1, C2	C1, C2	C1, C2	C1, C2	C1, C2
		CWA	C1, C2	C1, C2	C1, C2	C1, C2	C1, C2	C1, C2
		CWOA	C1, C2	C1, C2	C1, C2	C1, C2	C1, C2	C1, C2
LIGHT INCUBATOR	AMBIENT+	NCWA	C8, C9	C8, C9	C8, C9	C8, C9	C8, C9	C8, C9
		CWA	C8, C9	C8, C9	C8, C9	C8, C9	C8, C9	C8, C9
		CWOA	C8, C9	C8, C9	C8, C9	C8, C9	C8, C9	C8, C9
	AMBIENT	NCWA	C2, C4	C3, C4	C3, C4	C3, C4	C3, C4	C3, C4
		CWA	C3, C4	C3, C4	C3, C4	C3, C4	C3, C4	C3, C4
		CWOA	C3, C4	C3, C4	C3, C4	C3, C4	C3, C4	C3, C4

OSTREA EDULIS								
			Dissolved O2	NH4+	Alkalinity	Temperature	CO2	pH
DARK INCUBATOR	AMBIENT+	NCWA	O6	O6	O6	O6	O6	O6
		CWA	O6	O6	O6	O6	O6	O6
		CWOA	O6	O6	O6	O6	O6	O6
	AMBIENT	NCWA	O1, O2	O1, O2		O1, O2	O1, O2	
		CWA	O1, O2	O1, O2		O1, O2	O1, O2	
		CWOA	O1, O2	O1, O2		O1, O2	O1, O2	
LIGHT INCUBATOR	AMBIENT+	NCWA	O8	O8	O8	O8	O8	O8
		CWA	O8	O8	O8	O8	O8	O8
		CWOA	O8	O8	O8	O8	O8	O8
	AMBIENT	NCWA	O3, O4	O3, O4		O3, O4	O3, O4	
		CWA	O3, O4	O3, O4		O3, O4	O3, O4	
		CWOA	O3, O4	O3, O4		O3, O4	O3, O4	

(Table 1 and 2: tables including all the incubations that were performed throughout the research)

2.2 Collection of individuals

The individuals of *Ostrea edulis*, were collected at the pilot site of the MCSA "MAREA", grant agreement: 886037, located in an area in the south of Venice and close to Chioggia, near the *diga sottomarina* (Fig. 6). 30 specimens were collected by the MSCA fellow Camilla Bertolini, PhD. The individuals were brought to the scientific campus of Ca Foscari in Mestre and then placed in the canal close by, Canal Salso, to prepare the basins in the laboratory. After the oyster were placed in the two basins, a period of acclimation of 2 weeks started: half in the AMBIENT and half in the AMBIENT+ basin.



(Fig. 6: location of collection of *Ostrea edulis* individuals)

Crassostrea gigas individuals, have been collected directly from the canals of Venice city, specifically in the area of the *Teatro della Fenice*, using a *sandalo* boat with oars. The individuals were selected at random from the banks of the canal from the researcher and handpicked with gloves and a knife (fig. 7, 8, 9, 10), then placed inside a net-bag. Again, 30 oysters were collected and then brought to the scientific campus of Ca Foscari and placed in the canal Salso to prepare the basins in the laboratory. After the oyster are placed in the two basins, a period of acclimation of 7 days started: half in the AMBIENT and half in the AMBIENT+ basin.

The period of acclimation depends on the oyster species, but as (Thompson, et al., 2012) said in their paper, for *O. edulis* and *C. gigas* the acclimation time is the same, and

corresponds to at least 7 days, even if for C. Gigas it has been stated also that in some cases it takes shorter or longer to acclimate, depending on the conditions.

(Fig. 7, 8, 9, 10: collection of *Crassostrea gigas* from the canals of Venice, Credits: Ruggero Romano)

2.3 *Description of the lab*

The lab in which the experiments were carried out was inside of a container (Fig. 11). In it, oysters were kept, the incubations were performed, and the measurements were taken. The reason why the lab was developed inside the container was because the previous project for which it was used needed a space for cultivating vegetables, and the aquaculture agency that was affiliate was lacking such a space. In fact, in this case it was possible to decide all the technological solutions that where necessary for to develop an aquaponic system inside such a small space (Brigolin, 2020). The reason why the containers were moved to the campus of Ca Foscari was because the project was finished

and so they could be used in the future for different purposes, such as master thesis or other projects.

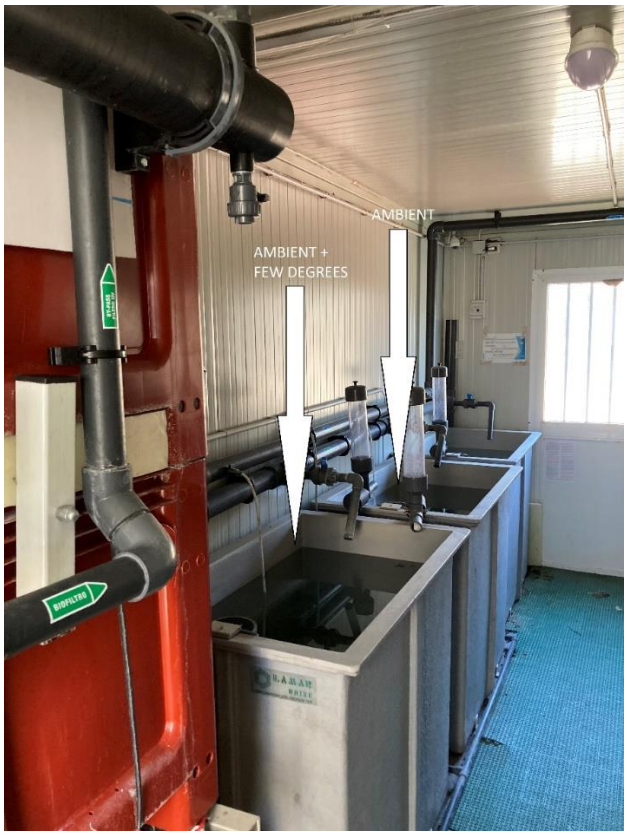
(Fig. 11: lab-containers at the scientific campus of Ca Foscari in Venice)

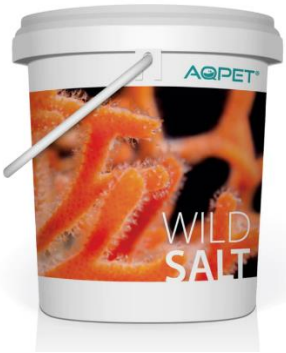
One tank was designated for the AMBIENT water and the other one for the AMBIENT+ few degrees water (Fig. 12). The basins shown in Fig. 12 were 50 cm for 100 cm at the base, and they were filled with water from the tap until 15 cm deep, because not too much water was needed to submerge the oysters. The total amount of litres in each basin was around 90. Then, the right amount of salt and minerals (Fig. 13) was added to make the water as similar as possible, from a salinity point of view, to the one found in the lagoon (between 34 and 36 PSU, or 30 mg/l). In order to reach that salinity level, around 2.7 kg of WILD SALT was added in each basin, because a total of 90 litres of water was placed in each basin. In both basins there was a pump in order to keep the water in movement. The pumps had also water dripping from the top in order to oxygenate the water constantly. Moreover, the AMBIENT+ basin will be provided with a small heater to increase the temperature in the water.

In the lab there was a table-like structure where measurements were taken, and the necessary equipment stored.

The basins where the oysters were kept, and the incubators were kept inside the container on the right where atmospheric temperature was kept a bit cooler, since it was

not a greenhouse-like structure. On the other hand, the working surface and the equipment were kept inside the left container (greenhouse-like).





(Fig. 12 and 13: basins where oysters were kept on the left, and prepared salt for the basins on the right)

Furthermore, inside the left container, a small culture of microalgae has been introduced in order to have a feed stock for the oysters that was self-produced and controlled. Unfortunately, the period in which the research has been carried out was April, May, and June, so the temperatures were too high to let microalgae grow inside a greenhouse-like structure. The small basins where it was thought to cultivate the microalgae (Fig. 14) would have been perfect for lower temperatures and a constant bubbling system to aerate the culture.

(Fig. 14: microalgae basins)

2.4 Animal husbandry

With animal husbandry is meant taking care of the individuals used for experiments in this case. In fact, for the experimental phase it is important to use healthy animals in order to get standardised results. It is imperative that the individuals are healthy also because they are kept in the same basin all together, which means that if even one individual result unhealthy in any way, could affect all the others creating problems for the schedule of the experiment. It is important then, to study the literature on the animals used for the experiments in order to treat them properly and to provide them with the right nutrients, temperature, and environmental conditions in general. In fact, every day the temperature and dissolved O_2 were measured in order to monitor them.

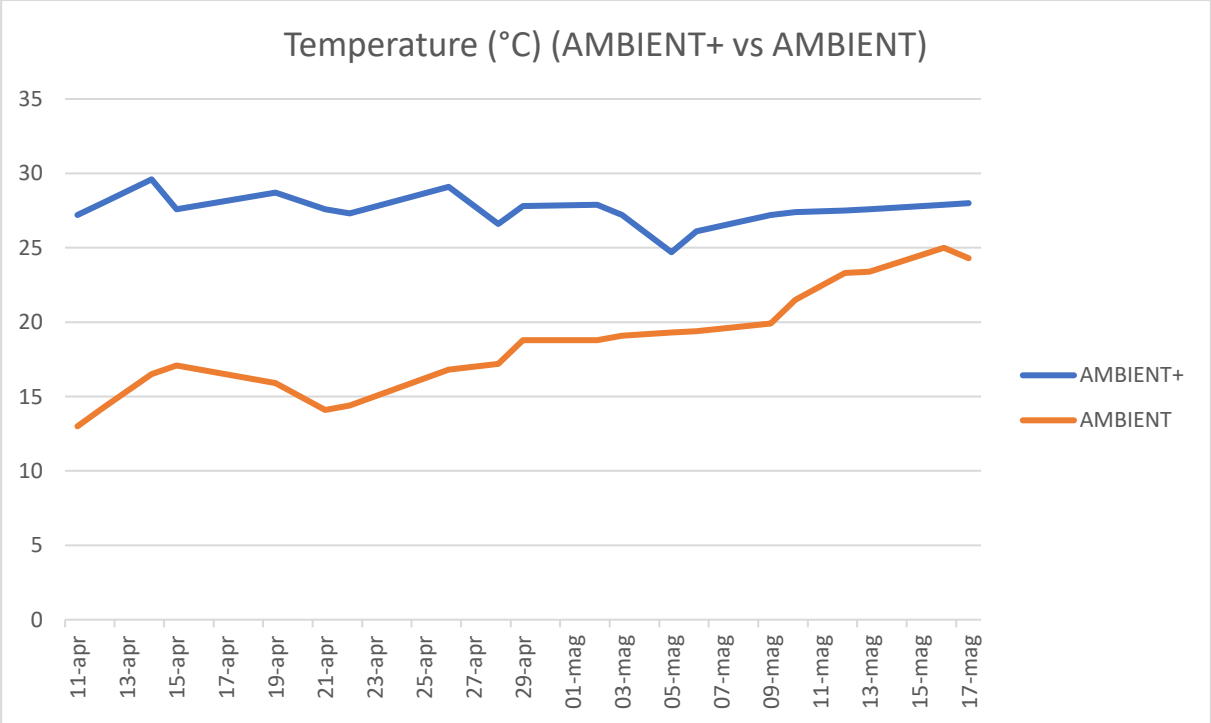
2.4.1 Feeding

Both *Ostrea edulis* and *Crassostrea gigas*, feed on plankton, filtering phytoplankton species for food, and also ingesting detrital particulate organic matter (CABI, Magallana gigas, 2022). As it has been described in the chapter about the lab set up, a small culture of microalgae was planned in order to be able to feed the oysters directly, but unfortunately the conditions of the lab were not ideal for this purpose and the culture died quite rapidly. If the culture worked properly, the oysters would have been fed with the cultivated microalgae every week, but since it didn't work an alternative had to be applied. The oysters were then fed with 6 beakers of 50 ml of water from the canal Salso close to the lab every Thursday, for each of the two basins. This was not the ideal solution but was the only way to not postpone the experimental phase and the whole experiment. Indeed, the concentration of phytoplankton in the water of the canal Salso was lower that the concertation of the in-lab cultivation of microalgae, and of course less controlled in terms of other microorganisms and debris.

This solution worked out perfectly for the AMBIENT basin but had some repercussions on the AMBIENT+ one, because probably the oysters would have needed more quantities because the water temperature was higher.

2.4.2 Temperature

The temperature in both basins was monitored every Monday, Tuesday, Thursday, and Friday, during the whole duration of the experimental phase. The temperatures measured were then transcribed on an excel sheet in order to have an overview of the changes with time. In theory, the temperature in the AMBIENT basin would increase with time because, since the experimental phase started in April, the atmospheric temperature would increase with the beginning of summer season. In fact, this was the case. As a matter of fact, the temperature increased of 10°C from the first measurement (22/04/2022) to the last measurement (09/05/2022). Moreover, these measurements were taken also to make estimations on the dissolved O_2 changes, and if they can be correlated to the changes in temperature.



(Fig. 15 temperature monitoring in AMBIENT and AMBIENT+ basins)

As can be seen from Fig. 15, the temperature rise in the AMBIENT basin is almost gradual in its increase. Starting from the first measurement on the 11th of April, with some fluctuations, the temperature in the basin increased of 11.3°C (from 13°C to 24.3°C). This is explained from the increase in atmospheric temperature, which increases because of the incoming summer season. It is important to say that the first set of incubations has been done using oysters kept in the AMBIENT basin because, during the experimental

planning, it has been hypothesized that the temperature would have increased, so in this way the oysters used for incubations would have been acclimated to a similar temperature throughout all the incubations.

As for the AMBIENT basin, also for the AMBIENT+ basin, temperature and dissolved O_2 were monitored throughout the time of experimental phase. Differently from the trend of temperature shown in AMBIENT basin, the temperature of AMBIENT+ basin fluctuates between 29.6°C and 26.1°C. This is because there was a heater inside the water set at 29.5°C, which switches on only when the temperature perceived by its sensor goes below that set temperature. As can be seen from the graph, on the 5th of May the temperature measured 24.7°C which is indeed very low compared to the other measurement. This was because that day the water was renewed, due to several dead oysters found that morning.

The ideal temperature in which the two species of oysters thrive differs from each other. For *Ostrea edulis*, fluctuates between 14°C and 24°C (Eymann, et al., 2020), but can manage to live in a larger range of temperatures. On the other hand, *Crassostrea gigas*, manages to thrive in a larger range of temperatures. In fact, can live properly from 3°C until 35°C (Palmer, et al., 2021).

2.4.3 Mortality

It is common in ecology experiments, were living organisms are involved, to have mortality of certain individuals. This is the case because usually, the experiments are done in contexts that are not natural for the organisms, even if the researchers typically try to recreate the environment in the best and more accurate way possible. Anyhow, even if all the parameters are set to be as close as possible to the natural world, there would always be an artificial factor that can cause stress among the organisms, or there could be external contamination that can cause illnesses, bring viruses, or just directly kill some of the individuals.

During the period in which the oysters were kept in the canal Salso there was no mortality at all among the individuals of both species. Also, during the acclimation period in the basins there was no mortality. During the acclimation periods it is more common to have mortality due to stress because the individuals have to adapt to a new environment, to new conditions and to other individuals that perhaps were not close to them previously.

On the other hand, during the course of the experimental phase, there was mortality among the individuals. The first set of incubations was undertaken with the oysters from the AMBIENT basin. This because, as it has been said before, the temperature in that basin would have theoretically increased with time and with the rise of atmospheric temperatures. In order to have a lower variation in temperatures it was wiser to do all the incubations from the AMBIENT basin rather that the AMBIENT+ basin where the temperature stayed more constant throughout time. This indeed resulted in the fact that the oysters that were kept in the AMBIENT+ basin stayed for a longer period of time waiting for the incubation period (13 days precisely). This extended time built up the stress and the higher temperatures in the basin increased it even more. This resulted in mortality, especially in *Ostrea edulis* individuals, that are more sensitive to higher temperatures compared to *Crassostrea gigas*. On the 29th of April 2022, 80% of *Ostrea edulis* individuals in the AMBIENT+ basin died and had to be removed. The water was refreshed on the 5th of May, and the internal borders of the basin were cleaned. The dead individuals (12 out of 15) were replaced with other individuals from the same species on the 5th of May and let them acclimate for a couple of days only because they already acclimated to the basins before, where they rested in case of back up. Unfortunately, on the 12th of May 6 more individuals from *Ostrea edulis* died again, and got replaced the day after. Lastly, on the 16th of May 2 more oysters from the same species died again.

Mortality could have been caused by a variety of factors. The one that is more plausible is indeed the water temperature that was at the limit for *Ostrea edulis* survival, but it could have been due also to an unknown pathogen in the water that hit only the individuals from the Ostrea edulis species.

CHAPTER 3 - Results

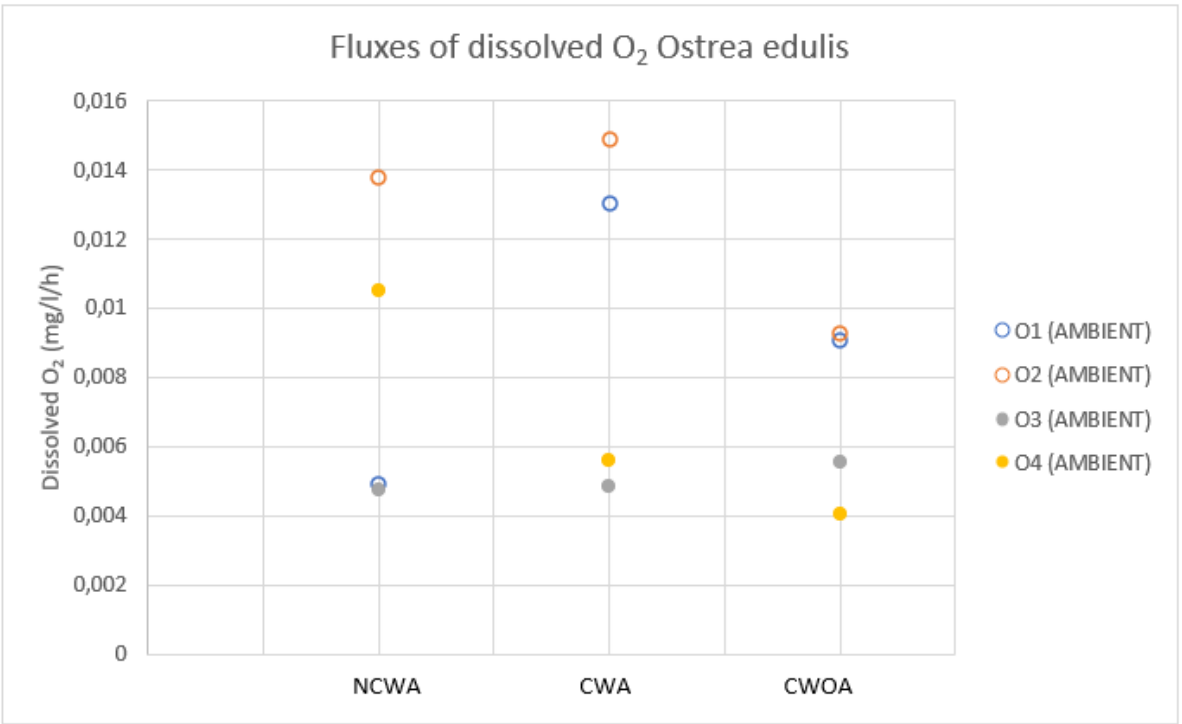
This chapter presents the results of the incubations. The results chapter is divided for species of oyster analysed, so, firstly the results from incubations of *Ostrea edulis* will be reported. Secondly, the same graphs will be displayed for the measurements of *Crassostrea gigas.*

Lastly, but of equal importance, the monitoring of temperature and dissolved O_2 will be shown.

All the data in the graphs representing the O_2, NH_4^+, and CO_2 fluxes in the result chapter have been standardized in the same way: the data has been transformed into a flux, dividing the percentage or concentration measured with the volume of the incubator and by 5 hours of incubation, in order to have it standardized for 1 hour only. Moreover, each specific measurement was divided again by the weight in grams of the oyster in question.

To clarify the graphs in this chapter, can be seen that half of the dots are full and half are empty, this is to indicate which measurements were taken from the incubation in the dark chamber (empty) and which ones from the light chamber (full).

3.1 *Ostrea edulis*

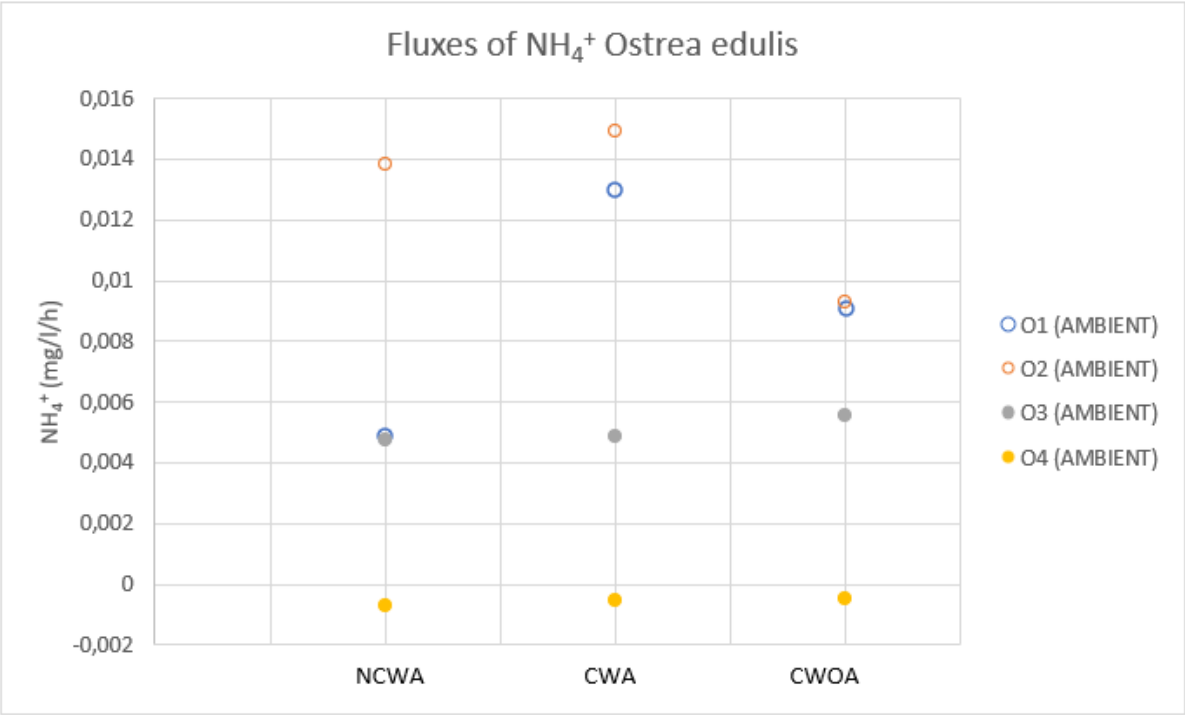


(Figure 16: Fluxes of dissolved O_2 *Ostrea edulis*)

In Fig. 16 it is displayed the measured dissolved O_2, which was taken in every situation mentioned from now on, with the portable dissolved oxygen meter HI9146 from Hanna Instruments.

The data acquired are in mg/l, which have been calculated from a percentage assuming that the salinity was 30 ppm, which is the most direct way to show results like the ones of dissolved O_2 fluxes.

For the incubation with oysters cleaned with animal (CWA), it is clear that, even if slightly, there is a difference between the oysters kept inside the dark incubator, in comparison to the ones in the light one. In fact, the flux of the oxygen is higher in the dark incubator, compared to the light one that is lower.



(Figure 17: Fluxes of NH_4^+ *Ostrea edulis*)

Regarding the NH_4^+, it is visible from the first glimpse at the appendix with all the measurement before and after the incubations, that during the time of incubation, the NH_4^+ concentration in the water increases, which is something that was hypothesized. This because, while filtering and transforming one type of nitrogen (nitrate), oysters are

also producing NH_4^+ as a by-product, or waste, which indeed increases its concentration in the water as shown in Fig. 17.

In Fig. 17 can be seen a distinction between oysters who underwent the dark incubation in comparison to the ones of light incubation. Without light it seems that oysters excrete more NH_4^+.

As can be seen in chapter 3.1.1, oyster O3 is the biggest one of the four used for the experiments in AMBIENT basin, which theoretically would be the one excreting the biggest quantity of NH_4^+, but apparently it is not the case, because O1 is smaller than it and still produces higher peaks of NH_4^+ excretions, especially when the oyster is still present inside the shell.

AMBIENT	DATE	temperature (°C)
O1 NCWA before incubation (DARK)	11/04/2022	13
O3 NCWA before incubation (LIGHT)	11/04/2022	13
O1 NCWA after incubation (DARK)	11/04/2022	18,6
O3 NCWA after incubation (LIGHT)	11/04/2022	18,8
O1 CWA before incubation (DARK)	12/04/2022	14,4
O3 CWA before incubation (LIGHT)	12/04/2022	14
O1 CWA after incubation (DARK)	12/04/2022	20,7
O3 CWA after incubation (LIGHT)	12/04/2022	18,7
O1 CWOA before incubation (DARK)	14/04/2022	16,9
O3 CWOA before incubation (LIGHT)	14/04/2022	16,1
O1 CWOA after incubation (DARK)	14/04/2022	23,9
O3 CWOA after incubation (LIGHT)	14/04/2022	21,8
AMBIENT		
O2 NCWA before incubation (DARK)	15/04/2022	17,3
O4 NCWA before incubation (LIGHT)	15/04/2022	16,8
O2 NCWA after incubation (DARK)	15/04/2022	22,6
O4 NCWA after incubation (LIGHT)	15/04/2022	21,2
O2 CWA before incubation (DARK)	19/04/2022	16
O4 CWA before incubation (LIGHT)	19/04/2022	15,8
O2 CWA after incubation (DARK)	19/04/2022	20,6
O4 CWA after incubation (LIGHT)	19/04/2022	18,9
O2 CWOA before incubation (DARK)	21/04/2022	14,2
O4 CWOA before incubation (LIGHT)	21/04/2022	13,9
O2 CWOA after incubation (DARK)	21/04/2022	18,7
O4 CWOA after incubation (LIGHT)	21/04/2022	16,8
AMBIENT+		
O6 NCWOA before incubation (DARK)	16/05/2022	27,4
O8 NCWOA before incubation (LIGHT)	16/05/2022	27,3
O6 NCWOA after incubation (DARK)	16/05/2022	30,8
O8 NCWOA after incubation (LIGHT)	16/05/2022	29,6

O6 CWOA before incubation (DARK)	17/05/2022	26,8
O8 CWOA after incubation (LIGHT)	17/05/2022	26,5
O6 CWOA before incubation (DARK)	17/05/2022	30,9
O8 CWOA after incubation (LIGHT)	17/05/2022	29,5

(Table. 3: Temperature incubations *Ostrea edulis* AMBIENT basin)

The temperature inside the incubators increases with time, especially if there is no exchange of air because the chamber is sealed. In fact, as can be seen from Table 3, the temperature increases from before the incubations to after. The increase is not very pronounced, but in most cases, there is an increase of 5°C between the beginning and the end of the incubation, which means more or less 1°C every hour of incubation. This is the case in the AMBIENT situation, but we will see that it would be a little bit different for the AMBIENT+ one.

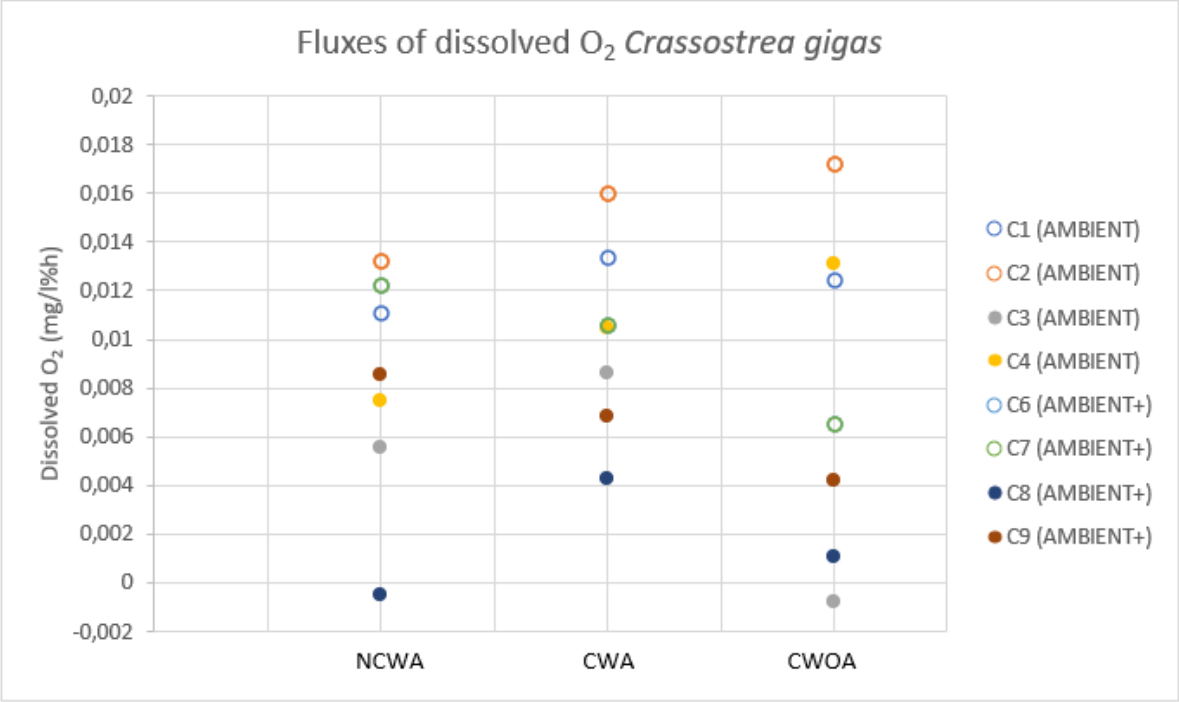
During the time of experiments, since the incubations started for the AMBIENT basin because of the reasons mentioned in the previous chapters, several oysters died in the AMBIENT+ basin, resulting in a shortage of individuals for the incubations of *Ostrea edulis* from the AMBIENT+ basin. As can be seen from Table 3, there are data only for two individuals instead of four like the other graphs. This is because there were no replacements easily accessible in the short term. The researcher decided to still undergo the incubations, even if only with two individuals.

3.1.1 Size and weight of individuals

OYSTER	weight (g)	length (cm)	width (cm)	volume (ml)
O1	45.86	8.7	5.5	35
O2	28.78	7.9	4.8	45
O3	62.45	8.4	5.8	25
O4	48.06	8.5	5.6	40

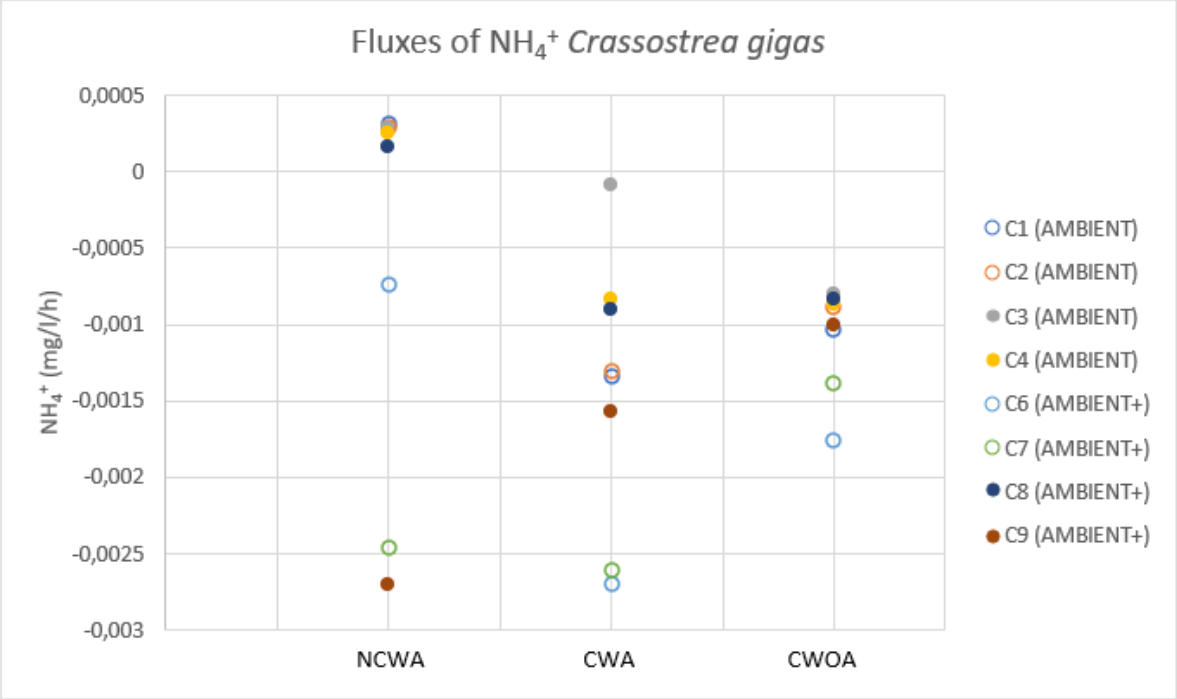
(Table 4: weight, length, width, and volume of *Ostrea edulis* individuals used for experiments)

3.2 *Crassostrea gigas*



(Fig. 18: Fluxes of O_2 *Crassostrea gigas*)

As for *Ostrea edulis*, the dissolved O_2 measured before and after the incubations seems to not change significantly between dark and light chambers, so this means that the light availability during the incubations is not a factor affecting the consumption of O_2 in the water. The only thing that can be said looking at Fig. 18 is that during the NCWA and CWA incubations, three out of fours oysters which undertaken the dark incubation, seems to have higher flux of O_2 compared to the other ones.



(Fig. 19: Fluxes of NH_4^+ *Crassostrea gigas*)

The peculiar thing about Fig. 19 is that, during the first incubation (NCWA) it seems that almost all the oysters analysed had positive flux for NH_4^+, which is something strange because usually oysters excrete NH_4^+ instead of uptake it. The other two incubations instead made the oysters behave more homogenously between each other. Furthermore, during dark incubations, even if slightly, there was more excretion of NH_4^+.

AMBIENT	DATE	temperature (°C)
C1 NCWA before incubation (DARK)	22/04/2022	14,6
C3 NCWA before incubation (LIGHT)	22/04/2022	14,2
C1 NCWA after incubation (DARK)	22/04/2022	16,4
C3 NCWA after incubation (LIGHT)	22/04/2022	15
C1 CWA before incubation (DARK)	26/04/2022	17
C3 CWA before incubation (LIGHT)	26/04/2022	16,6
C1 CWA after incubation (DARK)	26/04/2022	20,4
C3 CWA after incubation (LIGHT)	26/04/2022	20,1
C1 CWOA before incubation (DARK)	28/04/2022	17,3
C3 CWOA before incubation (LIGHT)	28/04/2022	17,1
C1 CWOA after incubation (DARK)	28/04/2022	23,8
C3 CWOA after incubation (LIGHT)	28/04/2022	22,7
AMBIENT		
C2 NCWA before incubation (DARK)	29/04/2022	18,9
C4 NCWA before incubation (LIGHT)	29/04/2022	18,6
C2 NCWA after incubation (DARK)	29/04/2022	22,1

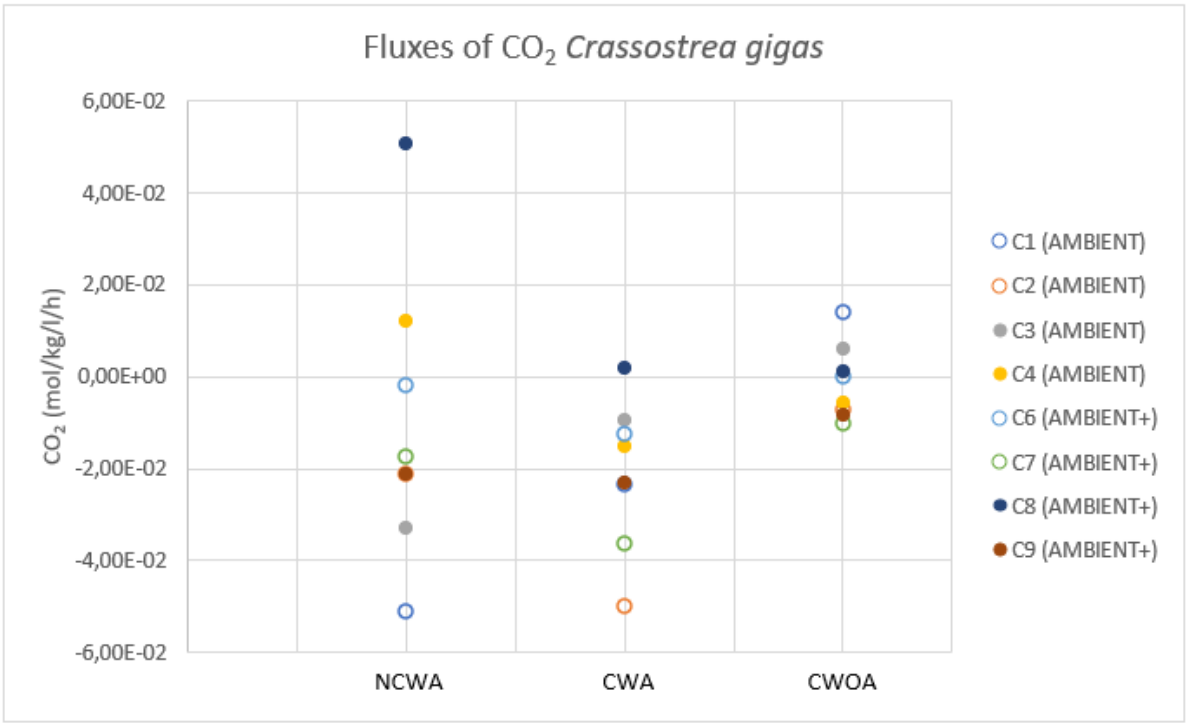
C4 NCWA after incubation (LIGHT)	29/04/2022	21,4
C2 CWA before incubation (DARK)	02/05/2022	18,7
C4 CWA before incubation (LIGHT)	02/05/2022	18,9
C2 CWA after incubation (DARK)	02/05/2022	24,9
C4 CWA after incubation (LIGHT)	02/05/2022	24
C2 CWOA before incubation (DARK)	03/05/2022	19,2
C4 CWOA before incubation (LIGHT)	03/05/2022	19
C2 CWOA after incubation (DARK)	03/05/2022	25,9
C4 CWOA after incubation (LIGHT)	03/05/2022	25,8
AMBIENT+		
C6 NCWA before incubation (DARK)	05/05/2022	24,4
C8 NCWA before incubation (LIGHT)	05/05/2022	24,5
C6 NCWA after incubation (DARK)	05/05/2022	22,7
C8 NCWA after incubation (LIGHT)	05/05/2022	21,5
C6 CWA before incubation (DARK)	06/05/2022	22,6
C8 CWA before incubation (LIGHT)	06/05/2022	22,7
C6 CWA after incubation (DARK)	06/05/2022	20,8
C8 CWA after incubation (LIGHT)	06/05/2022	28,9
C6 CWOA before incubation (DARK)	09/05/2022	24,2
C8 CWOA before incubation (LIGHT)	09/05/2022	24,3
C6 CWOA after incubation (DARK)	09/05/2022	27,2
C8 CWOA after incubation (LIGHT)	09/05/2022	26,1
AMBIENT+		
C7 NCWA before incubation (DARK)	10/05/2022	25,7
C9 NCWA before incubation (LIGHT)	10/05/2022	25,7
C7 NCWA after incubation (DARK)	10/05/2022	28,6
C9 NCWA after incubation (LIGHT)	10/05/2022	28,1
C7 CWA before incubation (DARK)	12/05/2022	25,4
C9 CWA before incubation (LIGHT)	12/05/2022	25,5
C7 CWA after incubation (DARK)	12/05/2022	28,3
C9 CWA after incubation (LIGHT)	12/05/2022	28
C7 CWOA before incubation (DARK)	13/05/2022	26,7
C9 CWOA before incubation (LIGHT)	13/05/2022	26,6
C7 CWOA after incubation (DARK)	13/05/2022	30,1
C9 CWOA after incubation (LIGHT)	13/05/2022	29,2

(Table 5: Temperature incubations *Crassostrea gigas* AMBIENT and AMBIENT+ basins)

Looking at the temperature measured (Table 5), can be seen that there is a distinction between C1 and C3, and C2 and C4 overall temperatures before and after the incubations. In fact, there is a difference of few degrees between the two couples of oysters. This is hard to explain because if this was the case for the couples of C1 and C2, and C3 and C4, would have made more sense because two of them underwent an incubation in the dark and the other two in the light, which could have given more direct answers. In this case it

is not easy to make an assumption on why there was a difference of temperatures in pairs like this.

Temperature in AMBIENT+ basin behaves in strange ways, because in the case of C6 and C8 during the NCWA incubation it decreases, whereas in C7 and C9 it increases. During the CWA incubation it decreases again for C6 and instead for C8 increases substantially. During the last incubation instead, for all four oysters it increases more or less the same amount.



(Fig. 20: Fluxes of CO_2 *Crassostrea gigas*)

In the case of *Crassostrea gigas*, was possible to also calculate the CO_2 concentrations. This because pH measures and alkalinity measures were usable. Alkalinity and pH measurements were inserted into an R-studio package called Seacarb, which was installed in the programme and the data inserted in it. Then running the programme was possible to get values of CO_2 (in mol/kg) for specific situations, with specific alkalinity, pH, temperatures, and salinity of the water.

In Fig. 20 data of CO_2 concentrations are displayed, but apart from C1 and C3 there was not a real trend to be described. Anyhow, CO_2 increased in the water during the incubation

in most cases, apart from C4 and C8 in NCWA incubation, and in C1 and C3 in CWOA incubations, in which decreased.

The fluxes of AMBIENT+ differ from each other quite a lot, even if the couples C7/C9 and C6/C8 follow more or less the same patterns. Again, during the incubation period the CO_2 concentrations should increase, and this is the case apart from C8 during all three incubations. This could be due to an error in the calculations.

3.2.1 Size and weight of individuals

OYSTER	weight (g)	length (cm)	width (cm)	volume (ml)
C1	32.86	5.7	4.5	25
C2	33.07	7.4	4.3	25
C3	44.71	8.2	4.4	45
C4	50.54	7.7	4.2	45
C6	29.68	6.0	4.4	25
C7	26.03	5.4	3.9	20
C8	55.34	6.8	5.4	45
C9	45.78	7.3	4.4	45

(Table 6: weight, length, width, and volume of *Crassostrea gigas* individuals used for experiments)

CHAPTER 4 – Conclusions and discussion

The outcomes of this research are going to be listed in this chapter, with everything that didn't go as planned, interruptions, setbacks, and any problem encountered along the way.

4.1 Discussion

As has been stated before, the research behind this masters' thesis and the manuscript itself, aim to understand in a better way certain aspects of the fluxes of two species of oysters in the lagoon of Venice. The two oysters that have been analysed are *Ostrea edulis* and *Crassostrea gigas.* The collection of individuals from the first species was made possible by the founder of project MAREA, where the oysters come from. In fact, the results concerning this species were made available to MAREA in order to be helpful for the project itself, as the previous chapters stated already. On the other hand, the individuals from *Crassostrea gigas* species were collected directly from the canals of Venice city by the researcher himself. The main aspects that have been analysed through incubations and various analysis in this masters' thesis are the fluxes of O_2, NH_4^+, and CO_2 mainly, in order to compare them between the two species of oysters. More specifically, the main objective of this research was to understand which of the two species would produce more NH_4^+ during metabolism processes, intake more O_2 during respiration, and excrete more CO_2 also during respiration processes. The reason behind this objective was to make assumption on the fact that the reintroduction of *O. edulis*, through MAREA project and perhaps through other future restoration projects, would be beneficial for the lagoonal ecosystem, and more specifically would have more positive impacts compared to the already existing and thriving species of *C. gigas*. Furthermore, another important reason for the analysis behind this masters' thesis, was to get insights on the role of epibionts (biofouling) on the fluxes of O_2, NH_4^+, and CO_2, which is something that, as it has been stated in the previous chapters, creates a lot of problem in the aquaculture sector and in other sectors concerning water systems.

The whole research focuses on the comparison between the two species from the point of view of the objectives, in fact, it is important now to remind the two main research questions:

1) “Which species consume more O_2, excrete more NH_4^+ and emits more CO_2, at the same temperature and light intensity level?”

2) “Do epibionts (biofouling) affect the consumption and production rates of O_2, NH_4^+, and CO_2?”

These two research questions were the nucleus in writing down the experimental design which guided the whole research. The measurements that were taken were all planned in order to be able to answer these two questions. On the other hand, scientific research has got many uncertainties, and problems can be encountered throughout the course of experiments. This was the case during the undergoing of this thesis. Several oysters died, then they were replaced and died again, which made everything more difficult. Fortunately, during the incubations and measurements of *C. gigas* no problems were encountered, so all the measurements that were planned were taken. Contrastingly, during *O. edulis* incubations and measurements, several oysters died in the AMBIENT+ basin, so unfortunately was not possible to take the measurements or even to incubate them. The reasons behind this could be due to many factors, but the one that is more plausible is that the individuals from *O. edulis*, have been stressed indeed more than the individuals from *C. gigas.* This because the individuals of C. gigas were collected from the canals of Venice, in which they have been established and adapted since a long time. On the contrary, the individuals from O. edulis, were transported to the Venice lagoon only around July of 2021and they were still adapting to the environment, and yet they have been transported again to a lab. Moreover, as literature states, and as the previous chapters already described, *C. gigas* is much more adaptable and resistant than *O. edulis*, which could also be the reason of the mortality in the basin.

Other problems that were encountered during the course of the experiment were that the pH meter was not working at the beginning of the research and the alkalinity test was not available. This made impossible the measurements CO_2 fluxes in *O. edulis*, in fact, as can be seen from the graphs in the results chapter, there is not data for CO_2 fluxes to be compared with *C. gigas*.

Going more into depth in the results, now that the problems encountered have been described, several comments can be made, and an analysis can be done.

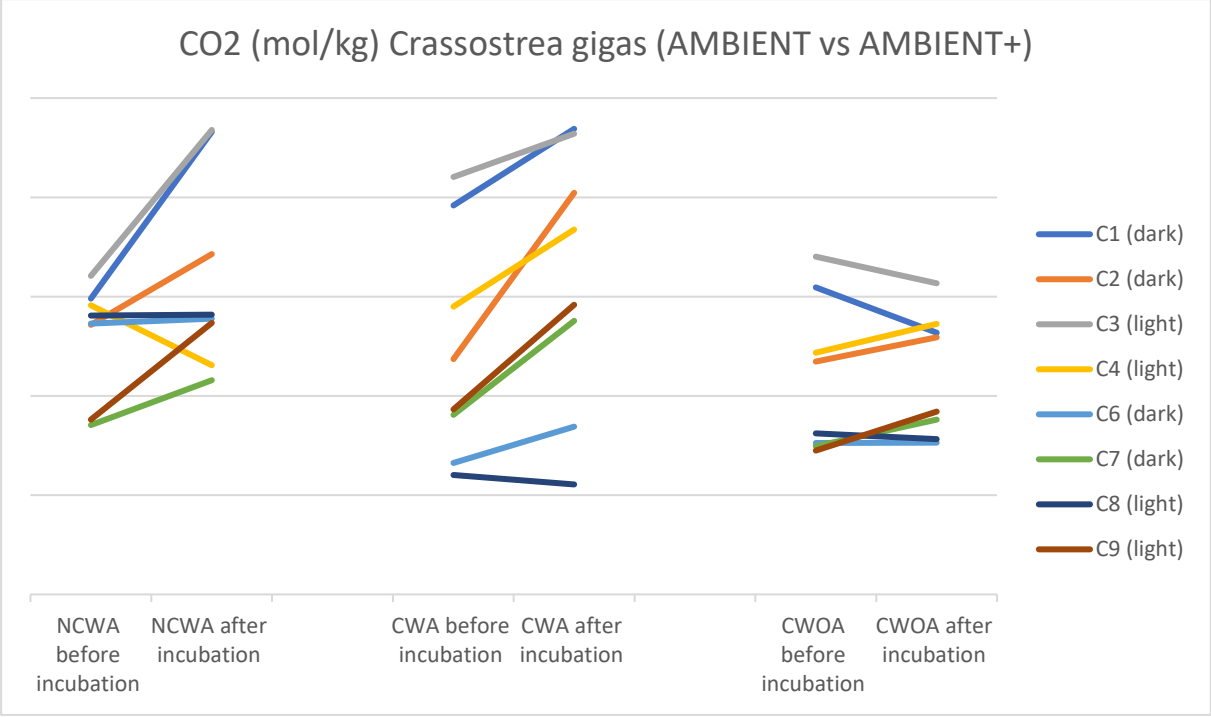
Regarding the dissolved O_2 fluxes measured for *O. edulis*, looking at the graph in fig. 17 can be seen that the fluxes of the oyster that underwent a dark incubation (CWA and CWOA) are in general higher than the ones in transparent incubators. Looking at the percentages in the raw data (see appendices) as well can be seen that the depletion of dissolved O_2 goes faster in dark chambers compared to transparent ones, apart from the incubation NCWA (not clean with animal). This could be due to lack of light, so more need of respiration. Looking at the data from the incubation CWA (clean with animal), can be seen that without epibionts on the shell, the consumption of O_2 is higher in dark incubators. This probably means that only the oyster consumes more O_2 when its dark even without epibionts on it. On the other hand, the O_2 fluxes in *C. gigas*, seems to be more heterogenous compared to the ones of *O. edulis*. During the CWA incubation, again the fluxes measured from the dark incubator seems to be slightly higher in 3 cases out of 4 compared to the ones measured in light incubator.

Moreover, talking about the NH_4^+ fluxes, can be said that for *O. edulis*, the measurements tend to be lower in the dark incubators again during the CWA incubation. In this graph it is clear to see that during the first two types of incubations (NCWA and CWA), every oyster analysed seems to excrete NH_4^+, whereas, during the last type of incubation where both animal and epibionts are not present, it seems that the NH_4^+ is up taken. This could be due to errors during the measurements. Looking at the graph from *C. gigas* instead, something strange seems to happen. During the incubation NCWA, it seems that only 3 oysters out of 4 from the AMBIENT group are excreting NH_4^+, which should not happen. The other oysters seem to uptake it. The thing is that NH_4^+ should be excreted because it is a by-product of metabolic processes. During the other two incubations it is clear that the measurements taken from the dark incubator are higher compared to the light one, which makes the researcher think that in dark situations there is more excretion of NH_4^+ compared to when light is present.

Talking about the temperature that has been measured before and after the incubations, it should be obvious that it increases with time, because the incubators, being completely sealed and with a magnetic stirrer inside that turns at all times, the temperature should increase. This is the case almost in every incubation that has been performed but it is not. In fact, during the first incubation type (NCWA), in AMBIENT+ for C. gigas, the temperature decreases of almost 4 degrees Celsius. There is no real explanation of this

behaviour, so it could be an error during the measurements, or a problem related to the instrument used.

Regarding the CO_2 fluxes, as it has already been stated before, it was possible only to take measurements for *C. gigas*, because during the incubation period of *O. edulis* the equipment was not available or was not working, so no data from pH and alkalinity were possible to be taken. That was unfortunate because it would have been ideal to be able to compare also the fluxes of CO_2 between the two species. It is then clear that only an analysis of the fluxes of CO_2 of *C. gigas* can be done. The data acquired and calculated through R-studio with pH and alkalinity were not as precise as it was hoped. In fact, the fluxes retrieved from R-studio using the sea-carb package did give several strange results. More specifically, with O_2 uptake by the oysters during the incubation period, there should be excretion of CO_2, but this was not the case in most measurements. In some cases, the stayed the same, in some cases increased, and in some other cases even decreased. Looking at fig. 30 for instance, can be seen the trend of before and after the 3 types of incubation, and can be also seen that there is no relation at all with the level of irradiance of the incubators (dark or light). This again, could be due to errors in the measurements or even errors during the R-studio calculations. In this case, in fact, it is impossible to draw conclusions.



(Fig. 21: comparison of CO_2 before and after incubation *C. gigas*)

Fig. 15 shows the trend of temperature in the basins where the oysters were kept throughout the whole period of experiments. The reason behind the monitoring this parameter was to have an overview of the two basins and to perhaps connect certain results to that, since that the oysters were kept for over a month inside these basins. The reason behind this thought was because if there were some inconsistencies in the results, the research would have looked at the trend from Fig. 15 in order to find answers. The real problem was that there were inconsistencies throughout the results which made impossible to use Fig. 15 to help understand the results better.

4.2 Conclusions

After the results have been discussed in the previous sub-chapter, it is now possible to draw final conclusions on the whole project of this masters' thesis. In order to be able to do that it is now important to remind ourselves about the objectives and research questions, which are at the foundation of the whole research.

The two main objectives of the thesis were:

1. To compare the respiration and ammonia excretion rates, under different temperature and irradiation conditions of the two oyster species (*Ostrea edulis* and *Crassostrea gigas*).
2. To assess the potential role of epibionts (biofouling) on the overall consumption and production of O_2, NH_4^+, and CO_2 of individual oysters from both species (*Ostrea edulis* and *Crassostrea gigas*).

Whereas the two main research questions were:

1. "Which species consume more O_2, excrete more NH4 and emits more CO2, at the same temperature and light intensity level?"
2. "Do epibionts (biofouling) affect the consumption and production rates of O_2, NH_4^+, and CO_2?"

In order to answer to the first research question, it is now important to focus on one element at a time:

Looking at the graphs representing the fluxes of O_2 in both species and comparing the results, it is clear that *C. gigas* consumes more O_2 compared to *O. edulis*. The individual of *O. edulis* which consumes more O_2, consumes around 0.1 %/l/h, instead, the individual which consumes more O_2 from *C. gigas* consumes more than 0.12 %/l/h. So, it is clear that in the specific situation of 5 hours incubation, *C. gigas* consumes more O_2.

Moreover, looking at the graphs representing the fluxes of NH_4^+, for both species the results seem strange. Looking at the data from *C. gigas*, it is clear that the incubations with AMBIENT individuals (so, coming from colder waters) there is almost no excretion of NH_4^+, whereas for *O. edulis* there is. On the other hand, since that for *O. edulis* was not possible to perform incubations for the AMBIENT+ situation (warmer waters), it is impossible to compare it, but can be said that C. gigas excreted NH_4^+ during all types of incubations (NCWA, CWA, AND CWOA).

Analysing the data from CO_2 measurements it is impossible to make a comparison between the two species because no data from pH and alkalinity were taken for the species *O. edulis* unfortunately, due to lack of equipment or malfunction. Moreover, the results from *C. gigas* were also not satisfactory.

Trying to answer to the first research question is challenging with the data acquired throughout the research but can be said that C. gigas is indeed the one species that consumes more O_2 and excrete more NH_4^+ between the two.

About the second research question more research should be done surely. At least calculating the weight of the epibionts and standardizing the results to that. But generally, looking at the data can be said that there is a slight decrease in O_2 consumption when epibionts are removed from the shell in both oyster species. On the other hand, for NH_4^+, there is no change in the data weather epibionts are present or not. Looking at the CO_2 instead, only for *C. gigas*, no conclusions can be drawn due to the inconsistency in the data.

From a general point of view, epibionts are microorganisms, which means that they have their own respiration and metabolic fluxes, which means that in theory they should have an impact on the overall fluxes in the water when attached to the oyster shells or other organisms or material.

4.2 Future research and recommendations

As can be seen from the results, discussion and conclusions of this masters' thesis, it is clear that further research should be done and the experimental design, material, and timing should be reviewed.

More specifically, more oysters should be analysed, which means making a bigger scale experiment overall. This would make possible to produce more results which will be important for a statistical analysis and more detailed graphs. Moreover, it would be fundamental to take the measurements from the beginning for both oyster species in order to be able to compare the parameters.

On another level, it would be very interesting to recreate a small-scale oyster reef and take the measurement directly from there in order to be as close as reality as possible and for longer periods of time in order to provide a seasonal profile of the fluxes.

Last thing that could be improved in future research is the development of a R-studio model with time series and projections in order to understand the fluxes in the long-term.

4.3 Acknowledgements

It took time, mental and physical strength to produce this masters' thesis and to undergo all the experimental phase that brought me to the results described in this manuscript. I would have never made it on my own, in fact many amazing human beings helped me directly and indirectly to accomplish this result. The end of my master studies at Ca' Foscari university of Venice.

Foremost, I would like to express my most sincere gratitude to my supervisors, who helped me throughout the whole process, Prof. Roberto Pastres and Camilla Bertolini. On this note, I would like to thank specifically Camilla, because she made me understand several things that were really hard for me to understand and helped me directly during the set-up of the lab, the writing of the research proposal, and the thesis itself.

Secondly, with the sincerest appreciation and love I would like to thank my life companion, my love, Vittoria, the one person who makes me think straight, who provide me the colours in life, who indicates me the way when there is no light or when the

storm is too difficult to handle. Thank you from the bottom of my heart. Without you none of this would have been possible.

Thirdly, I would like to thank with huge pride my mum Paola who made me the man I am now. Who always supported my decisions, and who was always there for me in dark times. Herbie, who is always been at her side, was fundamental as well in my "life career". When I needed to wake up from stupid moments during my student career, he was always there to help me. P&H were the ones who invested in me during my student life. The ones who really believed in me at all times. The ones that pushed me when I needed a push. Time will never be sufficient to thank you enough.

Then I would like to thank my dad, Andrea, who has always been there to have a conversation with me in front of a cold beer. Those moments are fundamental to think about something else. About when I was a young guy, when the road ahead of me was still taking shape. Thank you, dad, for helping me understand where to go and how to go.

Then, how can I forget my grandmother, Lalla, who was always there for me. No matter at what time. No matter for which reason. You are a role model for me. I wish that in the future I will love my family as you do with yours, and that my family will love me as they love you.

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I would also like to give a special acknowledgement to Chiara Facca, who helped me like if I was her student with several things.

I mean, there are many people more thank I could thank but if I do, the list will become long as the Divina Commedia…so I would like to throw out there a huge thank you to everyone that helped me in many different ways and for whatever reason.

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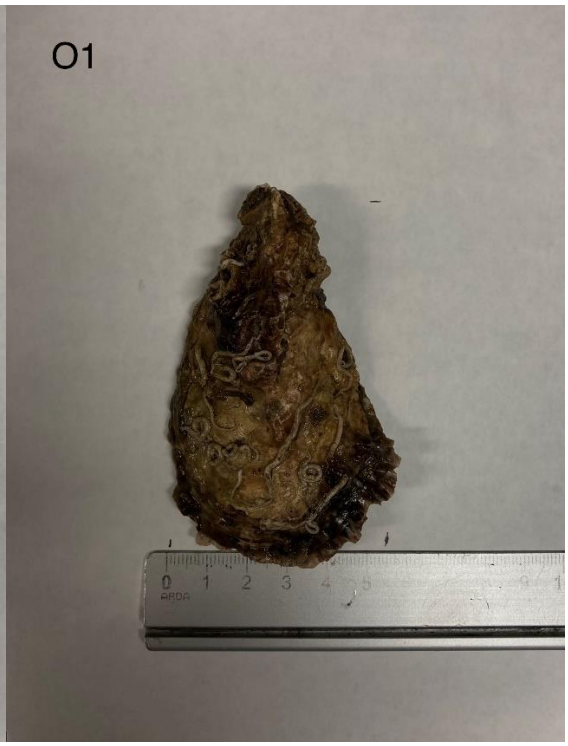
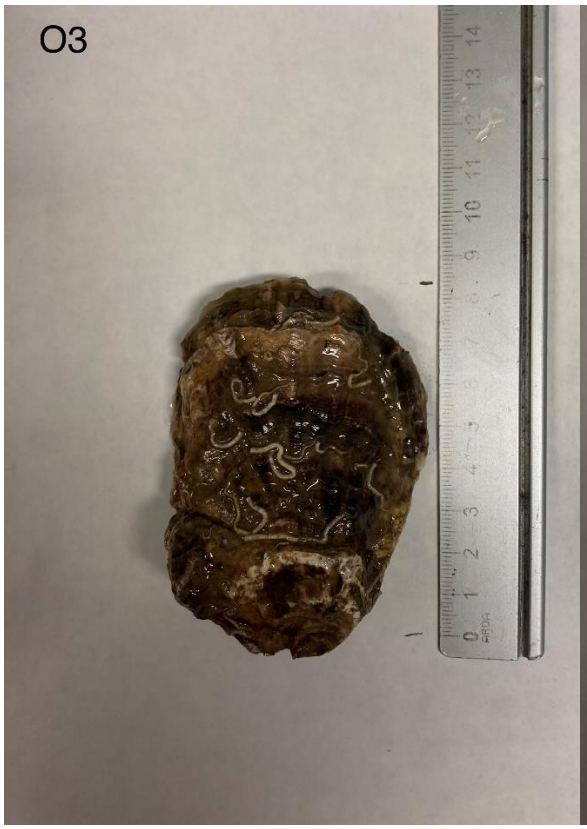
APPENDICES

1. *Photos oysters*

<u>Ostrea edulis:</u>

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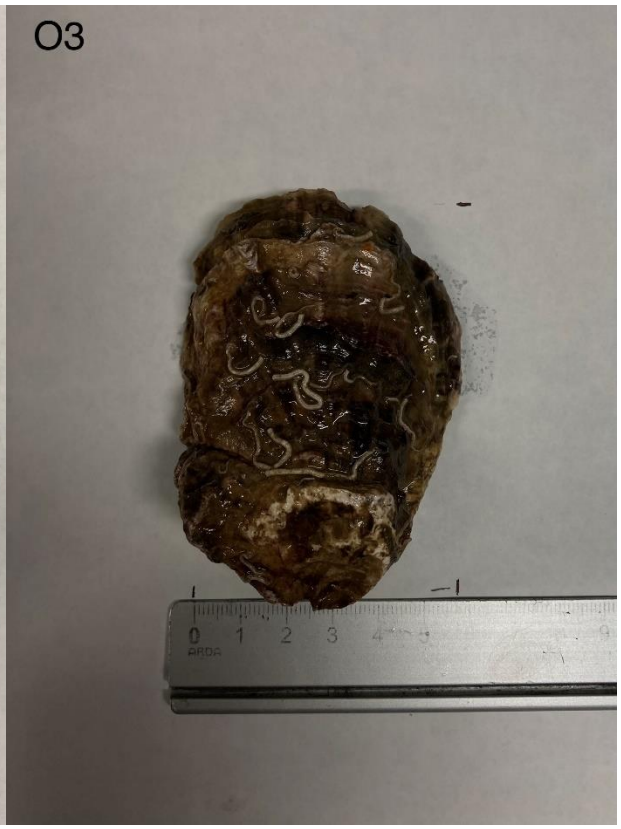
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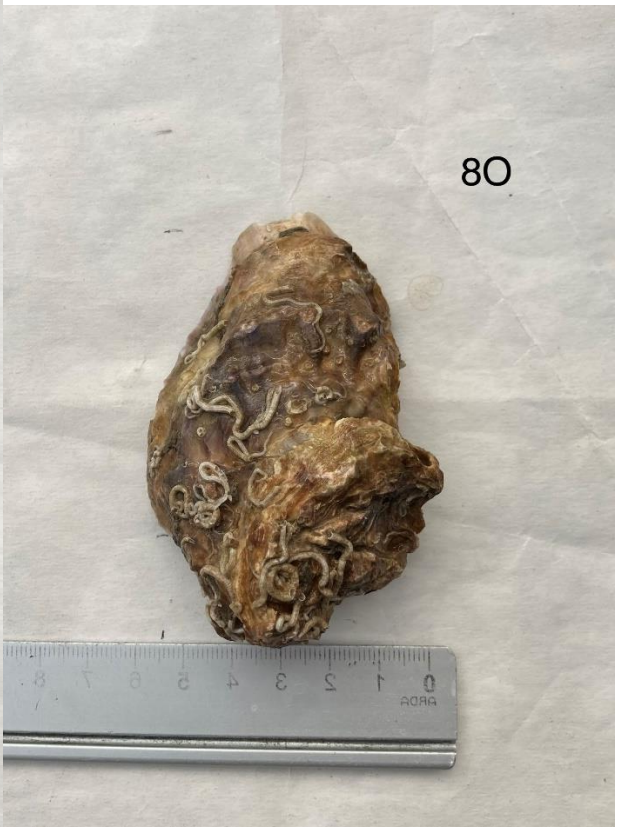
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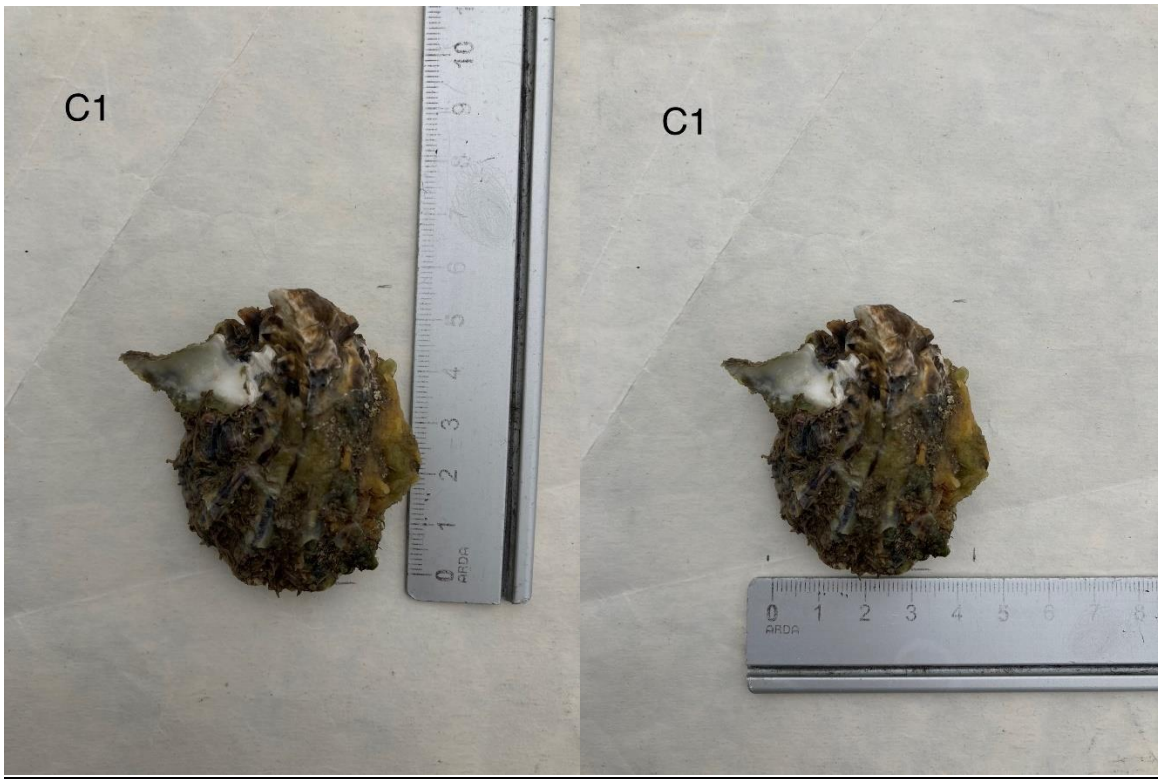

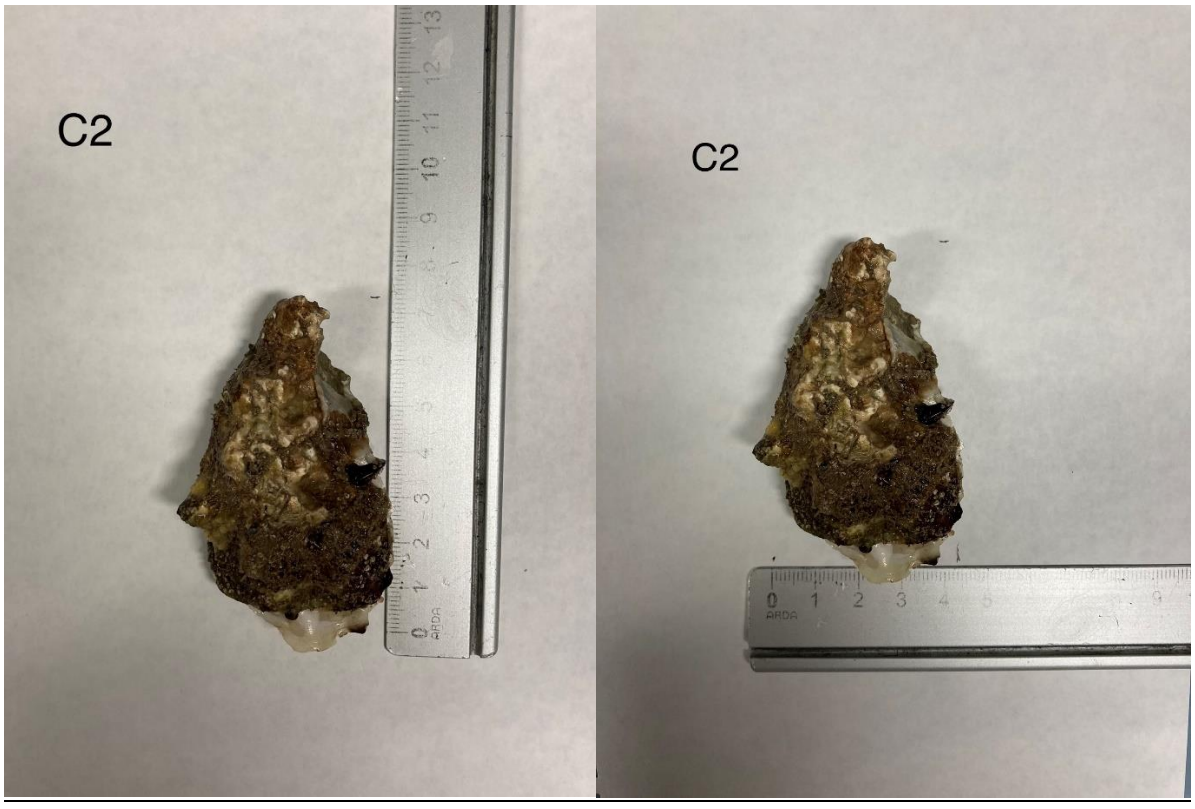


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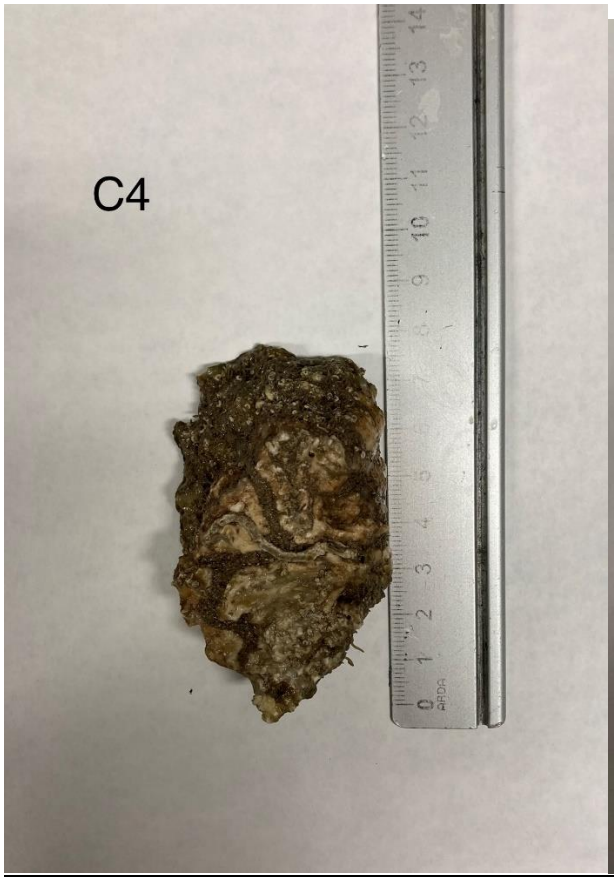
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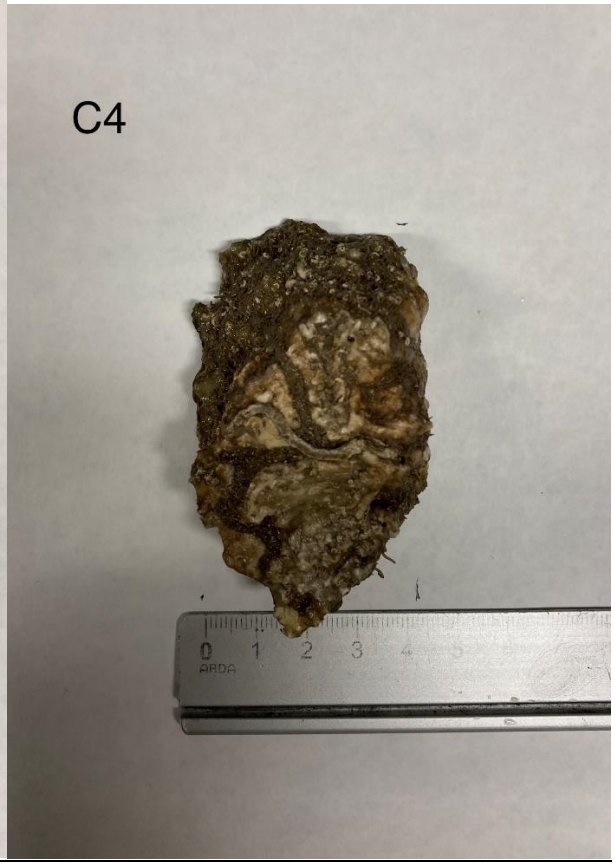
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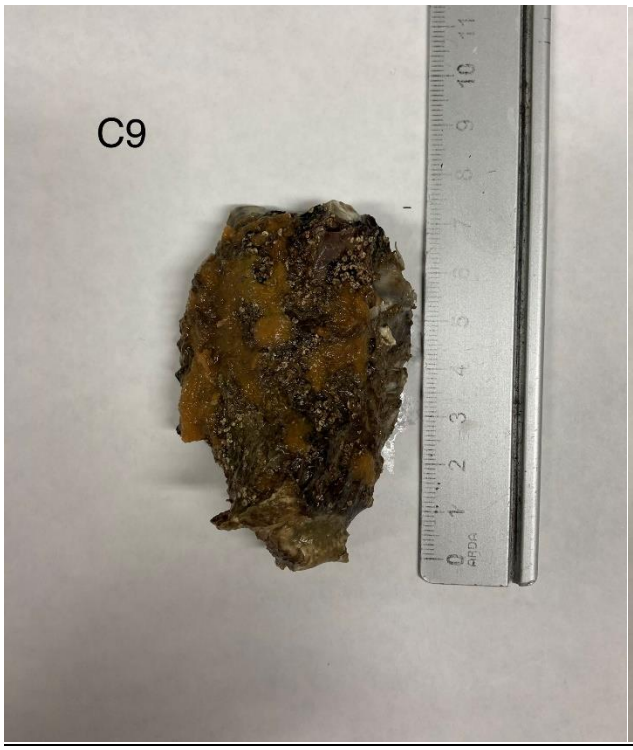
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2. *Meetings*

meeting	with	date	notes
1	prof, camilla	24th oct	first meeting at the lab with camilla and professor. They showed me the lab and the components of it. First real meeting about the thesis together.
2	camilla	3rd nov	discussion proposal with camilla
3	camilla	19th nov	discussion proposal/exp design with camilla
4	camilla	10th dec	discussion exp design with camilla (online)
5	camilla, daniele	12th jan	lab description more in details with daniele and camilla. We went through all the various components of the lab and daniele gave us some new ideas on the various processes
6	camilla, chiara	18th jan	meeting online with chiara (microalgae) and camilla. To get to know each other and to give us some first advice on the culture of microalgae.
7	chiara	19th jan	chiara showed me around the labs in the zeta building.
8	camilla		first batch of oysters from camilla and electricity in the lab turned on.
9			
10			

3. *Data collection*

<u>Ostrea edulis:</u>

AMBIENT	DATE	dissolved O2 (%)	ammonium (NH4+) (mg/l)	alkalinity (mmol/l)	alkalinity (mol/kg)	temperature (°C)	CO2	Ph
O1 NCWA before incubation (DARK)	11/04/2022	97,5	-0,006			13	0	
O3 NCWA before incubation (LIGHT)	11/04/2022	97,5	-0,004			13	0	
O1 NCWA after incubation (DARK)	11/04/2022	98,2	0,14			18,6	0	
O3 NCWA after incubation (LIGHT)	11/04/2022	94,8	0,07			18,8	0	
O1 CWA before incubation (DARK)	12/04/2022	104,3	-0,05			14,4	0	
O3 CWA before incubation (LIGHT)	12/04/2022	102,2	-0,051			14	0	
O1 CWA after incubation (DARK)	12/04/2022	86	0,18			20,7	0	
O3 CWA after incubation (LIGHT)	12/04/2022	97	0,06			18,7	0	
O1 CWOA before	14/04/2022	99,7	-0,082			16,9	0	

incubation (DARK)								
O3 CWOA before incubation (LIGHT)	14/04/2022	99,2	-0,083			16,1	0	
O1 CWOA after incubation (DARK)	14/04/2022	90,3	0,025			23,9	0	
O3 CWOA after incubation (LIGHT)	14/04/2022	92	0,08			21,8	0	
AMBIENT								
O2 NCWA before incubation (DARK)	15/04/2022	100	-0,069			17,3	0	
O4 NCWA before incubation (LIGHT)	15/04/2022	101,6	-0,06			16,8	0	
O2 NCWA after incubation (DARK)	15/04/2022	88,5	0,01			22,6	0	
O4 NCWA after incubation (LIGHT)	15/04/2022	82,9	0,11			21,2	0	
O2 CWA before incubation (DARK)	19/04/2022	101,9	-0,075			16	0	
O4 CWA before incubation (LIGHT)	19/04/2022	101,4	-0,078			15,8	0	
O2 CWA after incubation (DARK)	19/04/2022	88,4	0,053			20,6	0	
O4 CWA after incubation (LIGHT)	19/04/2022	93,9	0,05			18,9	0	
O2 CWOA before incubation (DARK)	21/04/2022	98	-0,074			14,2	0	
O4 CWOA before	21/04/2022	98,8	-0,073			13,9	0	

incubation (LIGHT)								
O2 CWOA after incubation (DARK)	21/04/2022	93,7	0,06			18,7	0	
O4 CWOA after incubation (LIGHT)	21/04/2022	95,3	0,05			16,8	0	
AMBIENT+								
O6 NCWOA before incubation (DARK)	16/05/2022	94,3	0,03	6,5	0,0065	27,4	0	8,51
O8 NCWOA before incubation (LIGHT)	16/05/2022	93,9	0,03	6,5	0,0065	27,3	0	8,51
O6 NCWOA after incubation (DARK)	16/05/2022	64,8	0,64	6,4	0,0064	30,8	0	8,34
O8 NCWOA after incubation (LIGHT)	16/05/2022	67,4	0,51	6,5	0,0065	29,6	0	8,29
O6 CWOA before incubation (DARK)	17/05/2022	94,1	0,03	6,9	0,0069	26,8	0	8,55
O8 CWOA after incubation (LIGHT)	17/05/2022	93,8	0,03	6,9	0,0069	26,5	0	8,56
O6 CWOA before incubation (DARK)	17/05/2022	88	0,23	6,4	0,0064	30,9	0	8,47
O8 CWOA after incubation (LIGHT)	17/05/2022	89,3	0,19	6,3	0,0063	29,5	0	8,48

Crassostrea gigas:

AMBIENT	DATE	dissolved O2 (%)	ammonium (NH4+) (mg/l)	alkalinity (mmol/l)	alkalinity (mol/kg)	temperature (°C)	CO2	Ph

C1 NCWA before incubation (DARK)	22/04/2022	99,6	0,03	6,7	0,0067	14,6	0	8,44
C3 NCWA before incubation (LIGHT)	22/04/2022	98,4	0,03	6,7	0,0067	14,2	0	8,42
C1 NCWA after incubation (DARK)	22/04/2022	85,1	-0,022	6,4	0,0064	16,4	0	8,25
C3 NCWA after incubation (LIGHT)	22/04/2022	87,9	-0,04	6,5	0,0065	15	0	8,27
C1 CWA before incubation (DARK)	26/04/2022	99,1	0,03	6,7	0,0067	17	0	8,32
C3 CWA before incubation (LIGHT)	26/04/2022	98,9	0,03	6,7	0,0067	16,6	0	8,3
C1 CWA after incubation (DARK)	26/04/2022	82,1	0,25	6,5	0,0065	20,4	0	8,21
C3 CWA after incubation (LIGHT)	26/04/2022	85,3	0,05	6,2	0,0062	20,1	0	8,2
C1 CWOA before incubation (DARK)	28/04/2022	98,8	0,03	6,2	0,0062	17,3	0	8,37
C3 CWOA before incubation (LIGHT)	28/04/2022	99,1	0,03	6,2	0,0062	17,1	0	8,34
C1 CWOA after incubation (DARK)	28/04/2022	88,6	0,2	5,8	0,0058	23,8	0	8,33
C3 CWOA after incubation (LIGHT)	28/04/2022	86,4	0,21	5,9	0,0059	22,7	0	8,29
AMBIENT								
C2 NCWA before incubation (DARK)	29/04/2022	101,6	0,03	6,1	0,0061	18,9	0	8,39

C4 NCWA before incubation (LIGHT)	29/04/2022	102,8	0,03	6,1	0,0061	18,6	0	8,37
C2 NCWA after incubation (DARK)	29/04/2022	82,9	-0,019	5,8	0,0058	22,1	0	8,26
C4 NCWA after incubation (LIGHT)	29/04/2022	87,5	-0,035	6	0,006	21,4	0	8,31
C2 CWA before incubation (DARK)	02/05/2022	99,8	0,03	5,8	0,0058	18,7	0	8,42
C4 CWA before incubation (LIGHT)	02/05/2022	100,2	0,03	5,8	0,0058	18,9	0	8,4
C2 CWA after incubation (DARK)	02/05/2022	80,4	0,25	6,6	0,0066	24,9	0	8,22
C4 CWA after incubation (LIGHT)	02/05/2022	79,5	0,24	6,2	0,0062	24	0	8,24
C2 CWOA before incubation (DARK)	03/05/2022	99,7	0,03	6,2	0,0062	19,2	0	8,44
C4 CWOA before incubation (LIGHT)	03/05/2022	100	0,03	6,2	0,0062	19	0	8,43
C2 CWOA after incubation (DARK)	03/05/2022	78,1	0,18	5,9	0,0059	25,9	0	8,32
C4 CWOA after incubation (LIGHT)	03/05/2022	73,6	0,25	6	0,006	25,8	0	8,31
AMBIENT+								
C6 NCWA before incubation (DARK)	05/05/2022	87,6	0,03	11	0,011	24,4	0	8,52
C8 NCWA before incubation (LIGHT)	05/05/2022	87,9	0,03	11	0,011	24,5	0	8,51

C6 NCWA after incubation (DARK)	05/05/2022	80,4	0,14	10,9	0,0109	22,7	0	8,53
C8 NCWA after incubation (LIGHT)	05/05/2022	84,4	-0,016	10,6	0,0106	21,5	0	8,53
C6 CWA before incubation (DARK)	06/05/2022	89,6	0,03	6	0,006	22,6	0	8,57
C8 CWA before incubation (LIGHT)	06/05/2022	89,4	0,03	6	0,006	22,7	0	8,56
C6 CWA after incubation (DARK)	06/05/2022	83,3	0,43	5,9	0,0059	20,8	0	8,51
C8 CWA after incubation (LIGHT)	06/05/2022	85	0,28	5,9	0,0059	28,9	0	8,55
C6 CWOA before incubation (DARK)	09/05/2022	94,3	0,03	6,4	0,0064	24,2	0	8,53
C8 CWOA before incubation (LIGHT)	09/05/2022	94,2	0,03	6,4	0,0064	24,3	0	8,51
C6 CWOA after incubation (DARK)	09/05/2022	92,4	0,29	5,5	0,0055	27,2	0	8,45
C8 CWOA after incubation (LIGHT)	09/05/2022	93,8	0,26	5,6	0,0056	26,1	0	8,46
AMBIENT+								
C7 NCWA before incubation (DARK)	10/05/2022	92,3	0,03	6,4	0,0064	25,7	0	8,48
C9 NCWA before incubation (LIGHT)	10/05/2022	92,2	0,03	6,4	0,0064	25,7	0	8,47
C7 NCWA after incubation (DARK)	10/05/2022	76,8	0,35	5,7	0,0057	28,6	0	8,34

C9 NCWA after incubation (LIGHT)	10/05/2022	71,3	0,65	5,9	0,0059	28,1	0	8,28
C7 CWA before incubation (DARK)	12/05/2022	95,7	0,03	6,3	0,0063	25,4	0	8,46
C9 CWA before incubation (LIGHT)	12/05/2022	95,5	0,03	6,3	0,0063	25,5	0	8,45
C7 CWA after incubation (DARK)	12/05/2022	83,2	0,37	5,8	0,0058	28,3	0	8,27
C9 CWA after incubation (LIGHT)	12/05/2022	79,9	0,39	5,9	0,0059	28	0	8,26
C7 CWOA before incubation (DARK)	13/05/2022	94,1	0,03	6,2	0,0062	26,7	0	8,5
C9 CWOA before incubation (LIGHT)	13/05/2022	93,9	0,03	6,2	0,0062	26,6	0	8,51
C7 CWOA after incubation (DARK)	13/05/2022	88,8	0,21	6,1	0,0061	30,1	0	8,41
C9 CWOA after incubation (LIGHT)	13/05/2022	85,9	0,26	6	0,006	29,2	0	8,4

4. Monthly LOG

Activity Log

Date	Total Time	Activity	Remarks/Follow-Up
1-october			
2-october			
2-october			
4-october			
5-october			
6-october			
7-october			
8-october			
9-october			
10-october			
11-october			
12-october		first contacts	
13-october		first contacts	
14-october		first contacts	
15-october		first contacts	
16-october		first contacts	
17-october		first contacts	
18-october		first contacts	
19-october		first contacts	
20-october		first contacts	
21-october		first contacts	
22-october		first contacts	
23-october		first contacts	
24-october	1.5h	First real meeting in person with Professor and Camilla to talk about the thesis. They showed me the lab for the first time and we started seriously talking about the thesis.	

25-october	2h	Reading first papers	
26-october	2h	Reading papers and writing down first ideas	
27-october	3h	Reading papers and writing down first ideas	
28-october	3h	Reading papers and writing down first ideas	
29-october	2h	Reading papers and writing down first ideas	
30-october	2h	Reading papers and writing down first ideas	
31-october	2h	Reading papers and writing down first ideas	

Date	Total Time	Activity	Remarks/Follow-Up
1-november	2h	Reading papers and writing down first ideas	
2-november	2h	Reading papers and writing down first ideas	
3-november	1.5h	meeting with Camilla to discuss first ideas	
4-november	3h	reading papers and started writing proposal	
5-november	3h	reading papers and started writing proposal	
6-november	2h	reading papers and writing prosal	
7-november	2h	reading papers and writing prosal	
8-november	2h	reading papers and writing prosal	
9-november	3h	reading papers and writing prosal	
10-november	2h	reading papers and writing prosal	
11-november			
12-november			
13-november			
14-november			

15-november			
16-november	2h	reading papers and writing prosal	
17-november	2h	reading papers and writing prosal	
18-november	2h	reading papers and writing prosal	
19-november	1.5h	meeting with Camilla to discuss proposal	
20-november	1.5h	correcting proposal	
21-november	1.5h	correcting proposal	
22-november	1h	started writing experimental design	
23-november	1h	started writing experimental design	
24-november	1h	writing exp. design	
25-november	1h	writing exp. design	
26-november	2h	writing exp. Design and gantt-chart	
27-november	1h	writing exp. Design and gantt-chart	
28-november			
29-november			
30-november			

Date	Total Time	Activity	Remarks/Follow-Up
1-december	1h	fixing gantt-chart	
2-december	1h	literature review	
3-december	1h	literature review	
4-december	1h	literature review	
5-december	1h	literature review	
6-december			
7-december			

8-december			
9-december	1h	last changes in gantt-chart	
10-december	1h	meeting online with Camilla to discuss exp. Design and gantt-chart	
11-december	0.5	literature review	
12-december	0.5	literature review	
13-december	0.5	literature review	
14-december	0.5	literature review	
15-december	0.5	literature review	
16-december	0.5	literature review	
17-december	0.5	literature review	
18-december	0.5	literature review	
19-december			christmas holidays
20-december			christmas holidays
21-december			christmas holidays
22-december			christmas holidays
23-december			christmas holidays
24-december			christmas holidays
25-december			christmas holidays
26-december			christmas holidays
27-december			christmas holidays
28-december			christmas holidays
29-december			christmas holidays
30-december			christmas holidays
31-december			christmas holidays

Date	Total Time	Activity	Remarks/Follow-Up
1-january			christmas holidays
2-january			christmas holidays
3-january			exams preparation full immersion
4-january			exams preparation full immersion
5-january			exams preparation full immersion
6-january			exams preparation full immersion
7-january			exams preparation full immersion
8-january			exams preparation full immersion
9-january			exams preparation full immersion
10-january			exams preparation full immersion
11-january			exams preparation full immersion
12-january	1.5h	lab description more in details with daniele and camilla. We went through all the various components of the lab and daniele gave us some new ideas on the various processes	
13-january			exams preparation full immersion
14-january			exam
15-january			exams preparation full immersion
16-january			exams preparation full immersion
17-january			exams preparation full immersion
18-january	1h	meeting online with chiara (microalgae) and camilla. To get to know each other and to give us some first advice on the culture of microalgae.	exam
19-january	1h	meeting in zeta building with Chiara Facca to have a look around the labs and the microalgae cultures.	
20-january			exams preparation full immersion

21-january			exams preparation full immersion
22-january			exams preparation full immersion
23-january			exams preparation full immersion
24-january			exams preparation full immersion
25-january			exams preparation full immersion
26-january			exams preparation full immersion
27-january	0.5h	Chiara came to the lab to check out the basins for the microalgae	exams preparation full immersion
28-january			exam
29-january	2h	literature review	
30-january	2h	literature review	
31-january	2h	literature review	

Date	Total Time	Activity	Remarks/Follow-Up
1-february	2h	incubations trials	
2-february	2h	incubations trials	
3-february	2h	literature review	
4-february	2h	literature review	
5-february			
6-february			
7-february	2h	literature review	
8-february	1.5h	meeting Green Sant'Erasmo	
9-february			
10-february	3h	went to mestre aquarium shop to get pump and heater for the basins	
11-february			
12-february			
13-february			
14-february			
15-february			

16-february	1.5h	meeting wit Chiara and Alessandro Bonetto to get the agitator for incubations and lab visit	darkened the incubator
17-february	4h	emptying the oyster basins and incubation trial	
18-february			
19-february	2h	literature review	
20-february			
21-february			
22-february	1h	understanding lab water flows (not successful), tried O2 measurement (not successful)	left 1 oyster over the weekend into incubator in order to see if the situation became anoxyc
23-february	5h	Day in uni. Literature review, chemical processes, checks in the lab.	Got the other agitator from Bonetto + call with Daniele for ossimetro
24-february	3h	6 hours incubation trial with measuremments	
25-february	1h	literature review	
26-february			
27-february			
28-february	1h	literature review	

Date	Total Time	Activity	Remarks/Follow-Up
1-march	2h	incubation trial and lit review	
2-march			
3-march	2h	incubation trial and lit review	
4-march			
5-march			
6-march			
7-march	5h	getting familiar with some of the lab procedures and kits	
8-march	2h	meeting with Camilla and Prof and literature review	
9-march	4h	experimental design and brainstorming	
10-march			
11-march			
12-march			
13-march			

14-march			
15-march			
16-march			
17-march			
18-march			
19-march			
20-march			
21-march			
22-march	2h	Got the 20kg of salt from Marghera and experimental design	
23-march	6h	experimental design and brainstorming	
24-march	6h	fill up the basins - check exp design with Camilla - pH meter	
25-march			Torino
26-march			Torino
27-march			Torino
28-march			Torino
29-march	4h	Salt in the basins, insert pumps and heater, insert oysters for acclimation, put biofilters into the canal, talked with camilla for exp. Design	
30-march	4h	hypothesis and procedure	
31-march			

Date	Total Time	Activity	Remarks/Follow-Up
01-apr			
02-apr			
03-apr			
04-apr	4h	meeting with matteo to try kits for measurments in lab + reading about procedures	
05-apr	2h	fixing hypothesis and reading articles about epibionts	
06-apr	4h	helping camilla in lab for recordings + meeting with camilla for exp design + fixing	
07-apr	2h	fixing exp design in the library	
08-apr			
09-apr			
10-apr			
11-apr	3h	preparation for first incubations (O1 andf O2 NCWA) and incubations	took photos and measurements of the oysters
12-apr	3h	incubations O1 and O3 CWA	
13-apr			
14-apr	3h	incubations O1 and O3 CWOA	

15-apr	3h	incubations O2 and O4 NCWA	took photos and measurements of the oysters
16-apr			
17-apr			
18-apr			
19-apr	3h	incubations O2 and O4 CWA and meeting with Camilla	
20-apr			
21-apr	3h	incubations O2 and O4 CWOA	
22-apr	3h	incubations C1 and C3 NCWA	
23-apr			
24-apr			
25-apr			
26-apr	3h	incubations C1 and C3 CWA and meeting with small presentation Prof and Camilla	
27-apr			
28-apr	3h	incubations C1 and C3 CWOA	
29-apr	3h	incubations C2 and C4 NCWA and measurements oysters	several oysters died from the ambient+ basin / oxygenator stopped working for microalgae PBR
30-apr			
Date	Total Time	Activity	Remarks/Follow-Up
1-may			
2-may	3h	incubations C2 and C4 CWA	
3-may	3h	incubations C2 and C4 CWOA	
4-may			
5-may	3h	incubations C6 and C8 NCWA	took photos and measurements of the oysters, and renewed water hot basin + added oysters
6-may	3h	incubations C6 and C8 CWA	
7-may			
8-may			
9-may	3h	incubations C6 and C8 CWOA	
10-may	3h	incubations C7 and C9 NCWA	took photos and measurements of the oysters
11-may			
12-may	3h	incubations C7 and C9 CWA	6 oysters died, and fed oysters
13-may	3h	incubations C7 and C9 CWOA	added 2 oysters in AMBIENT+
14-may			
15-may			
16-may	4h	incubations O6 and O8 NCWOA	2 oysters (ostrea) from AMBIENT+ died
17-may	4h	incubations O6 and O8 CWOA	finish and clean a bit the lab
18-may			exam period

19-may			exam period
20-may			exam period
21-may			exam period
22-may			exam period
23-may			exam period
24-may			exam period
25-may			exam period
26-may			exam period
27-may			exam period
28-may			exam period
29-may			exam period
30-may			exam period
31-may			exam period

Date	Total Time	Activity	Remarks/Follow-Up
1-june			exam period
2-june			exam period
3-june			exam period
4-june			exam period
5-june			exam period
6-june			exam period
7-june			exam period
8-june			exam period
9-june			exam period
10-june			exam period
11-june			little holiday
12-june			little holiday
13-june			little holiday
14-june			little holiday
15-june			little holiday
16-june			little holiday
17-june			little holiday
18-june	4h	working on the table of contents	
19-june			
20-june	3h	working on the table of contents and meeting with Camilla	
21-june	4h	working on the table of contents	
22-june	1h	fix exp. Design	
23-june	1h	fix exp. Design	
24-june			
25-june			
26-june			
27-june	1h	fix exp. Design and cover page	
28-june	3h	fix exp. Design and cover page	
29-june			trasloco

30-june			trasloco

Date	Total Time	Activity	Remarks/Follow-Up
1-june			exam period
2-june			exam period
3-june			exam period
4-june			exam period
5-june			exam period
6-june			exam period
7-june			exam period
8-june			exam period
9-june			exam period
10-june			exam period
11-june			little holiday
12-june			little holiday
13-june			little holiday
14-june			little holiday
15-june			little holiday
16-june			little holiday
17-june			little holiday
18-june	4h	working on the table of contents	
19-june			
20-june	3h	working on the table of contents and meeting with Camilla	
21-june	4h	working on the table of contents	
22-june	1h	fix exp. Design	
23-june	1h	fix exp. Design	
24-june			
25-june			
26-june			
27-june	1h	fix exp. Design and cover page	
28-june	3h	fix exp. Design and cover page	
29-june			trasloco
30-june			trasloco

Date	Total Time	Activity	Remarks/Follow-Up
1-july	1h	changes in exp. Design	
2-july	3h	changes in exp. Design and alk. Measurements	
3-july	2h	changes in exp. Design and alk. Measurements	
4-july	5h	preparing for meeting on Wednesday and alk. Measurements	
5-july			

6-july	3h	Meeting with prof Pastres	
7-july	1h	Theoretical framework	
8-july	2h	Meeting matteo and check results	
9-july			
10-july			
11-july	4h	Theoretical framework	
12-july			lavoro
13-july			lavoro
14-july			lavoro
15-july			lavoro
16-july			redentore
17-july			redentore
18-july			redentore
19-july			lavoro
20-july			lavoro
21-july			lavoro
22-july			lavoro
23-july			lavoro
24-july			lavoro
25-july	5h	methods	
26-july	5h	methods	
27-july	5h	methods	
28-july	5h	methods	
29-july	4h	methods	
30-july	5h	methods	
31-july	5h	methods	

Date	Total Time	Activity	Remarks/Follow-Up
1-august	5h	results	
2-august	6h	results	
3-august	5h	results	
4-august	6h	results	
5-august	6h	results	
6-august			hyke
7-august			hyke
8-august	6h	results	
9-august	6h	results	
10-august			work
11-august			work
12-august			work
13-august			work
14-august	6h	results	
15-august			ferragosto
16-august			ferragosto
17-august	6h	results and conclusions	

18-august	6h	results and conclusions	
19-august	6h	results and conclusions	
20-august	6h	results and conclusions	
21-august			holiday
22-august			holiday
23-august			holiday
24-august			holiday
25-august			holiday
26-august			holiday
27-august			holiday
28-august			holiday
29-august			holiday
30-august	2h	Fixing errors	
31-august	2h	Fixing errors	

Date	Total Time	Activity	Remarks/Follow-Up
1-September	3h	fixing thesis	applied for thesis
2-September	3h	fixing thesis	
3-September			
4-September			
5-September			
6-September			
7-September			
8-September	1h	Meeting prof + fixings	
9-September			cinema event venezia
10-September			cinema event venezia
11-September			travelling back to torino
12-September	3h	Call with Camilla + fixings	
13-September	3h	fixing thesis	sent back thesis to prof
14-September	3h	fixing methods	
15-September	3h	fixing methods	

16-September	4h	finishing thesis	
17-September	4h	finishing thesis	
18-September			
19-September	4h	finishing thesis	
20-September	4h	finishing thesis	
21-September			family event
22-September	4h	finishing thesis	
23-September	4h	finishing thesis	
24-September	4h	finishing thesis	
25-September	4h	finishing thesis	
26-September	4h	finishing thesis	
27-September	4h	finishing thesis	
28-September	4h	finishing thesis	
29-September	4h	finishing thesis	
30-September			Notte dei ricercatori