UNIVERSITY of STIRLING

# Characterisation of *Crassostrea gigas* (Thunberg, 1793) farming environment, physiological performances and challenges in shallow Mediterranean lagoons.

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## Abstract

The Italian territory is covered of approximately 384,000 ha of coastal lagoons and these could represent potential location for Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) farming. Approximately 10,000 ha of the coastal Italian lagoons are located in the Sardinian region, these are currently not utilized for shellfish farming, and present suitable environmental conditions that allow for good growth rates and optimal market quality for Pacific oysters farming. Therefore, these lagoons can become potential farming sites to increase this industry in the Italian territory, where the demand for this shellfish species cannot be met by domestic production alone.

The aim of this PhD study was to enhance Pacific oyster farming in Mediterranean coastal lagoons taking into account local aquaculture industry's ambitions and challenges, therefore different Pacific oyster culture aspects were investigated.

To achieve the goals of this PhD study, novel farming tools and their benefits to Pacific oyster farming were investigated. Moreover, the validation of ShellSIM® growth model in a new environment was carried out, providing a new validated tool to farmers and stakeholders to monitor oysters' performances and estimate productivity in local waters. Finally, a site selection methodology was developed to identify suitable sites for Pacific oyster farming, and potential risks to consumers linked to microplastics pollution in coastal areas and uptake in Pacific oyster were investigated.

Taken together, the results of this PhD study provide new insight on ways to improve *C. gigas* sustainable aquaculture industry in Mediterranean coastal lagoons, with particular focus on the Italian territory.

## Acknowledgements

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## List of Abbreviations

**AHP:** Analytical Hierarchy Process

**ANOVA:** Analysis of variance

**ASP:** Amnesic shellfish poisoning

**Cd:** Cadmium

**Cr (VI):** Hexavalent Chromium

**Chl-a:** Chlorophyll-a

**CI:** Condition Index

**Cu:** Copper

**DEB:** Dynamic Energy Budget model

**DDT:** dichlorodiphenyltrichloroethane

**DO:** Dissolved Oxygen

**dpf:** Day post fertilisation

**DSP:** Diarrhetic shellfish poisoning

**F:** Female

**FABM:** Framework for Aquatic Biogeochemical Models

**FB:** Floating Bags

**Fe:** Iron

**FLAG:** Fisheries Local Action Group

**GDP:** Gross Domestic Product

**GF/F:** Glass microfiber Filters retain particles down to µm.

**GIS:** Geographical Information System

**GPS:** Global Position System

**GSM:** Global System for Mobile communications

**HCB:** Hexachlorobenzene

**HCL:** Hydrochloric acid

**Hg:** Mercury

**hpf:** Hours post fertilisation

**hrs:** Hours

**IAS-CNR:** National Council of Research

**IMC:** International Marine Centre

**Ind.:** Individual

**LAORE:** Sardinian Government Agency

**M:** Male

**MCA:** Multi Criteria Analysis

**MCE:** Multi Criteria Evaluation

**mpf:** Months post fertilisation

**NEB:** Net Energy Balance

**NSP:** Neurotoxic shellfish poisoning

**OsHV-1µvar:** Ostreid herpes virus 1

**OU:** Ortac Units

**PAHs:** Polycyclic aromatic hydrocarbons

**Pb:** Lead

**PBDEs:** Polybrominated diphenyl ethers

**PCBs:** Polychlorinated biphenyls

**Pg:** petagram

**PIM:** Particulate Inorganic Matter

**PML:** Plymouth Marine Laboratory

**POC:** Particulate Organic Carbon

**POM:** Particulate Organic Matter

**POPs:** Persistent organic pollutants

**POS1:** Sampling position 1

**POS2:** Sampling position 2

**POS3:** Sampling position 3

**PSP:** Paralytic shellfish poisoning

**PVC:** Polyvinyl chloride

**PP:** Potential Production

**RMSD:** Root mean square deviation

**S:** Taylor diagram Skill score

**Sal:** Salinity

**Sb:** Biological Suitability

**SE:** Standard Error

**SIRA:** Sistema Informatico Regionale Ambientale

**Sl:** Logistic suitability

**St:** Total suitability

**T:** Temperature

**Temp:** Temperature

**TPM:** Total Particulate Matter

**UV:** Ultra Violet

**Vol. ext:** Volume of 90 % acetone used in the Chl-a extraction (ml)

**Vol. sample:** is the volume of water filtered (liters) for Chl-a analysis

**Zn:** Zinc

## Thesis Structure

The overall thesis structure is shown in figure a, while the chapters will be divided as follow:

- **Chapter 1:** General Introduction.
- **Chapter 2, 3, 4, 5:** are the experimental chapters **(**fig. a).
- **Chapter 6:** General Discussion and conclusions. This chapter summarises the key findings and the implications of the studies performed during this PhD. Moreover, limitations and further research are discussed.

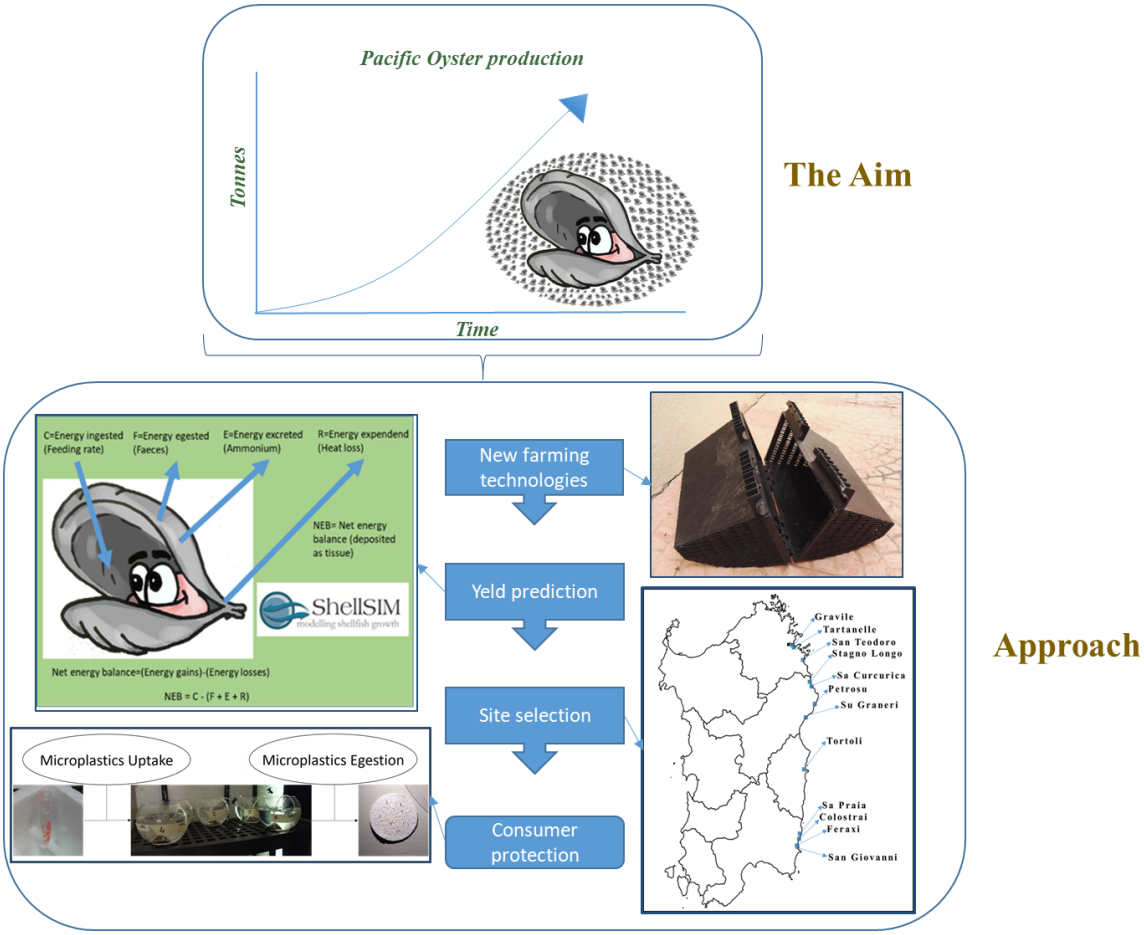


**Figure a:** Overall thesis structure. The Experimental chapter are divide as follow: New farming technologies = **Experimental chapter 2:** Improving Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) Production in Mediterranean Coastal Lagoons: traditional *vs* novel farming methods; Yeld prediction = **Experimental chapter 3:** Validation of the growth model "ShellSIM®" on traditional and novel farming methods; Site selection = **Experimental chapter 4:** Site Selection for Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), farming in Shallow Mediterranean lagoons. A case study using AHP process and DEB growth model in the east coast of Sardinia; Consumer protection = **Experimental Chapter 5:** Microplastics Uptake and Egestion Dynamics in Pacific oysters, *Crassostrea gigas* (Thunberg, 1793), Under Controlled Conditions.

# 1. General Introduction

## 1.1. Thesis Background and Motivation

Sardinian coastal lagoons have a very high naturalistic value and they are among the most extensive in Europe. Their origin is mainly linked to the particular geological history of the island. These lagoons are distributed along the island coastline, with the highest density in the Gulf of Oristano, in the Gulf of Palmas and in the Gulf of Cagliari (Sardegnaagricoltura.it, 2019).

Fishing in the lagoons for wild fish and bivalves, together with agriculture and hunting, were the most popular subsistence practices of the Sardinian people (residues of meals, as fish vertebrae and shells, of the ancient inhabitants of the island date back to the ancient Neolithic, around 6,000 years BC). The exploitation of the lagoon resources continues and is reported that during the Roman period the "arsellari" (clams harvesters), operating in the lagoons of Cagliari and Oristano, marketed their product all over the island: many are, in fact, the remains of molluscs shells found in inhabited areas of the internal part of Sardinia (Sardegnaagricoltura.it, 2019).

Due to the fact that in the Sardinian history fishing activities were mostly focused on the lagoons, that continued to guarantee abundant and renowned products for centuries, during the Middle Ages, these “water areas” were involved in various forms of appropriation and management by part of sovereigns and religious orders. Only in the last century the Sardinian government, with the Regional Law n. 39 of 2 March 1956, abolishes the exclusive and perpetual fishing rights and regulates its functioning in inland and lagoon waters (Sardegnaagricoltura.it, 2019).

Currently most of the lagoons are owned by the Sardinian Government, who assigns them in concession. Exceptions to this general rule are the San Teodoro lagoon, owned by the local council and the Mistras, Pilo and Casaraccio lagoons that are privately owned. Between all Sardinian lagoons, around 30 are exploited for fish and shellfish production. Their management is usually under cooperatives or fishermen's consortia and fishing activities are mostly carried out using traditional techniques. These techniques are based on the habitual migrations of fish (young or adult) from the sea to the lagoons and *vice versa*.

The most commercially relevant fish species in Sardinian lagoons, are mullets, *Mugil cephalus* (Linnaeus, 1958) and *Liza aurata* (Risso, 1810), eels, *Anguilla anguilla*

(Linnaeus, 1958), crabs, *Carcinus mediterraneus* (Czerniavsky, 1884), and more valuable species such as sea bream, *Sparus aurata* (Linnaeus, 1758) and sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). Among the bivalve molluscs, clams, *Ruditapes decussatus* (Linnaeus, 1758) and *Ruditapes philippinarum* (Adams and Reeve, 1850), are the most significant species being harvested from the wild.

Most of the lagoons, suffer from a strong presence of human activities (construction sites / civil uses / tourist activities) that can cause conflicts with the uses for primary production and environmental conservation. Nonetheless, many lagoons are still of considerable importance for professional fishing and aquaculture and thanks to their centuries long use, have been preserved from more environmentally impacting activities. Indeed, small scale traditional fishing and extensive aquaculture operations have *de facto* played an important role in the conservation of these sites (Cataudella *et al*., 2014). Due to the extensive nature of fishing and farming activities, actions aimed at optimizing production have often coincided with those necessary for environmental conservation such as control and maintenance of marine and continental water (i.e. rivers and streams) inflows to keep an efficient internal water circulation.

The Sardinian region in 2018 had a population of 1,648,176 with a density of 68 individual per $km^2$ and a general unemployment rate of 15.4 % and a 35.7 % youth unemployment rate. Furthermore, it is recorded a staggering 55 % youth participation rate failure, which is an indicator of the potential labour force not seeking employment (ec.europa.eu, 2019) mostly linked with a generalised lack of job offers in the more rural coastal areas. Therefore, the commercial activities carried out in the Sardinian lagoons can also offer important employment opportunities within this context.

In Italy there is a high demand for seafood products, and most Italian aquaculture companies are involved in shellfish farming, contributing to over 64 % of national total aquaculture production. However, demand is greater than supply, and in 2017 over 1.3 million tonnes of seafood were imported to the country (FAO, 2018; FAO, 2018a; Sardegnaagricoltura.it, 2019). One of the products in high demand, which cannot be met by domestic production alone, is Pacific oyster (Tamburini *et al.*, 2019).

Therefore, there is a need to plan and manage coastal activities, including shellfish aquaculture, to optimise the benefits from these lagoons in a sustainable way. In this scenario shellfish farming provides different ecosystem services to estuarine environments such as: improvement of water quality and sediment consolidation (Newell *et al.*, 2002;

Cressman *et al.*, 2003; Newell and Koch, 2004; Grizzle *et al.*, 2006; Piehler and Smyth, 2011). Indeed, it has been demonstrated that the environmental impacts of these cultured species are small, especially if compared with finfish farming, and can be minimised by using appropriate farming protocols (Crawford *et al.*, 2003). Moreover, considering that the Sardinian's Pacific oyster farming industry is still in its infancy, this local context was considered to be appropriate to perform this PhD study.

Sardinia has the potential to help meeting the Italian demand for Pacific oyster through domestic production, rather than relying on imports, if suitable locations for this development can be identified and coastal lagoons should be explored for this purpose.

This PhD study was performed with the aim to boost Pacific oysters' production in Sardinia using, for the first time, an experimental approach to gain new knowledge on the oyster farming in the Sardinian context. For this purpose in the first part of this PhD study, the best performing farming tools (novel *vs* traditional) where investigated and the validation of a growth model was performed in order to establish the potential productivity of different site and within each site, these two studies are described respectively in chapter 2 and 3 of this PhD thesis. Moreover, a methodology to select new potential farming sites is described in chapter 4. Potential impacts of microplastics on the cultured Pacific oysters and the relative potential risk for consumers are presented and discussed in chapter 5.

Results obtained here represent a stepping stone for further improvements in oyster production at farm level and, more widely, provide novel insights into the potential for Pacific oyster production in the region. The results of this study can also be applied in the wider context of other Mediterranean regions. Furthermore, the applied nature of this investigation is such that the findings can be relevant to a wide audience including, aquaculture scientist, farmers and policymakers.

## 1.2. The Pacific oyster, *Crassostrea gigas*

### 1.2.1. Anatomy and Morphology

The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) is a lamellibranch suspension-feeding bivalve mollusc. The valves are different in size and shape and this can change due to different environmental conditions, such as temperature and salinity (Quayle, 1969; Hèral and Deslus-Paoli, 1990; FAO, 2019). The right valve is concave, and the left valve is flat or slightly convex. They are protandrous hermaphrodite, and sexually mature Pacific oyster size can vary from 80 to 300 mm in length (FAO, 2019).

Inside the shell, there is the mantle, which is divided into two lobes, between these there is a cavity (pallial cavity) where the gills are located, these have the role of extracting oxygen and filtering the water to retain food particles, which enter the digestive system through the mouth. This is surrounded by the labial palps and is located near the hinge, dorsally to the mouth there are oesophagus, stomach, crystalline style sac which constitute the digestive gland. The intestine is composed of midgut, rectum and the anus. Inside the pallial cavity, there is also space for the gonads which, during the reproductive period can reach 70 % of the meat dry weight, the kidney or neprhidium and the heart (fig. 1.1) (Gerdes, 1983; Miossec *et al.*, 2009).

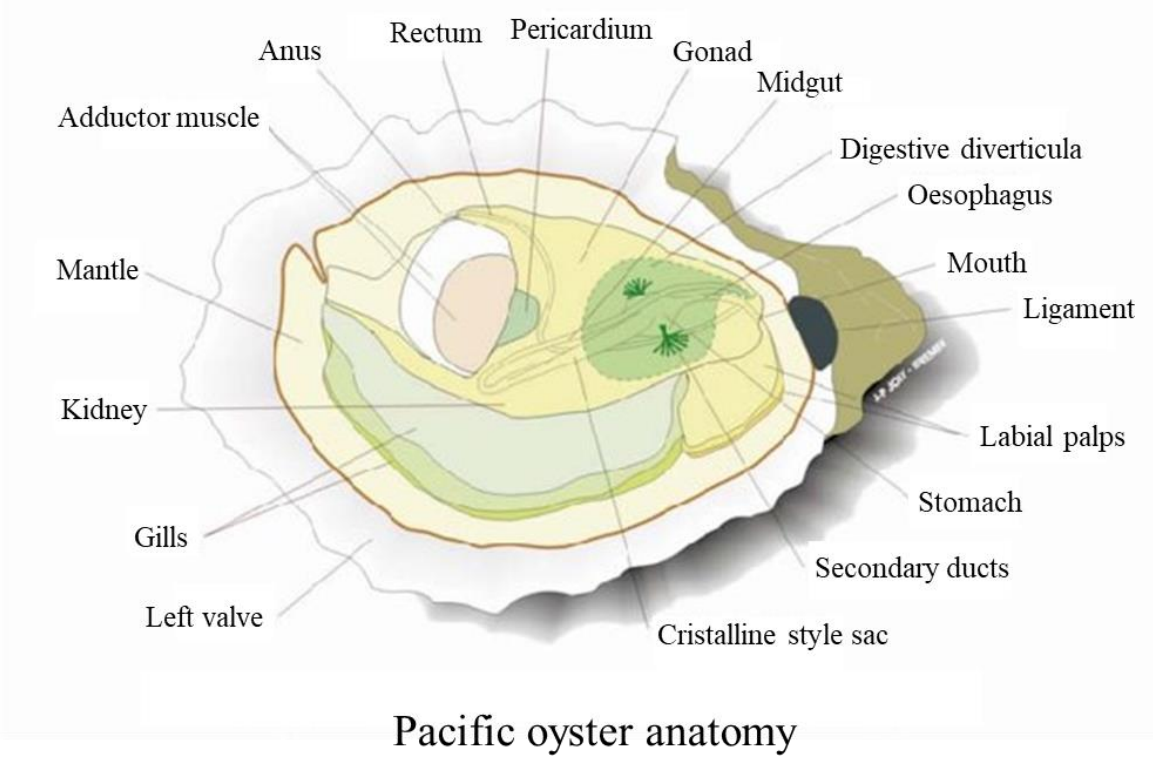


**Figure 1.1:** *Crassostrea gigas* anatomy (Modified from: Miossec *et al*., 2009).

The sex ratio in Pacific oyster mainly depends on environment condition (Baghurst and Mitchell, 2002), and in particular on food availability; in areas with a good food supply, there is a predominance of females but these can revert to male if food availability decreases and environmental conditions are unfavourable (Steele and Mulcahy, 1999; FAO, 2019). Guo *et al.* (1996), report that the sex is determined by a single gene locus with a maleness (M) allele that is dominant and an allele for protrandic females (F). Genotypes MF are true males and FF are protrandic females that in the juvenile stage are male and then they can change sex due to environmental effects, such as temperature or nutrition, or genes regulations (Hèral and Deslus-Paoli, 1990).

Temperature is a major regulator in gametogenesis in marine bivalves (Sastry, 1979). In Pacific oyster this starts at around 10 °C (Fabioux *et al*., 2005) and Lubet (1980) in his study reported that below 17-18 °C there is no emission of sexual product. Moreover, the optimum salinity range for gametogenesis was between 15 ‰ and 32 ‰ (FAO, 2019).

The Pacific oysters are oviparous and in a size of 8-15 cm in length can produce between 50 and 200 million eggs in one single spawn. Pacific oyster adults release the sperm and the eggs into the water column where fertilization takes place.

Once fertilised, the cells within the egg start to divide passing from the stage of morula to Gastrula. Depending on temperature and salinity, the stage of trochophore larva starts after 12 hours, D-shaped larva after 27 hours, veliger larva after 7 days and at 15 days pediveliger larva (fig. 1.2). Helm and Millican (1977) reports that the optimum salinity value for the larval development is 25 ‰, but this can successfully take place at salinities between 20 and 35 ‰, and the higher the salinity, the more important it is that the temperature is above 22 °C (Hèral and Deslus-Paoli, 1990).

The free-swimming stage terminates with metamorphosis and the planktonic larvae metamorphose in benthic spat (between 1- and 3-months post fertilization) that attaches to a substrate and becomes sessile (fig. 1.2) (Vogeler *et al.*, 2016).

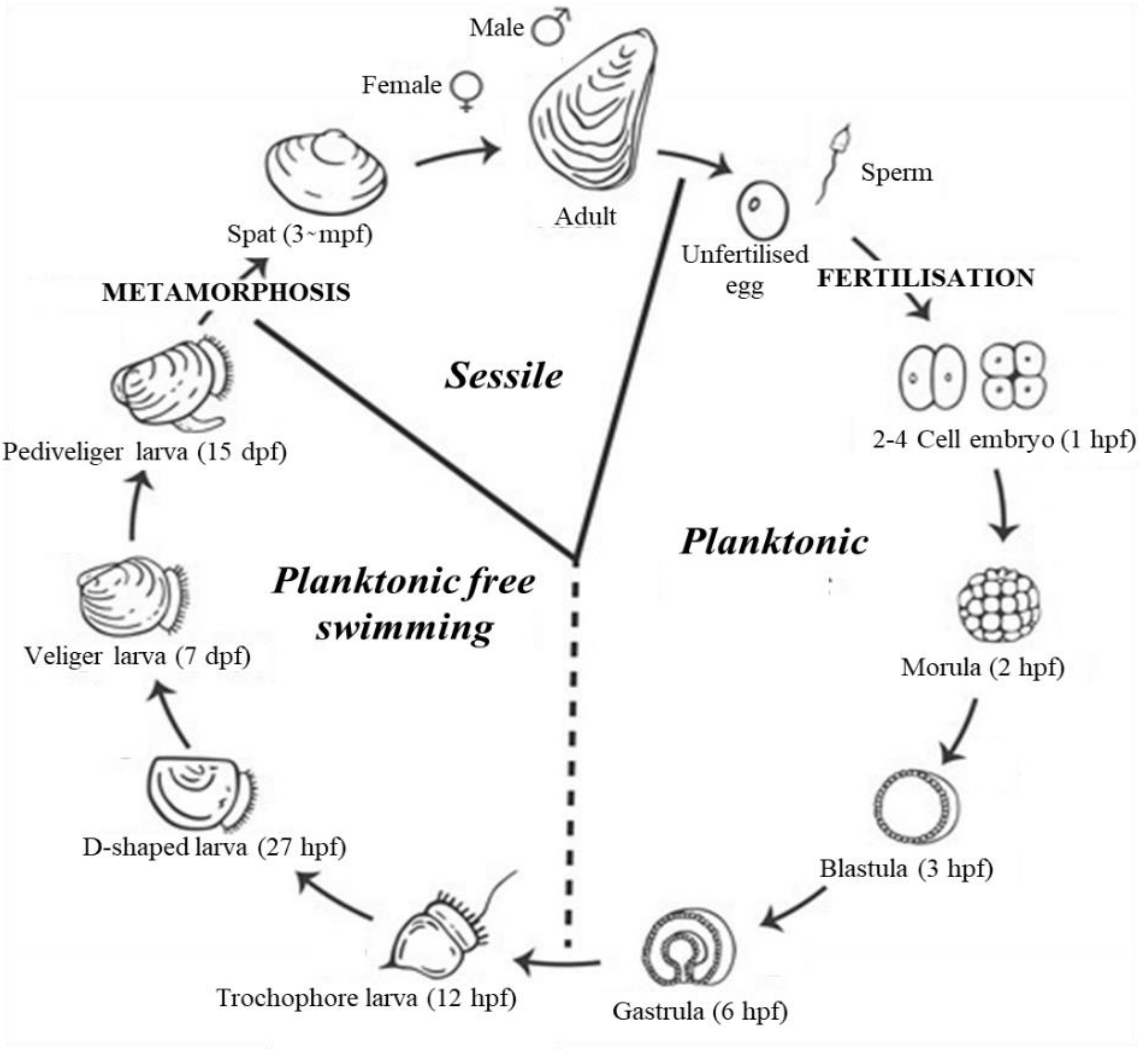


**Figure 1.2**: Pacific oyster life cycle (Modified from: Vogeler *et al*., 2016).

### 1.2.2. Environmental influence on Oysters Biology

In coastal areas as in Mediterranean lagoons, variation in temperature, salinity and food availability, can affect the metabolism of Pacific oyster (Sparks and Chew, 1959; Agius *et al*., 1978), particularly, the growth rate, condition index and survival rate are influenced by these environmental factors (Incze *et al.*, 1980; Bernard, 1983; Brown and Hartwick, 1988). The precise influence of each individual factor on the physiology of Pacific oysters is difficult to quantify, because these sometimes interact synergistically. For example, the food availability is temperature and salinity dependant, low temperatures and food availability are generally coincident while low salinity in coastal areas coincide with freshwaters inputs and therefore with higher amounts of nutrients and consequently with higher phytoplankton biomass. Moreover, there are also endogenous factors that influence

the response to the environmental conditions i.e. genotype and physiological status of the animal (Goosling, 2003).

Temperature is an important factor controlling oyster growth, reproduction and breeding success (Quayle, 1969; Hèral and Deslus-Paoli, 1990). The effects of this factor on bivalves are related to mechanical and physiological effects. For example, there is a change of seawater viscosity depending on the temperature (higher temperature lower viscosity and *vice versa*), and this fact influence the amount of water that can be filtered, the more viscous and les water can be pumped for a given energy expenditure, therefore limiting the clearance rates (Jørgenson *et al*., 1990; Podolsky, 1994). Marine bivalves living temperature range is between -3 and 44 °C (Vernberg and Vernberg, 1972) and for the Pacific oyster the range of temperature tolerance is between -1.8 and 35 °C (FAO, 2019).

Filtration rate increases with higher temperatures and therefore increases the amount of food captured by the gills (20 °C is the optimum water temperature for feeding efficiency) (Quayle, 1969). Spawning is also controlled by water temperature and this occurs with an optimum temperature around 20 °C (Magoon and Vining, 1981).

Salinity is an important limiting factor in mollusc bivalves, oysters are euryhaline, and therefore they can tolerate a wide range of salinity and live in different environments, from estuaries and bays (where the salinity can change rapidly due to rainfall and inflow from rivers) to fully saline oceanic waters. This wide range of tolerance to different salinity values is possible due to the mechanisms to adjust the concentration of intracellular osmolytes by which Pacific oyster regulate cell volume (Hosoi *et al.*, 2003).

For the Pacific oyster the optimum salinity range is between 25 and 35 ‰ (Wiltshire, 2007) but it is possible to find them in salinity lower than 10 ‰ and higher than 35 ‰. Oyster juveniles have the same salinity range tolerance as adults (Gosling, 2003; FAO, 2019). Hèral and Deslus-Paoli (1990) reports that over 50 ‰ a high mortality was observed and lower than 15 ‰ the growth was affected.

Similarly to temperature, salinity also has a role in the filtration rate. Loosanoff (1953) reports that when *Crassostrea Virginica* (Gmelin, 1791) was exposed to different salinities (from 27 to 5 ‰) and a marked decrease in filtration rates was observed with decreasing salinity, until this process stopped at the lowest salinity tested (Loosanoff, 1953). Studies on oyster salinity range show that Pacific oyster has a wide tolerance, and individuals can survive for relatively short period of time at salinities near to 0 ‰ by just closing their

valves (Hèral and Deslus-Paoli, 1990). The physiological mechanism by which salinity is related to growth is not fully explained, but energy losses due to osmoregulatory process must be considered as a significant energy cost that cannot be employed for growth and reproduction (Bayne and Newell, 1983).

Shellfish are filter feeders, and they can control feeding in different ways, such as using different rate of water filtration, sorting the particles filtered between edible and low nutritional value material, sorting particle size and varying digestion and absorption through gut and stomach (Gosling, 2003; Miossec *et al.*, 2009).

Bivalve feed on suspended particles (bacteria, phytoplankton, micro-zooplankton, detritus and dissolved organic material), and the relative proportions in which these are available to the animals can change between locations, environments and seasons (Gosling, 2003).

There is little information about Pacific oyster specific food item utilized in the wild, but it is assumed that the main energy source is derived from phytoplankton (Gosling, 2003) and that the organic particulate matter (detritus) is an important part of the natural diet (Carboni *et al.*, 2016; Newell, 2016).

### 1.2.3. Biogeography

The Pacific oyster is native to Japan and coastal regions of Asia, and due to its wide adaptation range at different environmental conditions, is the most widespread oyster in the world (fig. 1.3) (Shatkin *et al*., 1997).

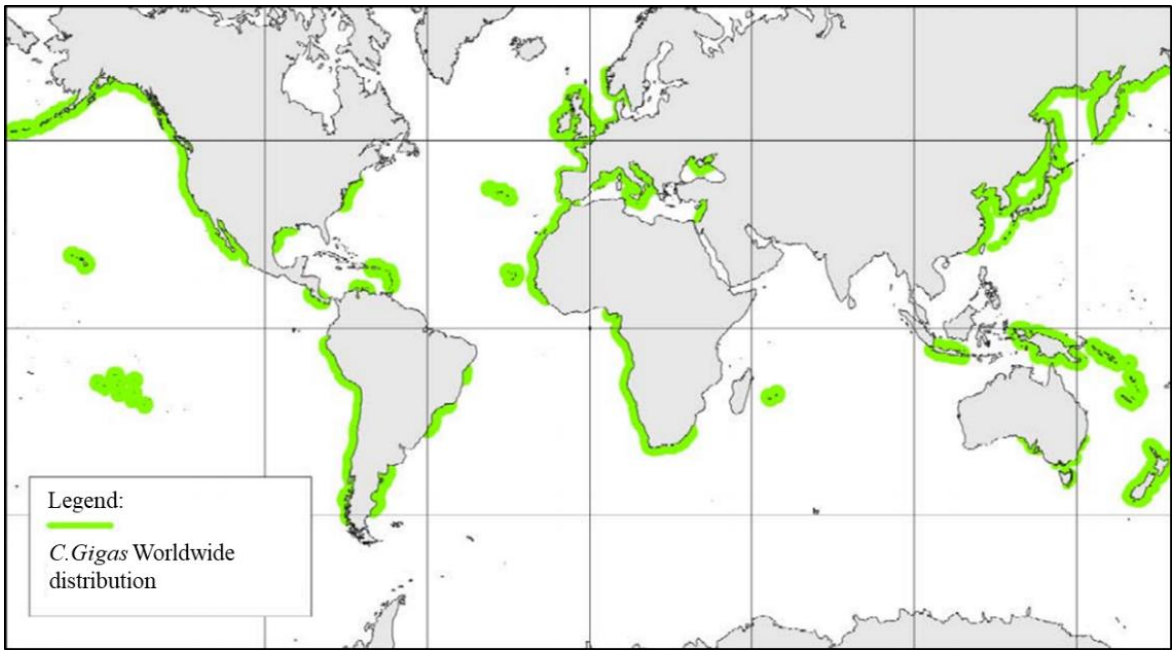


**Figure 1.3:** Worldwide distribution of *Crassostrea gigas* (Modified from: Miossec *et al*., 2009).

Pacific oysters were introduced in different parts of the world such as the United States of America, France, UK, Korea, China, New Zealand, Australia, South Africa and South America and Italy, mainly to replace native oysters in areas where these were overfished or stocks were depleted due to disease, but also to create an industry. Due to the wide adaptability to different environments, Pacific oyster's wild population are now established in different parts of the world (Buck *et al*., 2006; Cardoso *et al*., 2007; Wrange *et al*., 2009; Miossec *et al*., 2009).

The Pacific oyster is an estuarine species that prefers shallow rocky seabed bottoms (where they live attached to the rocks), but it is also possible to find them on mud or mud-sand bottoms down to a depth of 40 m (Arakawa, 1990; Reise, 1998; Dupuy *et al*., 1999; Ernande *et al*., 2003; FAO, 2019).

There are no official records of first introduction of *C. gigas* in Sardinia beyond the relatively recent establishment of private ventures such as "Compagnia Ostricola Mediterranea" in 2007. Nonetheless, several old large shells are regularly found in

thanatocoenosis around the Island indicating that spread of this species on its shore precedes commercial farming (*pers. obs.*). *Ostrea edulis*, is a European native species that can be found in whole the Mediterranean Basin. In Italy the production of the *O. edulis* is negligible and most of the marketed flat oysters are imported or harvested from natural banks (Carlucci *et al*., 2010). In recent years in Italy due to the reduction of natural populations, there is a growing interest on farming this species and several growth trials have been performed along its coasts. In Sardinia, growth and survival trials have been performed in different lagoons (Porto pozzo, San Teodoro and Calich) and the authors of these trials reports low survival rates but with growth rates higher than other central-western Mediterranean farming areas (Pais *et al*., 2007, 2012; Carlucci *et al.*, 2010; Saba *et al.*, 2013).

### 1.2.4. Ecosystem services

The ecosystem services are the benefits that humans derive from ecosystems. These services, as reported from the United Nations Millennium Ecosystem Assessment (http://www.millenniumassessment.org/en/index.html), can be divided into four categories: Provisioning services, regulating services, supporting services and cultural services (Vaughn, 2017).

The provisioning services are those one that provide material or energy from ecosystems, these includes different resources as for example food (agroecosystems, marine and freshwater systems and forests provide food for human consumption), row materials (for example material for construction, biofuel and plants oils), freshwater, medicinal resources (plants are used for traditional medicines and also provide row materials for the pharmaceutical industry) (Vaughn, 2017; http://www.millenniumassessment.org/en/index.html).

The supporting services are those one that provide living spaces for plants or animals (habitats for plants and animals provide everything that these needs to for their life cycles) maintaining a genetic diversity between, and within, species populations (Vaughn, 2017; http://www.millenniumassessment.org/en/index.html).

The regulating services by the ecosystems are those one provided by regulating e.g. the quality of air and soil (plants can play a role on improving the air quality by removing pollutants from the atmosphere) or providing flood and disease control (trees can stabilize

slopes and microorganism in the soil through their biological activity can break down the waste, in this way microbes are eliminated and level of nutrients and pollution are reduced). Moreover, climate control, carbon storage and pollination (Vaughn, 2017; http://www.millenniumassessment.org/en/index.html).

The cultural services, are the benefits that people obtain trough the contact with the ecosystem as aesthetic, spiritual and psychological benefits. In these cultural services are included the important different roles that the ecosystems play in the tourism industry (Vaughn, 2017; http://www.millenniumassessment.org/en/index.html).

The Pacific oysters, as other filter feeders can improve water quality by reducing suspended solids, turbidity, phytoplankton and bacterial biomass, moreover can increase the denitrification (Nitrogen removal) and the biomass of benthic algal via improved light penetration due to lower turbidity (Newell *et al*., 2002; Cressman *et al*., 2003; Newell and Koch, 2004; Grizzle *et al*., 2006; Piehler and Smyth, 2011). In addition to improving water quality, the ecosystem services provided by the oysters include: seashore stabilization, carbon sequestration, increasing habitat for fish, invertebrates and epibenthic fauna, diversification of the landscape and ecosystem (Wells, 1961; Bahr and Lanier, 1981; Meyer *et al*., 1997; Lenihan *et al*., 2001; Peterson *et a*l., 2003; Grabowski and Peterson, 2007).

The supporting services provided by *C.gigas* include the cycling of nutrients, creation of sediment, increasing seabed roughness and providing habitats for other organism. The oysters as others shellfish can impact on water flow at different scale, a micro scale, by the waterflow created by the exhalant siphons and by increasing bed roughness via the shell shape and a macro scale, for example the alternations of patches of oyster sea bed and patches of sediments. This affect the water mixing that is important for the cycling of nutrients, alteration of turbidity, and creation of sediments and decreasing of the wave's energy (Van der Schatte Olivier *et al*., 2018). Oysters forms reefs, these have different ecological functions as providing refuge food and substrate for other species. Herbert *et al*. (2012), reports that Pacific oysters reefs can show wide species diversity and Shumway *et al.* (2003) reports that the farming of these shellfish can provide, trough the farming equipment and the shells, new habitat for different organisms. Moreover, oyster reefs as reported from Coen *et al.* (2007) can increase the presence of finfish and invertebrate that are important for fisheries and recreational fishing therefore they can produce an economic benefit (Van der Schatte Olivier *et al*., 2018).

The Provisioning services provide by the Pacific oysters can be divided in two parts: the provision of nutrition (e.g. oyster meat) and the provision of raw materials as for fertilizer (e.g. ground flesh), constructions (e.g. shells for building or shoreline protection), jewellery (mother of pearl). Moreover, the crushed oyster shell can be used to improve acid soils (through the use of lime or other calcareous materials) and to stimulate the growth of soil and rhizospheric microorganism (Van der Schatte Olivier *et al*., 2018).

The Regulating services: Pacific oysters can filter a large amount of water and due to their filtration system are able to modify biogeochemical cycles filtering organic matter from the water (Kellog *et al*., 2013). The eutrophication of water environments caused by an excess of nutrients is a worldwide issue, Pacific oysters mitigates environmental changes by removing nitrogen and phosphorous from the environment (for example filtering Phytoplankton) and using them for shell and tissue growth (and these are then removed from the water environment once these shellfish are harvested) (Kellog *et al*., 2013; Van der Schatte Olivier *et al*., 2018), they can also remove these nutrients trough the production of bio-deposits (these are anoxic environments for denitrifying bacteria)(Newell *et al*., 2005). Moreover, by the water filtration system Pacific oysters can remove from poor quality waters bacteria, protozoa and viruses, this will lead to the inedibility of the product but at the same time, pacific oyster as other shell fish could be used to improve water quality in area where finfish are farmed or bathing waters (Roslev *et al.*, 2009; Clements *et al*., 2013; Van der Schatte Olivier *et al*., 2018). Pacific oyster farming it is an important industry for human consumption but at the same time there is a gaining attention on the rule that these species have in the carbon cycle. The Calcium Carbonate is involved in the shell production, during this process carbon dioxide is formed, therefore potentially leading to increase the pCO2 in the first layers of waters and evasion of CO2 into the atmosphere, therefore the calcification process is considered by some authors as a source of Co2 in to the atmosphere other consider that the shell represent a deposit for carbon (Fodrie *et al.*, 2017; Hickey, 2009; Higgins *et al.*, 2011). However, Van der Schatte Olivier *et al*. (2018) reports that further studies need to be done to understand the potential of Pacific oyster and other shellfish as a store fore CO2. Into the category of regulating services Pacific oysters' reefs by providing protective structure act as biological barriers helping to reduce shorelines erosion (Scyphers *et al.*, 2011; La Peyre *et al.*, 2015).

Pacific oyster beds include different cultural services. These shellfish beds can attract a wide diversity of birds therefore creating important environments for birdwatching activities (tourism). Van der Schatte Olivier *et al.* (2018) reports that shellfish as Pacific

oyster are often use for scientific experiments indeed a bibliographic research made in the years between 1918 and 2018 showed 196.000 research made on different Oysters species. These shellfish are important also as traditional food, for example Christmas period in France and often they are the attraction of some seafood festivals that attract tourists (Buestel *et al.*, 2009; Van der Schatte Olivier *et al*., 2018).

## 1.3. Bivalve Culture

### 1.3.1. Industry and global production

Marine bivalves in 2016, accounted for 15 % of the total global aquaculture food production (110.2 million tonnes), with a mean (2010 - 2016) annual production of 15 million tonnes (FAO, 2018a). The total production depends on the interaction between the market demand and production capacity which could depend on different physical, ecological, and social factors (FAO, 2018). In Europe, from the 800 thousand tonnes of bivalves produced in the 1999 production decreased to 600 thousand tonnes in 2016, accounting for the 3.6 % of the global production of bivalves (fig.1.4) (FAO, 2018a).

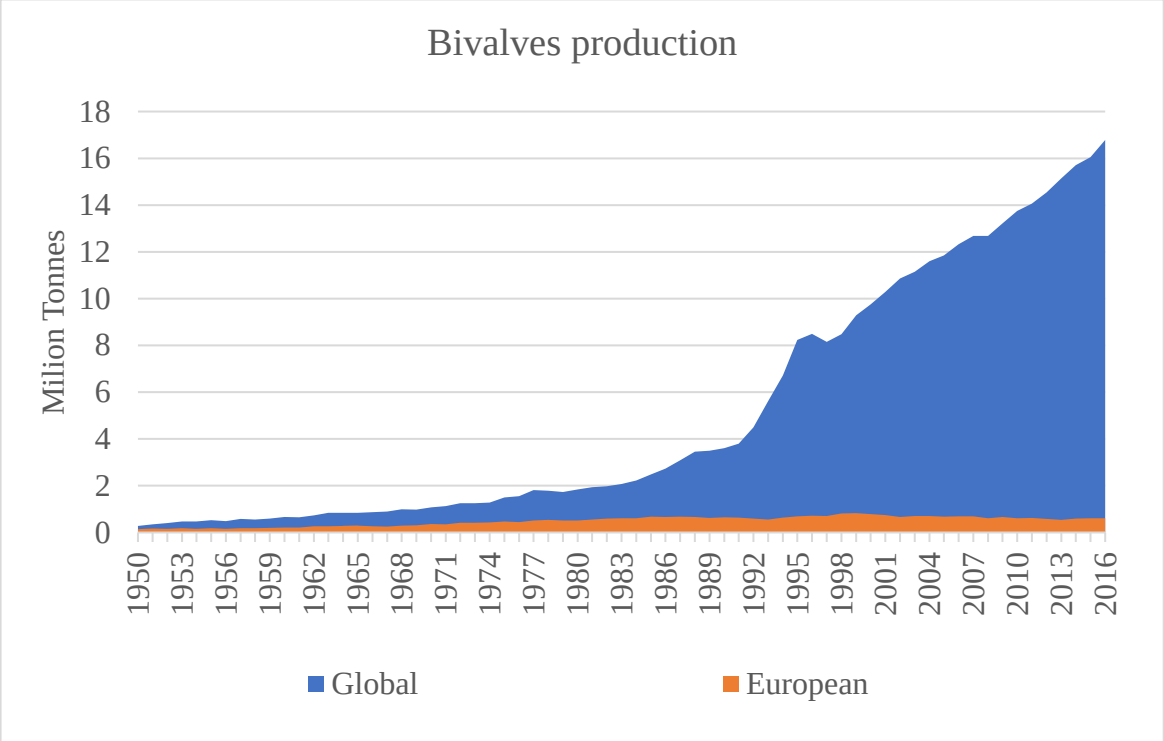


**Figure 1.4:** Global and European production of Bivalves (FAO, 2018a).

In the FAO statistic database are reported a total of 79 farmed marine bivalves' species, and among these the mussels, clams, scallops and oysters are the most commonly farmed species. In 2016, China was the largest producer of bivalves with 14 million tonnes (FAO, 2018).

The Pacific Oyster is currently amongst the major farmed bivalves' species in the world. In 2016, 573.6 thousand tonnes where produced and 22.6 thousand tonnes where captured. In Europe the production of Pacific oyster in 2016 was 77 thousand tonnes with France being the largest producer (64 thousand tonnes) (fig. 1.5) (FAO, 2018a).

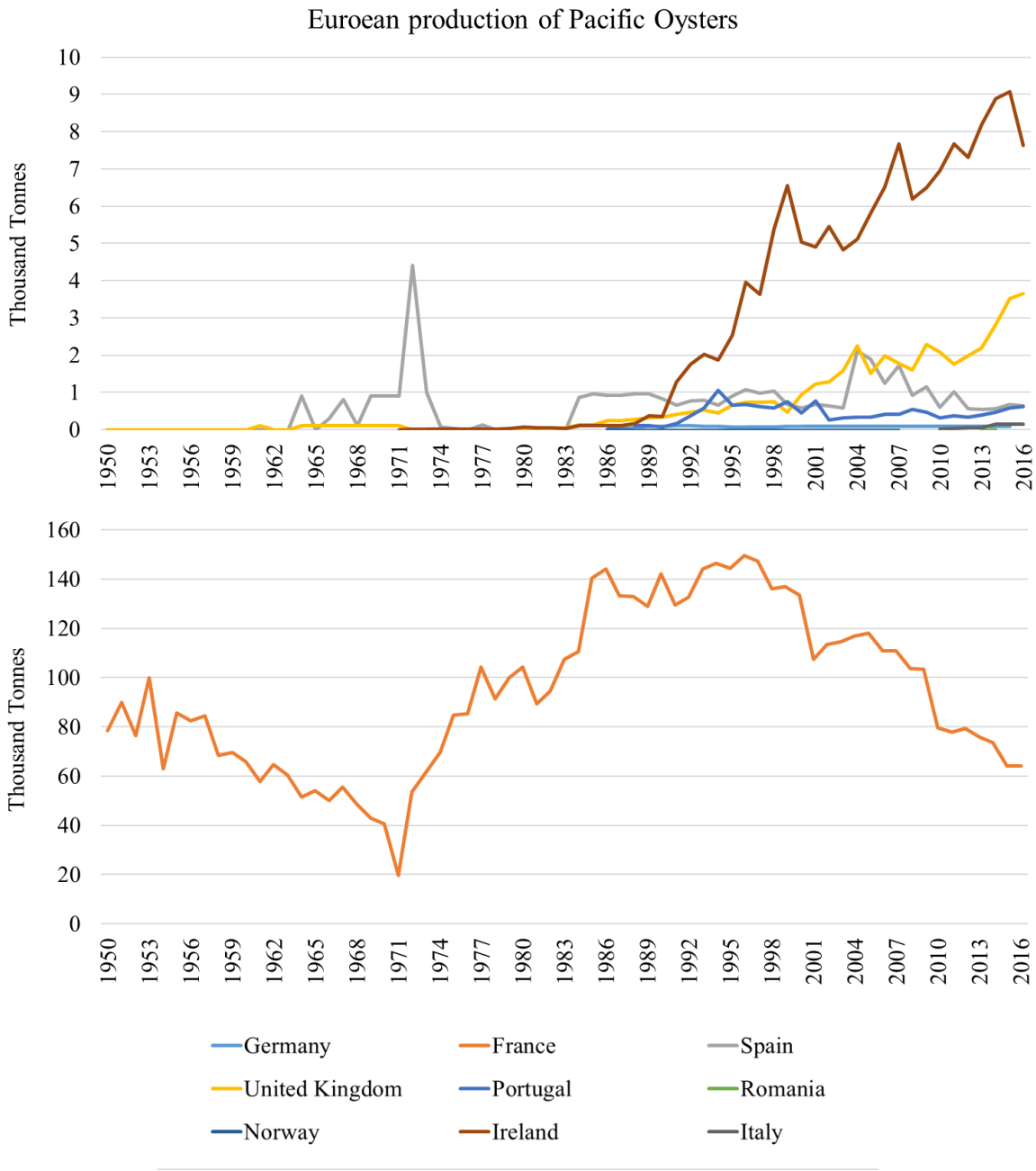


**Figure 1.5:** European production of *C. gigas* (FAO, 2018a). French production is kept separate to highlight its significantly higher production compared to the other European countries.

### 1.3.2. Pacific Oyster Aquaculture

The first culture of Pacific oyster recorded was conducted in Hiroshima bay (Japan) in the 16$^{th}$ century (FAO, 2019; Miossec *et al.*, 2009).

Supply of spat for aquaculture at the beginning, was obtained by collecting juveniles from the natural environment. The high demand of spat in the 70's evolved in the increased development of Pacific oyster's hatcheries including the production of triploid seed and

consequently breeding programs focused on improving Pacific oyster and qualities disease resistance and growth performance (FAO, 2019).

Records of the Pacific oysters culture in Europe, starts with the "Resur campaign" that consisted in a massive introduction of the alien species *Crassostrea gigas*; In 1966-1970 small scale production was conducted and hundreds of tonnes of Pacific oyster were imported into France from Canada and from Japan to be placed in different French areas including the Mediterranean Sea. This was a successful plan and the Pacific oyster demonstrated fast growth and good survival rates, enough to allow for the collection of sufficient spats from the wild to supply French production sites without importing any more spat from oversea (Grizel and Hèral, 1991; Buestel, 2009; Miossec *et al.,* 2009).

Due to this massive introduction into the French area and due to the wide adaptability to different environments, Pacific oyster wild population are now established in different parts of Europe, from Portugal in the south as far as Norway and Sweden (Buck *et al.,* 2006; Cardoso *et al.,* 2007; Wrange *et al.,* 2009; Miossec *et al.,* 2009). Moreover, it is reported that Pacific oysters in Italy were imported from France around the 1966 (Fabioux *et al*., 2002), and feral populations are now established along the Italian coasts (Burioli *et al*., 2016).

### 1.3.3. Cultivation Techniques

In the past, Pacific oyster seed was obtained mainly from the wild but, nowadays, the role of commercial hatcheries is becoming of primary importance in supplying oyster seed due to the increasing demand of seed from the industry and the reduction of natural stocks (Helm *et al*., 2004).

Many factors must be taken into account for the construction of a bivalve hatchery, first the site selection, government regulations and the quality of the seawater.

The design of an oyster hatchery depends on different needs. The hatchery can be small and supply seed for their own on-growing system, or larger to supply seed for sale. Some hatcheries may include a nursery (Helm *et al*., 2004).

In hatcheries for Pacific oyster, microalgae are used to replace or as a supplement to the natural suspended particles. It is important that the microalgae used have a high nutritional value and permit a rapid growth rate of the oysters. Brown *et al.* (1998) indicates a few

microalgae species that have these characteristics and are used commonly in oyster hatcheries: *Thalassiosira pseudonana*, *Isochrysis sp.* (T.ISO), *Chaetoceros calcitrans* and *Pavlova lutheri*.

A general layout of a bivalve hatchery has several areas:

- Algal culture facility

The algal culture area is one of the most important parts of a bivalve hatchery due to the use of algae in all the phases of production; it should provide large quantities of algae when needed. The size of the algal culture area, depends on the volume of algae culture required, and by the cultured method used such as batch, bags or greenhouse (Helm *et al*., 2004).

- Broodstock holding and spawning area

In this area the broodstock is held during the year, it is important to have heated or chilled seawater, and to have the possibility to isolate the tank to control the photoperiod that can affect gonad maturation (Helm *et al*., 2004).

- Larval rearing

The larval culture area dimension depends on the amount of seed produced and the larvae culture method. In some hatcheries, larvae are reared at low densities as 2-3 per ml, in large tanks 40000-50000 L, in other hatcheries larvae are reared in smaller tanks (5000 L volume) with higher densities (Helm *et al*., 2004).

- Juvenile culture area

The larvae after the metamorphosis and until a size of 2 mm in length, are moved and kept in the juvenile culture room (Helm *et al*., 2004).

Another space that can be useful is a dry laboratory, for storage of scientific equipment and chemical preparations to examine cultures.

It is important that the various spaces of the hatchery can be isolated if there is a disease outbreak (Helm *et al*., 2004).

Depending on environmental conditions such as water depth, tidal range, water exchange rates and substrates, three main oyster farming methods are used: off-bottom culture, on-bottom culture and suspended culture (Buestel *et al*., 2009).

The on-bottom culture is the culture of oysters on the sea floor; oyster growth is comparable to the growth of wild oysters (pangeashellfish.com, 2016). In this farming method oyster shells are spread on the sea bed as a substrate for the spat to attach to or spat

are positioned inside cages and trays. Once the oyster reaches market size (80 g), they are harvested by hydraulic dredge or by hand picking during low tides (pangeashellfish.com, 2016). The advantage of this method is that oysters grow with a robust and hearty shell and therefore with a higher commercial value, but on the other side it is easier to loose production due to predators and natural disasters or mortality could occur due to sudden increase of sedimentation rate in the culture area which could cause suffocation (pangeashellfish.com, 2016).

The off-bottom cultures are the most common methods for oyster farming. There are different off-bottom methods used depending on the characteristic of the farm site and farmer preferences. In these farming methods oysters are enclosed and protected in plastic mesh bags set on trestles. The major advantage of this method is that there are less losses compared to the bottom culture, due to predators and weather, but the major disadvantage is increased costs for equipment and labour (for example to clean the gears from fouling) (Buestel *et al.*, 2009; pangeashellfish.com, 2016).

Suspended and floating culture involves attaching oysters onto ropes or deploying them inside hanging baskets, lanterns or inside floating bags. In this last method, the oysters are constantly under the action of waves and the sea current and they never dry due to tides; however, the farmers can still dry them by turning the bags to facilitate cleaning from fouling. There are not many differences between suspended and the off-bottom culture but the former is normally used in deeper waters (Gosling, 2003; Buestel *et al*., 2009).

When farming tools are chosen it is important to consider the deposition of fouling on these. Biofouling is one of the problems that affect world aquaculture production, and consequently high economic costs are held to control biofouling, it is estimated that the cost can reach 5-10 % of production costs (Fitridge *et al.*, 2012).

Biofouling can cause physical damage due to the organism that grows on the shell and affect the aesthetic quality (therefore reducing marketability), mechanical interference when fouling is around the hinge and lip (therefore affecting the feeding rate), competition for food and space. Moreover, the biofouling can reduce water flow, oxygen levels in the farming gear and can increase production costs due to extra maintenance of the equipment (Fitridge *et al.*, 2012).

There are different methods to control biofouling in shellfish aquaculture. One of these is the use of chemical antifoulants but this method can have adverse effects (such as

environmental pollution), and therefore methods, such the air exposure, are preferred (although this treatment is often not the most efficient) (Fitridge *et al.*, 2012).

Oyster in intertidal zones are exposed to air every day during tides, and this helps to control the biofouling, but in areas where oysters are not subject to air exposure, oyster are left to dry cyclically to minimize the adverse effect of biofouling on the production. Pacific oysters have a wide range of temperature tolerance compared to other shellfish species therefore the air-drying procedure is an effective method to control biofouling especially when mechanical cleaning is not possible (due to logistic constraints) and cleaning shells by hand is time consuming (i.e. in large scale oysters' farms). Air drying take several hours, depending on the air temperature, therefore to make this method effective the hours of exposure to air need to be adjusted depending on season, hours of the day and location (Watson *et al*., 2009). In many Sardinian Pacific oysters farms a common practice is to leave the shellfish air-drying overnight once per week (Alessandro Gorla, *personal communication 2019*).

In the Off-bottom category of farming methods there is a "new" type of system, the Ortac units (fig. 1.6) that was developed by jersey-based shellfish farmer Tony Legg. These are a type of baskets made of polypropylene and divided in two halves. They work attached to a trestle, and the particularity is that due to their shape they have a forced up welling flow system, that according to the manufacturers of these tools permits the improvement of growth rates without compromising the shell quality, and thanks to the constant movement under the action of currents, they require less handling due to less fouling deposition (therefore cleaner oyster shells).

**Figure 1.6:** Ortac units in San Teodoro lagoon.

During trials (performed by the company that supply these farming tools) on European native oyster, *Ostrea edulis* (Linnaeus, 1758), this system was able to reduce the production cycle from 3-4 to 2.5 years (Fusionmarine.com, 2017).

The choice of the farming tool is an important step during *C. gigas* production, due to the fact that these can influence oyster growth characteristics, among which the shell shape. Indeed, Pacific oyster are mostly sold alive and shell shape is one important characteristic, used to identify their marketability and commercial value, this must be hard, clean (sediments, debris, epibionts and blister) and with a teardrop shape (thick, deep and wide) (BIM, 1996; Heath and Wilson, 1999; Handley, 2002; Brake *et al*., 2003; Buestel, 2005; Doiron, 2008; Mizuta and Wikfors, 2018).

Since the 80's, it has become common to use triploids Pacific oysters. Triploids oyster brings several benefits to the production cycle: as they have reduced fecundity, with a very small or now possibility to spawn, therefore keeping a good meat quality and marketability all year round. Due to the reduced fecundity, it is also possible to preserve biodiversity from genetic pollution by escaped animals from farms. Triploid oysters have a higher

growth rate and become larger compared to diploid at the same age (Stanley *et al*., 1981; Allen and Dowing, 1991; Guo *et al*., 1996; Kang *et al*., 2013).

Triploidy in oysters was induced for the first time in 1980 in America by chromosome manipulation to produce sterile oysters (Stanley *et al.*, 1981; Allen and Dowing, 1991). Triploidy can be induced by suppressing meiosis I or II using chemical compounds (cytochalasin B (Nell, 2002)) or by crossing tetraploid males with diploid females. This last method is now the most commonly used to produce triploid oysters due to the fact that induction is not done using physical and chemical stress, and that these triploids shows a faster growth compared to triploids produced using chemicals or physicals stress (Beaumont and Fairbrother, 1991; Wang *et al*., 1999; Nell, 2002; Kang *et al*., 2013 ).

### 1.3.4. Growth performances of Pacific oyster

Usually the growth of a bivalve mollusc is measured by the increase in size and weight of the shell because it is easier to measure the whole animal instead of measuring the flesh weight which would result in terminal sampling preventing observations on individual growth. The shell is considered a record of the growth history of the bivalve as the growth line can be used to date back metabolic rates (Gosling, 2003).

It is important to say that in most bivalves, shell and flesh growth are not correlated. For example, in blue mussel, *Mytilus edulis* (Linnaeus, 1758), the shell growth is faster in spring-summer compared to the winter, while the flesh weight can increase or decrease depending on the reproductive cycle (Gosling, 2003).

Growth in Pacific oyster mostly depends on environmental characteristics, and genetic factors that influence growth by influencing different adaptation to different environmental conditions. Water temperature and food are the main factors that influence the bivalve's growth; salinity can be a limiting factor having a negative influence on the growth and mortality of the shellfish (Brown and Hartwick, 1988). Therefore, oyster will grow at different rates in geographical areas with different climatic conditions. Indeed, on the north coast of France and the west coast of Scotland, the Pacific oyster can reach the market size of 80 g in 3-5 years. In Mediterranean lagoons such as San Teodoro Lagoon (Sardinia, Italy) and Thau lagoon in France, the market size can be reached in 1-1 ½ year (Gangnery *et al.,* 2003). One more factor that can affect growth rate is farming density (total biomass per unit area) (Hèral and Deslus-Paoli, 1990).

### 1.3.5. Growth Modelling

Pacific oyster culture is expanding and it is an economically important industry in Europe, therefore many studies on Pacific oysters farming have been conducted and as a part of these many energetic models focused on interaction between bivalves and environment have been developed (Pouvreau *et al.,* 2006).

The majority of the energy budget models were developed for estimating growth, and most of them assume that some of the energy acquired by feeding is immediately utilized for physiological maintenance, and the rest is used for growth and is accumulated as metabolic reserve (Ren and Ross, 2001; Beadman *et al.,* 2002; Pouvreau *et al.,* 2006). Other models are based on the dynamic energy budget (DEB), this theory was proposed from Kooijman (2000). In the DEB models it is assumed that the energy is stored in reserves, and then utilized for the different metabolic process (Beadman *et al.,* 2002).

Most of shellfish energy budget models can simulate growth only in the locations where they were calibrated, therefore without giving the possibility to use them in different locations with different habitats and this would limit application for the decision making process during site selection for new farms and for expansion of existing farms (Dowd, 1997; Hawkins *et al.,* 2013).

Hawkins *et al*. (2013) has validated a shellfish growth model "ShellSIM®" to predict growth in different habitats for two species *Mytilus edulis* and *Crassostrea gigas.* At the moment this growth model has been validated for 14 species (4 species of Mussels, Oysters, Clams and 2 species of Scallops) in different sites, and different locations from Europe to the U.S.A, China, New Zealand, Malaysia and Australia (Shellsim.com, 2011).

The ShellSIM® growth model was developed in Plymouth Marine Laboratory by Dr. A. J. S. Hawkins, and the software is commercially available. This growth model is based on principles of energy balance (net energy balance = Energy ingested – (energy egested + energy excreted + energy expended)) (fig. 1.7) and simulates the relations between shellfish and the environment giving information about potential production outputs (Hawkins *et al.,* 2013). It was developed as a tool to be used by farmers, Scientist and Regulators (Shellsim.com, 2011).

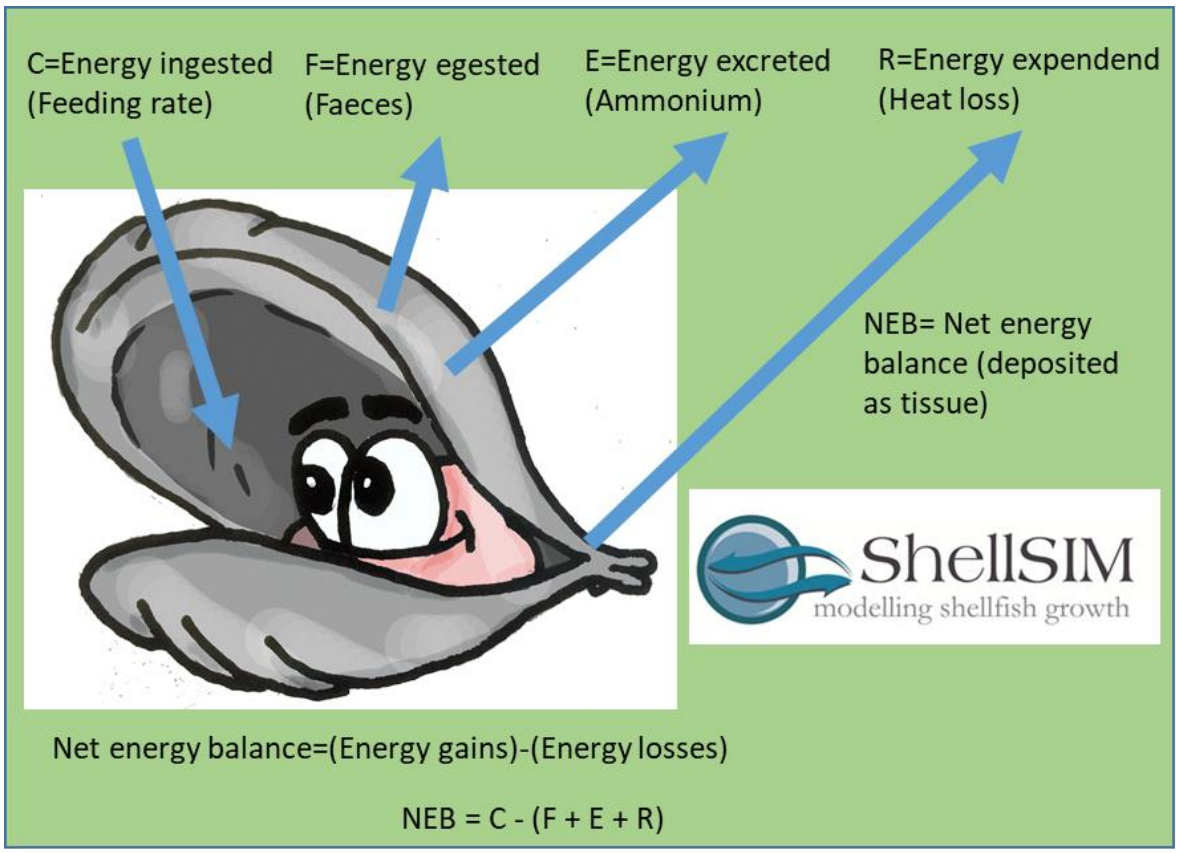


**Figure 1.7:** ShellSIM® is based on principles of energy balance and simulates the relations between the shellfish and the environment (Modified from: Shellsim.com, 2011).

## 1.4. Environmental impacts on oyster Aquaculture

### 1.4.1. Pollution

Coastal areas of many countries are used for aquaculture but at the same time these are subject to other human activities, which are often the cause of: pollution, floral and faunal changes and physical alteration of the environment (Vitousek *et al*., 1997; Epstein, 1998).

Due to human presence and activity sewage discharge are often present in the same coastal area utilised for shellfish farming. These can have different effect on the environment and therefore on the aquaculture of the bivalve's molluscs. Some organic and inorganic materials in sewage can be toxic, and can contribute to an increase amount of nutrients, metals and pathogenic organism. The discharge of sewage can cause eutrophication and algal blooms: these can be harmless but can become harmful when the bloom is so dens to cause anoxic conditions therefore resulting in death of fish and invertebrates (Hallegraeff, 2003). In other cases, algal bloom can be of algal species that produce toxins that if enter in the human food chain, for example through oysters, cause gastrointestinal and neurological illnesses (i.e. Paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP). There are also some algal species that are non-toxic for human but harmful to fish and invertebrates, these can block and create damage to the gills (Hallegraeff, 2003). Moreover, the sewage discharge can cause microbiological pollution due to the presence of faecal coliform and pathogens (for example Salmonella) (Grimes *et al*., 1984; Xuemei and Hawkins, 2002). The shellfish farmed in polluted waters, being filter feeders, can accumulate metals, and some of these as Mercury (Hg), Cadmium (Cd), Lead (Pb) can be toxic at low doses (Stanković *et al.*, 2012), other metals as Iron (Fe), Copper (Cu) and Zinc (Zn), that are essential for life, can become toxic at high concentrations, therefore the accumulation of these in shellfish species farmed for human consumption can become a public health problem (Jovi and Stanković. 2014).

A part of the pollution mention above, sewage discharge of water treatment plants, are believed to be one of the main contributors of microplastic pollution in coastal environments (Cole *et al*., 2011; Duis and Coors, 2016). Microplastics are becoming ever more present in marine environments due to human population increase, therefore a growing pollution deriving from discharge of human activities. Plastics > 5 mm and micro-plastics < 5 mm are part of everyday life. It is possible to find them in many products used

daily such as packaging for food and drinks, shopping bags, pens, toothbrushes and cosmetics (Cole *et al*., 2011; Browne *et al*., 2011).

Microplastics can be dangerous for aquatic organism health due to the fact that the ingestion of these can be a way to transfer pollutants. Indeed, microplastics in a marine environment can accumulate persistent organic pollutants (POPs) as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides as dichlorodiphenyltrichloroethane (DDT) or hexachlorobenzene (HCB) (Mayo *et al*., 2001; Rochman *et al.*, 2013; Smith *et al*., 2018). Moreover, monomer and additives leaching from plastics can have toxic impact both on human and wildlife (Hugo *et al.*, 2008; Oehlmann *et al*., 2009). Brown *et al*. (2013) and Rocheman *et a*l. (2013) reports that the ingestion of plastics lead to the accumulation of Polybrominated diphenyl ethers (PBDEs) in fish and lugworms. Different authors report the presence of microplastics in bivalves (Cauwenberghe and Janssen 2014; Li *et al.*, 2015; Cho *et al*., 2019) and these through the transfer of pollutants can have negative consequences on the immune system in marine bivalves (Renault, 2015), for example was seen that exposition of Pacific oysters haemocytes to mercury caused their mortality after 24h in vitro incubation (Gagnaire *et al*., 2004), Ciacci *et al*. (2011) expose *Mytilus galloprovincialis* to different concentration of hexavalent Chromium, Cr (VI), that is a contaminant in aquatic environments released from both domestic and industrial effluents, their result showed that this contaminant can modulate functional and molecular immune parameters in this shellfish species, even when exposed to non-toxic concentrations. Moreover, Bado-Nilles *et al*. (2008) studied the effect of some PAHs on the immune system of the Pacific oyster, reporting that some of these impacted both cellular mortality and phagocytic activity, suggesting that PHA pollution may be related to a lower resistance to diseases.

Microplastics can have negative consequences in the bivalves normal physiological activities as food uptake and therefore growth and survival of the organisms (Fendall and Sewell, 2009; Browne *et al*., 2011; Cauwenberghe and Janssen, 2014; Sussarellu *et al.*, 2016). Duis and Coors (2016) reported that physical effects of microplastics on marine species had a significant impact when present at high concentration. The negative impacts were mainly attributed to reduction in food intake and the consequent lower energy reserves available for physiological functions. Von Moos *et al*. (2012) studied the effect of exposure and ingestion of microplastic in *Mytilus edulis* (Linnaeus, 1758), reporting that these were present in the gills and in the digestive gland where they were accumulated, and as a consequence there was a strong inflammatory response and a lysosomal membrane

destabilization, therefore concluding that microplastics bioaccumulation can responsible for significant negative effects on tissues' functions.

Van Cauwenberghe and Janessen (2014) investigated the presence of microplastic in *Mytilus edulis* and *Crassostrea gigas*, showing that these were present in the two species observed respectively 0.36 ± 0,07 and 0,47 ± 0,16 particles $g^{-1}$ per soft tissue, and pointing out that the consumption of seafood cultured for human consumption could have potential risk for human health. Cole *et al*. (2015) on the other hand investigated the presence of microplastic and their effect on food intake and growth on Pacific oyster larvae, finding that microplastics were ingested by the oysters' larvae but they found only limited impact on feed intake and no consequence on growth rates.

Sussarellu *et al*. (2016) studied possible influence of microplastics on the physiology of the Pacific oysters, finding that oyster exposed to microplastics showed lower fecundity. They also saw that there was no accumulation in the gut therefore suggesting a wide egestion of microplastics. This may indicate that reproductive pathways are potentially disrupted by the substances leached by the micro plastic during digestion process and not necessarily only linked with their physical presence and accumulation in the digestive gland.

In a recent study, Ward *et al*. (2019) investigated the assumption of using eastern oysters, *Crassostrea virginica*, and blue mussels, *Mytilus edulis*, as bio-indicators for microplastics pollution. They demonstrated that microplastics size and shape can affect the ingestion and egestion of plastic particles in mussels and oysters. In their experiment, they exposed the shellfish to polystyrene microspheres (19-1000 μm) and to nylon microfibers (length 75-1075 μm x diameter 30 μm). The results show that 10 to 30 % of the smallest and 98 % of the larger microspheres were rejected. Despite, the similar proportion of large microfiber and microspheres ingested, there was a lower number of large microfibers rejected (~ 60 %) compared to the large microspheres rejected (98 %) and that both species rejected plastics particles with a diameter over 1000 μm. They report that there was also a different egestion of the microplastics and that the number of microplastics found in the shellfish gut depends on the different physical characteristic of the microplastics particles. The results of the Ward *et al.* (2019) study suggest that bivalves are poor bio-indicators for microplastics pollution.

Nonetheless, Cauwenberghe and Janessen (2014) reported that oyster exposed to microplastics could create a potential risk for human health and that wastewater effluent is a reality in many coastal areas and rivers, it will be important to do further studies on

accumulation and excretion time of microplastics in cultured shellfish especially on the most consumed species as the Pacific oyster.

### 1.4.2. Disease

Despite its wide distribution around the world, Pacific oyster seems to be affected just from a few major disease problems (e.g. Denman Island Disease, Nocardiosis, Herpes-type virus disease, Oyster velar virus disease) (FAO, 2019) but disease problems can result in massive losses therefore being a major issue in oyster production. Paul-Pont *et al*. (2013) reported that disease in mollusc can be caused by different pathogens such as protozoan and metazoan parasites, bacteria, and viruses. These last one and in particular Herpes viruses are of particular interest due to the fact that massive mortalities have recently been attributed to them.

Garnier *et al.* (2007) reported that Pacific oyster is subject to high rates of mortality especially in the summer and this was observed in different counties (e.g. Japan, USA, France). These summer mortalities are mostly attributed to the interaction of pathogens such as Herpesvirus 1 micro-variant (this is a strain of the Herpes virus OsHV-1 responsible of Pacific oyster mortality since 1992), bacteria belonging to the genus *Vibrio* and environmental parameters (Paul-Pont *et al*., 2013a).

As a consequence of the massive mortalities that have occurred over the past years, different studies have been conducted to further understand pathogenicity and mitigation strategies, but the epidemiology and the influence of aquaculture practices on the level of mortalities are still poorly understood (Garcia *et al.,* 2011; Paul-Pont *et al*., 2013; Paul-Pont *et al*., 2013a). Pernet *et al*. (2012) reports that farming practices such as density, depth and equipment have an important role in disease outbreaks and mortalities, therefore it will be important to reduce disease outbreaks with increased biosecurity and modifications to the farming practices.

Different approaches including breeding programs on production of OsHV-1 resistant Pacific oyster have been attempted (Dégremont, 2011, 2013; Paul-Pont *et al.,* 2013; Dégremont *et al.,* 2015; Whittington *et al.,* 2015; Camara *et al.,* 2017). Breeding programs have shown some success contributing to increase the survival during grow-out. Moreover, family selection studies indicate significant genetic variability for herpes virus resistance and that heritability estimates range between 0.2 and 0.4, strongly suggesting rapid genetic

gain through selective breeding programmes (Camara *et al.,* 2017). However, resistant families still show mortalities in a percentage of 5-19 % for juvenile oysters and 86 % for larvae (Dégremont, 2011; Dégremont, 2013; Dégremont *et al*., 2016). A recent study (Pernet *et al*., 2018) reports that to reduce OsHV-1 disease outbreak, it is important to maintain a good ecological status of waters were the shellfish are farmed. In this study, increases in food availability and food nutritional qualities (therefore growth rate and energy reserves) were associated with higher survival rates, while mortality rates increased with increased turbidity, terrestrial inputs and poor food quality (stress factors).

### 1.4.3. Climate change

Nowadays, there is an increasing trend on mass mortalities in cultured bivalves and often these are caused by the synergistic effects of different factors. Amongst these factors, ocean acidification and global warming are accused of increasing the frequency and severity of bivalves' mortality outbreak (Soon and Zheng, 2019).

Since the industrial revolution, emission released into the atmosphere due to human activities (Burning coal, oil and natural gases), has increased the percentage of carbon dioxide ($CO_2$) resulting in the atmosphere and ocean warming (Soon and Zheng, 2019). The Oceans play a fundamental role in the exchange of the carbon dioxide with the atmosphere, it is estimated that over the past 200 years ocean have absorbed the 50 % of the $CO_2$ produced by fossil fuel burning and cement production, and the global annual uptake is estimated to be between 1.4 and 2.5 Pg C $yr^{-1}$(Raven *et al.,* 2005; Bates *et al.,* 2012). Once absorbed, the gas is converted into carbonic acid, and this absorption, result in a chemistry change on the sea surface, more specifically altering the pH balance and making the oceans become more acidic (Caldeira and Wickett 2003, 2005; Feely *et al*., 2008; Le Quéré *et al*., 2009). In addition, the acidification can occur also in coastal waters, where pH is reduced by leaching from acid sulphate soils, humic and tannic acids from ground waters (Duarte *et al*., 2013; Jiang *et al*., 2017; Fitzer *et al*., 2018).

The change in acidity of the seawaters make difficult for marine life to adapt, carbonate ions in the ocean become less abundant in a more acidic ocean, making difficult for shellfish (clams, oysters, mussels) to build their shells. Gazeau *et al.* (2007) and Fitzer *et al*. (2018) reports that calcification rate in bivalves decrease due to effect of ocean and coastal acidification. Nonetheless, Fitzer *et al.* (2019) report that selective breeding for fast

growth and disease resistance had positive effect on the mechanism of biomineralization and therefore, can be used as strategy to produce oyster resistant to the ocean acidification also demonstrating that this favourable trait are highly heritable suggesting the potential for rapid genetic gain in breeding programmes and potential for rapid evolution in wild population.

Due to the fact that bivalve's production makes about 14 % of the total global marine food production (average period 2010-2015) (Wijsman *et al*., 2019), if both ocean and coastal acidification will increase due to climate change, the decrease of calcification in commercial bivalves will probably cause important economic losses (Gazeau *et al.*, 2007;). Moreover, there could be important losses in ecosystem services, because as previously mentioned, the Pacific oysters as other shellfish provide different ecosystem services as: improved water quality, seashore stabilization, carbon sequestration, increasing habitat for fish, invertebrates and epibenthic fauna, diversification of the landscape and ecosystem. The carbon sequestration is an important service to buffer global climate change, and bivalves are involved into the $CO_2$ fluxes trough: respiration (net release of $CO_2$), biocalcification (net sequestration of carbon), food ingestion, rejection of uningested food and egestion of unabsorbed food, these last three process are non-directly involved in the inorganic carbon cycle but are important process for phytoplankton dynamics which are involved in the $CO_2$ cycle (Filgueira *et al.*, 2019). Different studies (Chauvaud *et al*., 2003; Lejart *et al.*, 2012; Mistri and Munari, 2012; Munari *et al.*, 2013; Jiang *et al.*, 2014) report that the balance between sequestration, biocalcification process and respiration are negative therefore suggesting that bivalves are net generators of $CO_2$, except a study by Hily *et al*. (2013) in which it was reported that *Crassostrea gigas* and *Mytilus edulis* can efficiently sequester $CO_2$.

### 1.4.4. Conflicts for sites

Aquaculture produces 46.8 % of the total consumed fish and shellfish in the world, and is one of the fastest growing food production industries (FAO, 2018). The problem with the fast expansion of this industry is the fact that available space for new aquaculture site is becoming increasingly limited, due to competition with other human activities such as tourism (Hall, 2001), offshore renewable energy generation (Douvere and Ehler, 2009) and capture fisheries. Other activities such as agriculture and sewage discharge, although taking place on land, have the potential to further complicate the selection process of

aquaculture sites as their impact will still reach the aquatic environment even if these occur on land (Diaz *et al.*, 2012). Therefore, availability of suitable sites is one of the most significant limiting factors for industry expansion. (Hovik and Stokke 2007; Dempster and Sanchez-Jerez, 2008).

A key factor to increase the expansion of aquaculture industry is therefore the ability to establish which areas are most suitable for the development of this activity. Site selection methodologies can be applied to most human activities, such as choosing site for waste disposal (Şener *et al*., 2010) or choosing the location for schools and hospitals (Bukhari *et al*., 2010). In the aquaculture industry these methodologies have been used to assess the suitability of different locations to support farming activities in all aquatic environments such as: inland aquaculture (Aguilar-Manjarrez and Nath, 1998), marine fish cages (Perez *et al*., 2005) and shellfish farming (Vincenzi *et al*., 2006).

Different methodologies have been developed, for Site selection, to support decision-making. Maps produced using Geographical Information System (GIS) modelling using Multi Criteria Analysis (MCA) are used for site selection based on environmental, socio-economic and logistic criteria. Employing such methodologies support the decision-making process by using either contributing factors, which enhance suitability or constraints, which instead limit the potential use of a given location. These factors are weighted based on importance, and the most used method for this process is the analytical hierarchy process (AHP) described by Saaty (1988) and since used by several studies (Nath *et al*., 2000; Buitrago *et al*., 2005; Radiarta *et al*., 2008; Longdill *et al*., 2008; Silva *et al*., 2011; Micael *et al*., 2015; Falconer *et al.*, 2016).

In the recent study of Theuerkauf *et al*. (2019), they conducted a global spatial analysis using biological factor as nutrient pollution status, socioeconomic factor as governance quality and human health factors as wastewater treatment prevalence, to identify potential areas for the development of shellfish and seaweed aquaculture. They performed this study, due to the fact that these aquaculture industries are growing and because represents an opportunity to provide the ecosystem services, to remediate potential damage to the environment.

Many growth predictions tools have been developed to predict and explain the growth of shellfish according their environment (Pouverau *et al*., 2006). Once growth predictions are transferred from a single model individual to a farmed population, the output of growth

model could be used to study the potential productivity of different sites, therefore significantly contributing to the site selection of farming activities.

### 1.5. Study Area

Sardinia is the second largest island in the Mediterranean Sea (fig. 1.8). This island has a total surface of 24.100 $km^2$ (Sardegnastatistiche.it, 2019), with a population density of 69 inhabitants per $km^2$ (Demo.istat.it, 2019).

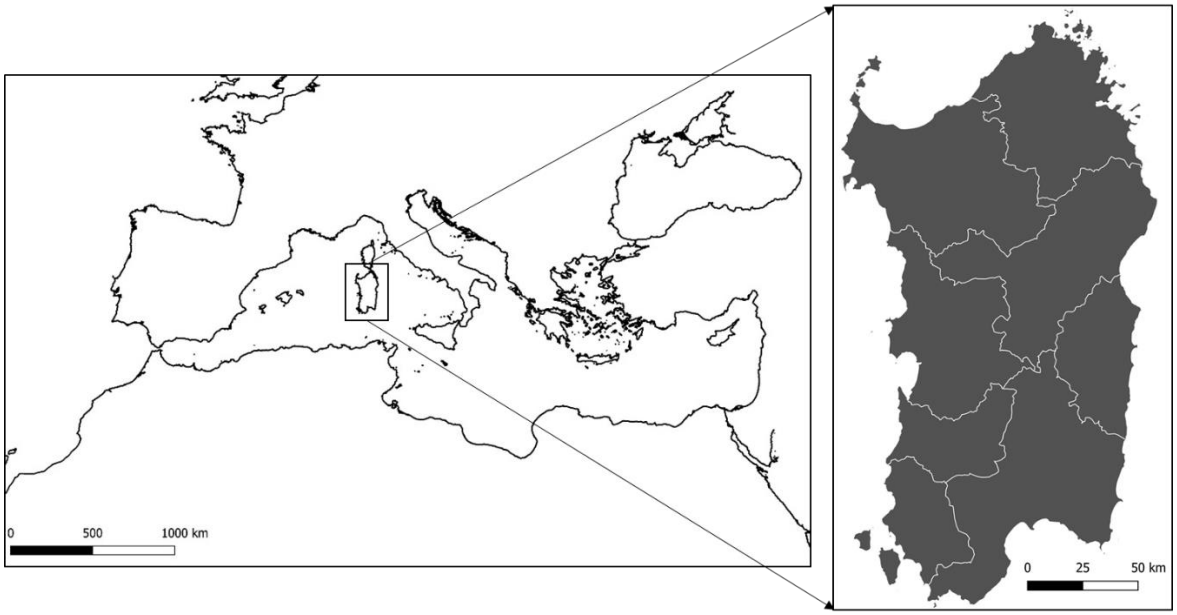


**Figure 1.8:** Sardinia island.

Sardinia is covered by approximately 10,000 ha of coastal lagoons that constitute 2.6 % of Italian lagoons. These lagoons are amongst the largest in Europe (Bazzoni *et al.,* 2013). These environments suffer from a strong presence of human activities that can cause conflicts between maintaining their environmental integrity and economic development needs. However, activities of professional fishing and aquaculture are well integrated with tourist-recreational activities (Sardegnaagricoltura.it, 2019).

These coastal lagoons have for centuries provided employment to local communities and most of them are still utilized for extensive fish farming (Sardegnaagricoltura.it, 2019). Most of these key transitional waters are used for extensive fish farming mainly of mullets (*Mugil cephalous* and *Liza aurata*), sea bass (*Dicentracuhs labrax*) and sea bream (*Sparus aurata*). In some of these lagoons shellfish farms of blue mussel are present and in the last twelve years Pacific oyster farming activities have begun.

In this type of environment, the Pacific oyster growth rate, reported by stakeholders, ranges from 4 to 12-14 months to reach the commercial size of 80 g, therefore making these types of environment possible sites for the expansion of this industry.

Most of the field trials for this PhD study (experimental chapter 2 and 3) were performed in the San Teodoro lagoon, with a small part performed in the Santa Gilla lagoon (the whole trial of the experimental chapter 2 was performed in the San Teodoro Lagoon while the trials of the experimental chapter 3 were performed in both San Teodoro and Santa Gilla lagoons). Moreover, in one of the studies of this thesis (experimental chapter 4), twelve lagoons situated in the East coast of Sardinia were involved in a site selection case study.

### 1.5.1. San Teodoro

San Teodoro Lagoon is situated on the north east coast of Sardinia (fig. 1.9), this is a eutrophic shallow lagoon with a mean depth of 0.7 m, and occupies approximately an area of 22 $km^2$ (Munari and Mistri, 2007).

This lagoon, managed by the municipality, is divided by the traditional fishing system "lavorieri" in two main parts (North and South) (fig. 1.9). To the North east side of the study site there is the mouth of the lagoon which is open to the sea all year round. Located in this area of the lagoon there is a Pacific oyster farm (~ 3 ha).

**Figure 1.9:** San Teodoro Lagoon and the traditional fishing system "lavorieri", that split the lagoon into two main parts (modified from: Google Earth. July, 2019).

The southern part is used mostly for fishing (Sea bream, Sea Bass, Mullets, eels, sole and clams) and for touristic activities. In fact, this part is close to the San Teodoro village, one of the most important tourist centres in the north of Sardinia. Due to the vicinity of this village, the lagoon receives municipal wastewaters and nutrients rich freshwater from the Rio san Teodoro and Rio Filicani (Munari and Mistri, 2007; Sardegnaagricoltura.it, 2019).

Most of the field studies of this PhD (Experimental chapter 2 and part of experimental chapter 3) were performed in the Pacific oyster farm in this lagoon. The farm "Compagnia Ostricola Mediterranea" started their activities in 2007 becoming in a couple of years the most important oyster farming company in the Italian territory, producing up to ~ 60 % of the total Italian production. To date the production of this company is decreasing, due to sanitary reason (declassification of water quality) and due to regulations on the use of space of this lagoon.

### 1.5.2. Santa Gilla

The Santa Gilla lagoon is situated in the south part of Sardinia island (fig. 1.10) and covers a wide area of 15 $km^2$. The lagoon's borders are within four different municipalities- Cagliari, Assemini, Capoterra and Elmas (Frontalini *et al*., 2009).

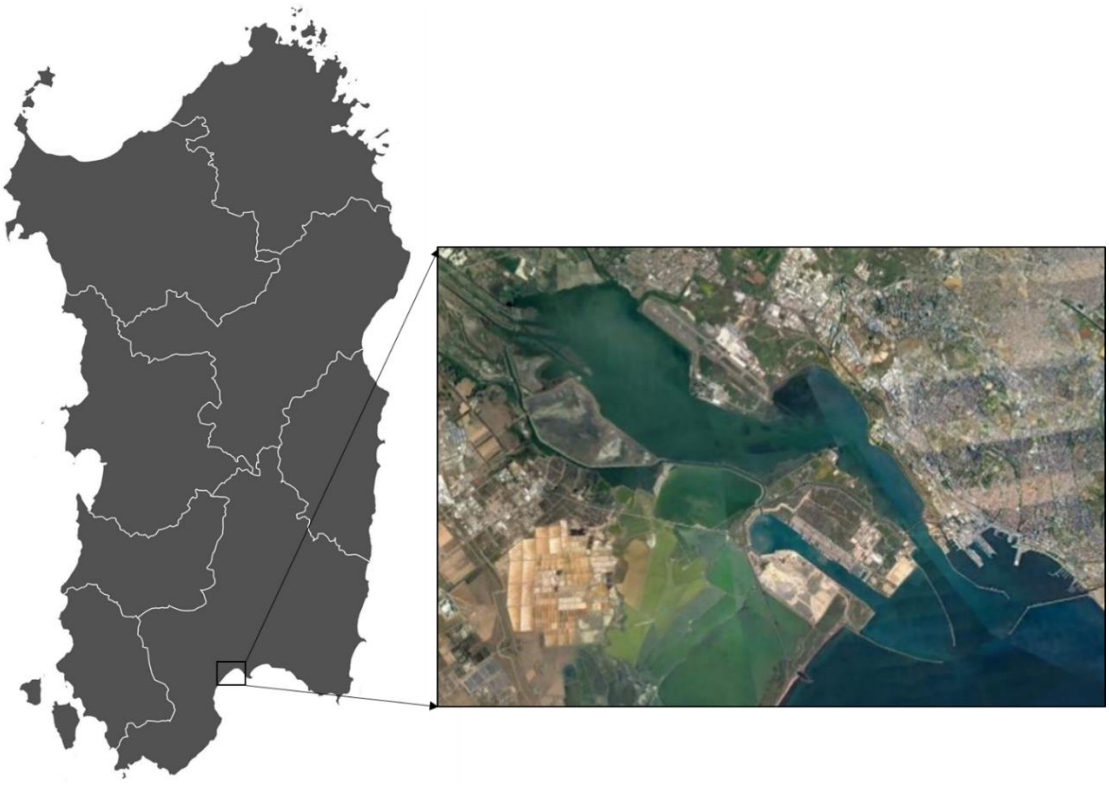

**Figure 1.10:** Santa Gilla lagoon (modified from: Google Earth. July 2019).

The catchment area that feeds the flow of fresh water to the lagoon is very wide, two important rivers the Flumini Mannu and the Rio Cixerri provide the lagoon with large quantities of fine inorganic materials and organic nutrients (apmolentargius.it, 2019).

The main exchange of fresh water and marine water take place in the south part of the lagoon. The mouth has an underwater section of 280 $m^2$ that permits a good water exchange. Smaller mouths are present in the South West part of the lagoon, but these are often subject to occlusion by sand deposits (apmolentargius.it, 2019).

This lagoon is subject to many human activities that were the cause of massive intervention of hydraulic engineering, roads and industrial activities. Among human activities, fishing for centuries has provided employment to local communities, until the 70's when, due to chemical and industrial pollution, fishing and mollusc harvesting was prohibited. In 1994, following environmental restoration, fishing activities started again (apmolentargius.it, 2019).

To date fishing and extensive aquaculture activities are carried out. Moreover, in the main channel that connects the lagoon to the sea mouth, mussel and oyster farming activities are performed by different companies. In this lagoon, on the contrary to other Sardinian lagoons, due to the depth of the channel where these activities are carried out, oyster farming involves the use of lanterns attached to ropes.

In this PhD study the growth model ShellSIM® was tested and the growth performance of Pacific oyster were monitored, in the oyster farm "Lo Squalo", that is situated near the sea mouth of the lagoon.

# 2. Improving Pacific Oyster, *Crassostrea gigas* (Thunberg, 1793) Production in Mediterranean Coastal Lagoons: traditional *vs* novel farming methods


## Abstract

Bivalve farming is a major European aquaculture activity, representing 48.5 % of total biomass produced. Italy is one of the largest consumers of oysters but local production does not meet the market demand. Italy has approximately 384,000 ha of shallow lagoons in its coastal area, already devoted to extensive aquaculture activities, which could also represent potential locations for Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) farming.

The aim of this study is to enhance Pacific oyster farming in shallow coastal lagoons by testing novel farming technologies (Ortac units). Therefore, the commercial performance of Pacific oysters and associated environmental parameters were monitored in a Sardinian coastal lagoon (San Teodoro). Oyster growth and survival were compared during a production cycle for two rearing systems: traditional systems (floating bags) and Ortac units. The latter has not been previously tested in coastal lagoons.

Results showed that at the end of a six months production cycle the oysters mean weight and Condition Index were significantly higher ($p$ value < 0.05) in floating bags than in Ortac (55.8 ± 0.9 g and 50.1 ± 1.3 g; 4.6 ± 0.1 and 3.9 ± 0.1 respectively). Also, the minimum commercial size (40 g) was reached by 98 % and 68 % of the oyster farmed in floating bags and Ortac units respectively, while the oysters reared in the floating bags showed a lower survival than in the Ortac units (82.1 ± 3.4 % and 95.8 ± 0.9 %, respectively).

Results of this study indicate that both floating bags and Ortac system should be employed during the production cycle to maximise oysters' survival and growth performances.

## 2.1. Introduction

Italy is one of the main seafood consumers in Europe and amongst the World's top 10 importers, estimated at 5.6 million US dollars in 2016 (FAO, 2016). Different species of shellfish, crustaceans and fish are farmed using both extensive and intensive methods.

In 2016 shellfish farming was the main aquaculture industry, contributing to over 64 % of the total Italian production. This country is the largest producer of Manila clam, *Venerupis philippinarum* (Adams and Reeve, 1850) and the third producer of Mediterranean mussel, *Mytilus galloprovincialis,* (Lamarck, 1819) in Europe. A smaller production includes grooved carpet shell, *Ruditapes decussatus* (Linnaeus, 1758) and Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) (Eurofish.dk, 2016; FAO, 2016). Pacific oyster is native to Japan and coastal regions of Asia, and due to its wide adaptation range at different environmental conditions, is the most widespread cultured oyster species in the world (Shatkin *et al*., 1997).

In 2016, Europe produced 77,000 tonnes of Pacific oysters, 145 of which were of Italian origin (30 tonnes by a single Sardinian company (FAO 2011-2018, Fishstat.J)). Italy is one of the largest consumers of oysters in Europe importing 6,500 tonnes per year primarily from France; this could represent an opportunity to diversify Italian shellfish farming in the future (Sardegnaagricoltura.it, 2016; FAO, 2016). Sardinia has approximately 10,000 ha of shallow coastal lagoons. This surface represents 2.6 % of the total lagoons area in Italy (Bazzoni *et al.*, 2013). Many of these lagoons are used for extensive finfish farming, but could be potential sites for Pacific oyster farming.

Currently, in the world, three main oyster farming methods are used depending on environmental conditions such as water depth, tidal range, water exchange rates and bottom substrates: off-bottom culture, on-bottom culture and suspended culture (Buestel *et al*., 2009). In Sardinian lagoons suspended culture is the most commonly used method due to the local environmental conditions. More specifically, floating bags are designed to keep the oyster growing at the water surface where most of the food is available. These are manufactured in square and diamond mesh patterns (from 4 to 23 mm), suspended on the surface thanks to two floaters which allow periodic exposure of the oysters to the air to reduce biofouling and strengthen the adductor muscle.

Amongst suspended oyster culture methods, several new farming tools have been recently developed, for example Ortac units (ABBLOX), OysterGro© (OysterGro) and Zapco

Tumbler (Zapco Aquaculture). These systems aim at improving oyster production by reducing manual labour, increasing growth rates and improving oysters' quality (i.e. shell shape). The Ortac system has been employed in this study. The Ortac system consists of baskets made of polypropylene plastic and divided in two halves. These operate attached to a trestle and, due to their shape, an up-welling water flow is passively generated by the surrounding water currents. Furthermore, thanks to the constant movement under currents actions, this system has been designed to reduce fouling therefore requiring less handling.

Aside from environmental conditions, the use of different grow-out gears affects oyster performances as suggested by the recent study from Rankin *et al.* (2018).

To date, only one independent trial has been conducted in Scotland to compare growth, survival and physiological performances of *Ostrea edulis* between Ortac and traditional bag systems (Francouer, 2017). Results of this study indicated that there were no significant differences in growth between *Ostrea edulis* reared in the two different systems (Ortac units and traditional bags) but higher survival was observed within the Ortac units. The study presented here is the first investigation and comparison of the performance of the Ortac system in warmer climates with a smaller tidal range.

The aim of this study is to compare the production efficiency between the traditional and new farming tools (Ortac and floating bags).

## 2.2. Materials and Methods

### 2.2.1. Growth Trial: Ortac *vs* floating bags

In this trial the performances between the floating bags (that are the most used farming equipment in Sardinian shallow lagoons) and the Ortac units was investigated. The reason why this tool was chosen over other farming equipment is because most other off-bottom oyster farming tools had been previously trialled under coastal lagoon conditions by the farmers. Ortac units are a type of baskets made of polypropylene and divided in two halves. They work attached to a trestle, and the particularity is that due to their shape they have a forced up welling flow system that permits the improvement of growth rates without compromising the shell quality, and thanks to the constant movement under the action of currents, they require less handling due to less fouling deposition (therefore cleaner oyster shells). Moreover, due their manufacture in light weight and that can be stored one inside the other storage and transportation is improved compare other Pacific oyster farming equipment as the floating bags (FIS, 2019). There are several farming tools have developed for suspended oyster culture, Ortac unit not having been tested yet, coming from a European manufacturer (therefore available faster and cheaper), and being suitable for use in shallow lagoons seemed to be the best choice for the aims of this study.

This trial was performed between June 2017 and December 2017 in the lagoon of San Teodoro (north-east Sardinia: 40°48’ 38.08’’N, 9°40’26.99’’E). A total of 2,400 triploid Pacific oyster seeds (1.7 ± 0.1 g, 2.9 ± 0.2 cm), from a French hatchery located in the Loire region of France, were randomly divided between 6 Ortac units and 6 Floating bags (200 individual per unit, mean total biomass per unit was 260.7 ± 5.6 g). Thirty oysters from each unit were tagged with an underwater curing epoxy resin (AquaScape) and biometric parameters were measured every two weeks (i.e. weight, length, depth and width) using a portable scale (Steinberg SBS-LW-2000A, 0.01 g) and callipers (METRICA, 0.05 mm). At each sampling point, total biomass and mortality were also recorded and 5 oysters per unit were selected for dry weight measurements (Mo and Neilson, 1994) and Condition Index (CI) calculations using the protocols described by Mo and Neilson (1994) for the dry weight and Davenport and Chen (1987) and Walne and Mann (1975) for CI calculations:

$$\mathrm{CI} = (\text{Dry weight meat (g)}/\text{Dry weight meat (g)} + \text{Dry weight shell (g)}) \times 100$$

To measure the Condition Index (CI), the Pacific oysters were opened and the soft tissues were separated from the shell and placed in different, properly labelled and weighted,

aluminium trays. Finally, the dry weight was measured placing the oysters inside the aluminium trays within an oven (Thermo scientific, HERATERM Oven) at a temperature of 80 °C for 60 hrs (Davenport and Chen, 1987). At the end of the drying period, the weights were then measured using an analytical scale (Gibertini E50S).

The oyster culture systems were positioned in two rows of three Ortac units and three floating bags (fig. 2.1). Ortac units were mounted onto two trestles (3 units per trestle), floating bags were attached to ropes as in the usual commercial setting of the Compagnia Ostricola Mediterranea, host of these trials.

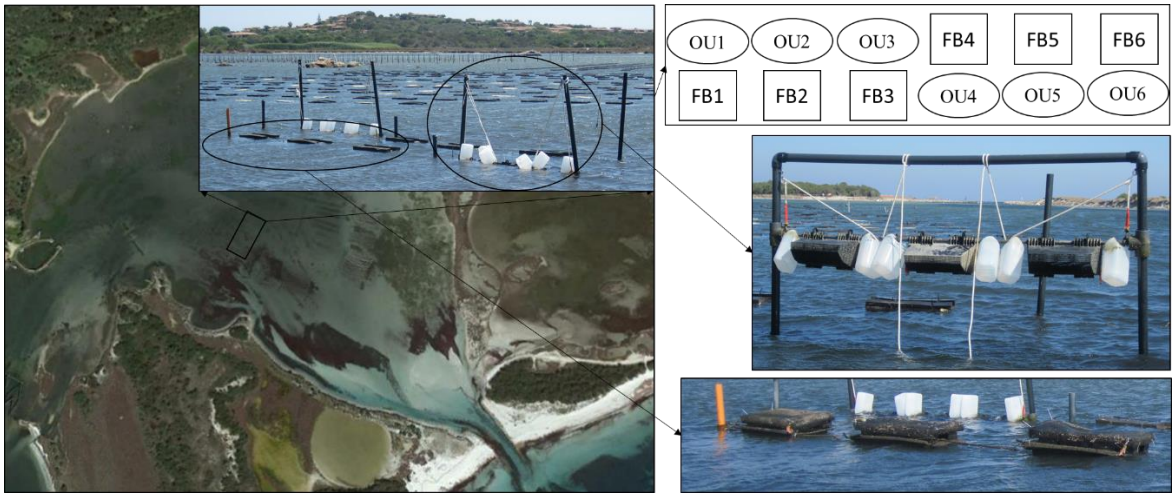


**Figure 2.1:** Position and diagram of the experimental layout of the Ortac units (OU n=6) and Floating Bags (FB n=6) in the San Teodoro Lagoon (modified from: Google Earth. July 2019).

The trestle used for attaching the Ortac units was manufactured on purpose to adapt this farming gear to Mediterranean tides conditions. This was a built with water pipes with a central horizontal tube (where Ortac units were attached) that runs on two lateral vertical tubes. The horizontal tube was provided of buoys in order to keep the Ortac units on the water surface where more nutrients are present (fig 2.1). Moreover, this structure, through the use of pulleys and ropes, permits keeping Ortac units out of the water in order to simulate ocean tides.

Oysters in both systems were cultured following the standard conditions of the company "Compagnia Ostricola Mediterranea" that hosted this trial, with 24 hrs of air exposure every two weeks to prevent biofouling, changing of the floating bags mesh (4, 9, 14 and 19 mm) according to oysters' size, and based on the increasing Pacific oyster's biomass. Grading was performed when the biomass in each farming unit reached about 4 kg live

weight and generally once every three months for both Ortac units and floating bags in order to keep similar biomass in both systems.

### 2.2.2. Statistical Analysis

Prior to analyses, data were tested for normality (Shapiro's test using minitab v.18) and homogeneity of variance (Lavene's test using minitab v. 18). Biometrics measures (weight, shell length, shell depth and shell width) were analysed by General Linear Model (GLM) followed by a Tukey post-hoc test where significant differences occurred. Shell depth and width data were transformed before statistical analysis to improve normality. Survival rate data were arcsine and log transformed before being analysed with a GLM followed by a Tukey post-hoc test where significant differences occurred. Condition index was also arcsine transformed before statistical analysis and was analysed by general linear model followed by a Tukey post-hoc test where significant differences occurred.

End points of all biometrics measures, survival rate and condition index, were analysed by one-way ANOVA followed by post-hoc Tukey's Multiple Comparison tests where significant differences occurred. All statistical analysis, including analysis of variance, data normality and homogeneity were performed using Minitab v.18.

## 2.3. Results

### 2.3.1. Growth Trial: Ortac *vs* floating bags

At the end of the production cycle (October to December), the Pacific oysters farmed in the floating bags had a significantly higher weight and shell depth (*p* value = 0.001; DF = 11, F = 8.89; *p* value = 0.001; DF = 11; F = 5.28 respectively) to those in the Ortac units (55.8 ± 0.9 g, 50.1 ± 1.3 g; 26.6 ± 0.2 mm, 24.2 ± 0.3 mm) (figs. 2.2a, c). Oysters farmed inside the Ortac units showed instead a significant higher growth in shell length (86.9 ± 1 mm, 75.4 ± 0.6 mm, *p* value = 0.001; DF = 11; F = 21.38, Ortac and floating bags respectively), and shell width (46.2 ± 0.5 mm, 44.6 ± 0.4 mm, *p* value = 0.017; DF = 11; F = 15.45) (figs. 2.2b, d).

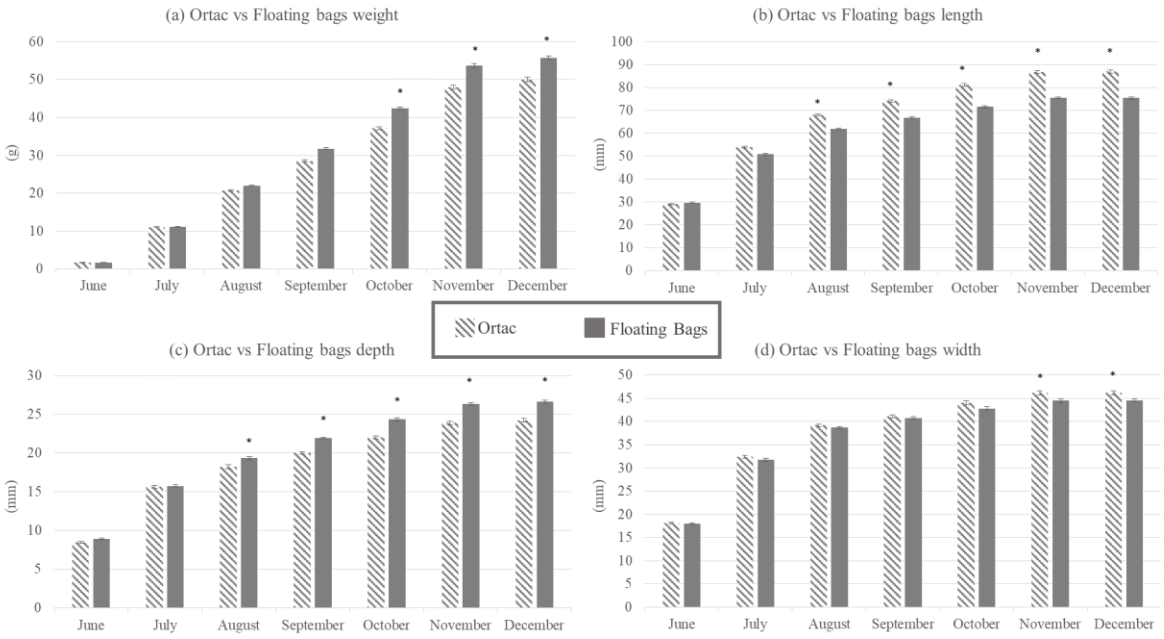


**Figure 2.2: (a)** Difference growth in weight between *C. gigas* farmed in two different tools (Ortac units and floating bags). **(b)** Difference growth in length between *C. gigas* farmed in two different tools (Ortac units and floating bags). **(c)** Difference growth in width between *C. gigas* farmed in two different tools (Ortac units and floating bags). **(d)** Difference growth in depth between *C. gigas* farmed in two different tools (Ortac units and floating bags). Stars indicate where significant difference occurs (*p* value < 0.05). Data are presented as mean ± SE; n = 6.

Survival was significantly higher (*p* value = 0.001; DF = 11; F = 6.50) in the Ortac units compared to the floating bags (95.8 ± 0.9 %, 82.1 ± 3.4 %) (fig. 2.3). The highest mortality occurred between June and July (3.8 ± 1 %, 16.3 ± 3.3 % Ortac units and floating bags respectively).

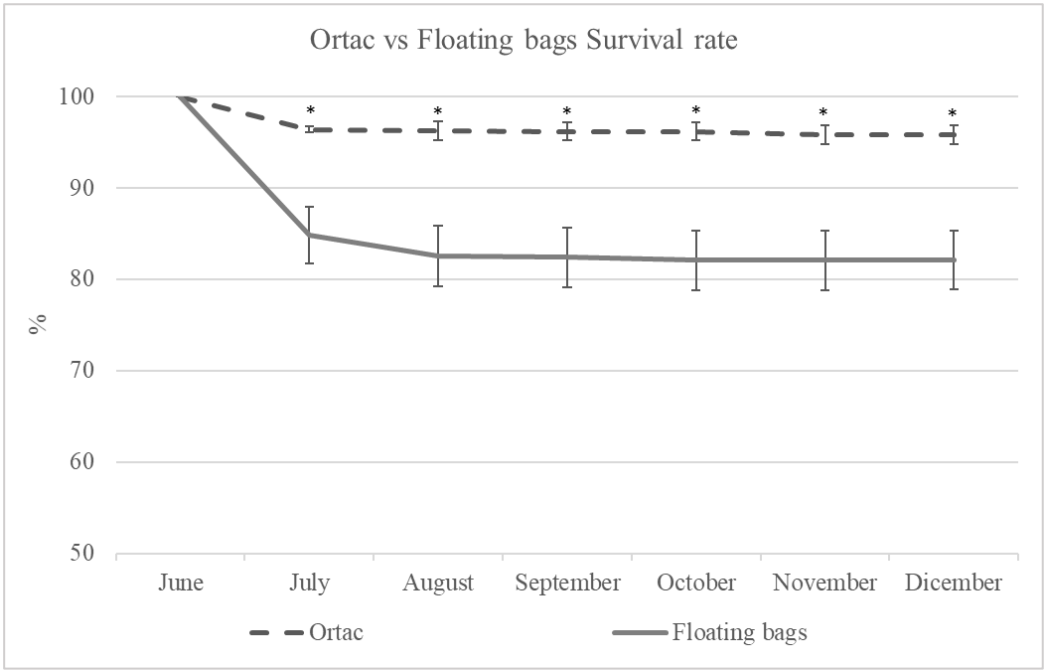


**Figure 2.3:** Comparison of survival rate between *C. gigas* farmed inside the Ortac units and floating bags. Stars indicate where significant difference occurs. (*p* value < 0.05). Data are presented as mean ± SE; n = 6.

The condition index at the end of the production cycle was significantly higher (*p* value = 0.001; DF = 11; F = 4.47) in the floating bags compared to the Ortac units (4.6 ± 0.1, 3.9 ± 0.1) (fig. 2.4) and the smallest commercial size (40 g) was reached by the 98 % and 69 % of the oyster farmed in the floating bags and Ortac units respectively (fig. 2.5).

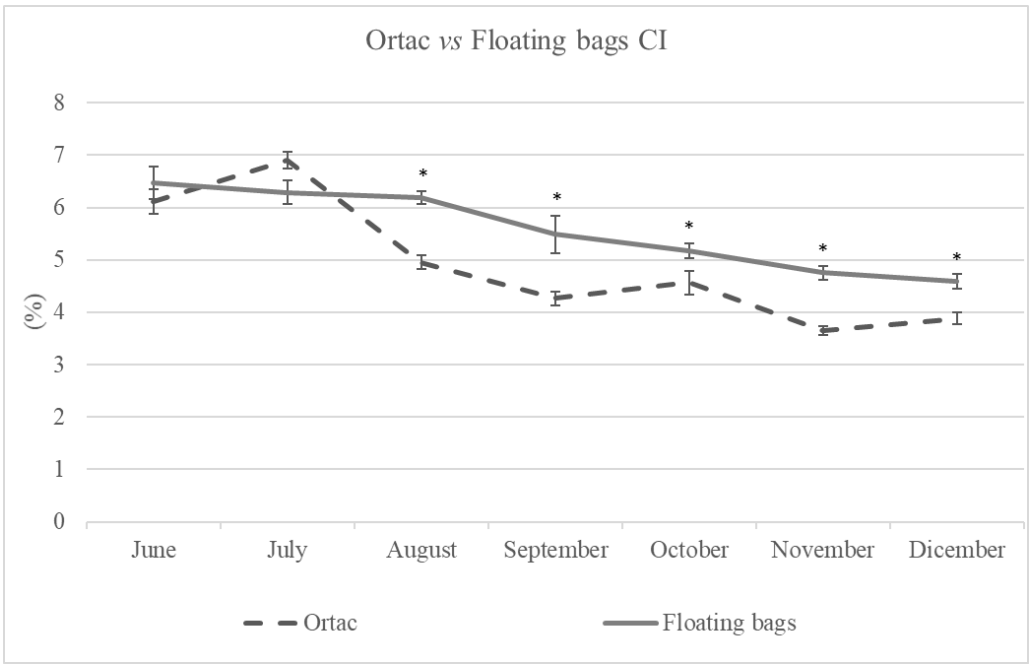


**Figure 2.4:** Comparison of condition index (CI) calculated as (Dry weight Meat (g) / Dry weight Meat + Dry weight shell) *100, between *C. gigas* farmed inside the Ortac units and floating bags. Stars indicate where significant difference occurs (*p* value < 0.05). Data are presented as mean ± SE; n = 6.

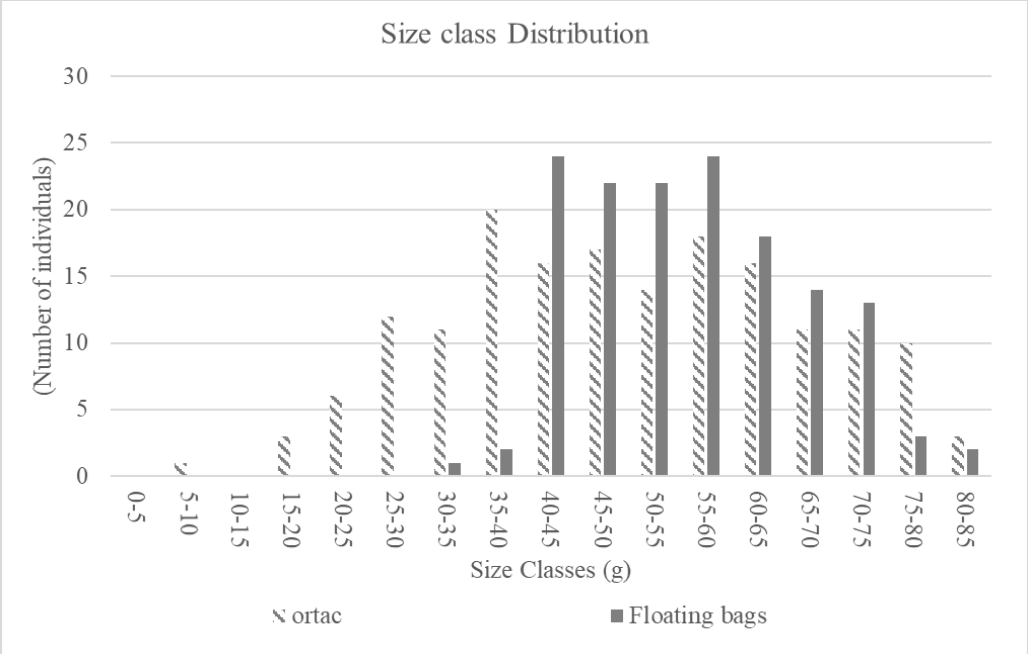


**Figure 2.5:** Comparison of size class distribution between *C. gigas* farmed inside the Ortac units and floating bags.

## 2.4. Discussion and conclusions

The results of this study provide new information to improve *C. gigas* farming in coastal lagoons. The higher survival rate in the Ortac units for the first two months and the higher growth in weight and CI in the floating bags, suggest a potential mixed use of the two systems during the production cycle. Specifically, the Ortac units may be employed when Pacific oysters are more susceptible to stress and during the stressful period (e.g. smaller size and hottest periods) and floating bags thereafter. As a result, by combining the two farming gears within one production cycle, it would be possible to reduce the capital costs of equipment by reducing the need for several meshes sizes in the floating bag system, and achieve a significantly higher survival rate from seed to market size individuals.

There was no statistically significant difference in growth between Ortac units and floating bags, but at the end of the production cycle there was a significant higher mean weight in the floating bags than in the Ortac units. Comparison of these results with previous studies is difficult due to difference in culture techniques, local environment, species used, initial oyster size and the production season.

Many studies report that the shell morphology in bivalves is influenced by population density, predation responses, handling and grow-out methods (Seed, 1968; Griffiths and Buffenstein, 1981; Van Erkom Schurink and Griffiths, 1993; Sheridan *et al.*, 1996; Bayne, 2000; Brake *et al*., 2003; Kube *et al*., 2011; Telesca *et al*., 2018). As in these studies, we observed a difference in shape between the animals reared inside the floating bags or Ortac units, with the latter showing longer and wider shells compared to the former which were instead thicker and with a higher CI.

The morphological differences found between individuals farmed in Ortac and floating bags are probably due to the shape of these different tools, and consequently the different interaction of these with the currents. Under low current speed typical of shallow lagoons, the shape of the Ortac units may have not promoted the rocking motion required to generate enough rubbing between oysters and the farming gear, causing less shell chipping, which is widely recognised as a factor promoting shell depth and a higher meat content (Holliday, 1991; Robert *et al*., 1993; O'Meley, 1995; Brake *et al*., 2003).

Moreover, the fact that the animals did not move enough inside the Ortac units probably induced those in the innermost part to grow more in length and width in order to increase the filtering surface. Mortality may depend on the farming system (Pernet *et al.*, 2012).

Improved survival in the Ortac system could be due to the shading effect provided by a more solid structure, which would shelter farmed individuals from direct sunlight and desiccation, particularly during the earlier part of the growth cycle and during air exposure periods (Spencer-Davies, 1970; Potter and Hill, 1982). Moreover, different studies report that one of the stress factors associated to mortalities is temperature, and sudden small changes may have a large effect on the survival of bivalves (Kennedy and Mihursky, 1971; Le Deuff *et al*., 1994; Le Deuff *et al*., 1996; Sauvage *et al*., 2009; Pernet *et al*., 2012; Petton *et al*., 2015; Pernet *et al*., 2018). Again, the more solid structure of the Ortac, which allows to keep the oysters in the shade, may have promoted more stable temperature and reduced stress.

In conclusion, the results of this study indicate that Ortac units improve the oyster's survival in the production early stage, therefore suggesting that both floating bags and Ortac units should be employed during the production cycle to maximise oyster's survival and growth performances. The use of Ortac units also reduces reliance on multiple mesh bags therefore simplifying production protocols. This study was performed in one area of one lagoon and during one production cycle (therefore, with one initial Pacific oyster size), therefore the results of this study may not apply to other lagoons, especially if these have different environmental characteristics as currents and depths.

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# 3. Validation of the growth model "ShellSIM®" on traditional and novel farming methods


## Abstract

The aim of this study is to enhance Pacific oyster farming in shallow coastal lagoons by validating an existing bioenergetic growth model (ShellSIM®).

Commercial performance of Pacific oysters and associated environmental parameters were monitored in two Sardinian coastal lagoons (San Teodoro and Santa Gilla, Italy) and on novel and traditional farming tools (Ortac units, floating bags and lanterns). Measured performances were compared with ShellSIM® predictions to evaluate the model's ability to predict growth and the potential production in other coastal lagoons.

ShellSIM® growth predictions were highly correlated with the observed data in both lagoons. However, high values for root mean square deviation (RMSD) indicated that ShellSIM® predictions were validated for San Teodoro lagoon but not for Santa Gilla suggesting further tailoring to some environmental conditions to produce more realistic growth predictions.

Results of this study provide a new validated tool to farmers and stakeholders to monitor oysters' performances and estimate productivity in local waters.


## 3.1. Introduction

This experiment has been conducted within the same context as Chapter 2, and focuses on the validation and use of ShellSIM® growth model for Pacific oyster production in two coastal Mediterranean lagoons.

The growth of bivalves is driven by the complex interaction between this species physiology and the environmental conditions in which they grow. These interactions can be simulated by growth models; indeed, these can explain growth and development as functions of environmental conditions (i.e. temperature, salinity, dissolved oxygen food concentration) (Rico-villa *et al*., 2010). Models in aquaculture are developed with the aim of answer question as economic feasibility, site suitability, to investigate the optimal

system design and farming protocols (Leung, 1986). Much effort has been dedicated to generate and validate growth models for bivalves (Pouvrerau *et al.,* 2006) over the past 20 years. Most of the energy budget models predicting growth are net production models, which assume that energy is immediately available for the animal maintenance while the rest is used for growth or deposited as a reserve. In this type of models Bochenek *et al*. (2001) and Hofmann *et al*. (2004) developed biochemically based models to simulate the growth and development of Pacific oyster larvae. Others models are based on a dynamic energy budget approach (DEB) where energy is first stored as a reserve and then used for different metabolic processes at a catabolic rate (Kooijman, 2000; Ren and Ross, 2001; Beadman *et al.,* 2002; Pouvreau *et al.,* 2006). Different DEB model has been developed and successfully used in shellfish aquaculture (Guyondet *et al*., 2010; Sarà *et al*., 2012; Stavraskidis-Zachou *et al*., 2019) but most shellfish energy budget models are only able to simulate growth for locations where they have been calibrated, therefore restricting their use in areas with different environmental conditions (Dowd, 1997; Hawkins *et al.,* 2013). ShellSIM® growth model has been calibrated for 16 shellfish species in different locations throughout Europe, the U.S.A, China, New Zealand, Malaysia and Australia and successfully simulates growth in different coastal and estuarine habitats. This includes *Mytilus edulis* and *Crassostrea gigas* (Shellsim, 2011; Hawkins *et al*, 2013).

ShellSIM® is based on the principles of energy balance:

$$\text{Net energy balance} = \text{Energy ingested} - (\text{Energy egested} + \text{Energy excreted} + \text{Energy expended})$$

This was developed as a tool to be used by farmers, scientist and environmental regulators (Hawkins *et al.,* 2013). Consequently, this growth model was considered to be appropriate to provide growth forecasts in Sardinian coastal lagoons with suitable validation for local conditions.

The aim of this study was to validate this existing bioenergetic growth model in two ecologically different Mediterranean coastal lagoons and for three different oyster farming systems: the Ortac units, the traditional floating bags and the lantern nets.

## 3.2. Materials and Methods

### 3.2.1. Study Design and Overview

The trial on the validation of ShellSIM® was performed in two different lagoons (San Teodoro and Santa Gilla).

In the San Teodoro lagoon, the validation of the growth model was performed during two different production cycles. In the first production cycle the growth model performance was tested in three different areas of the lagoon from the sea mouth to the internal part of the farming area (respectively sampling position 1 (POS1), sampling position 2 (POS2) and sampling position 3 (POS 3)) (fig. 3.1).

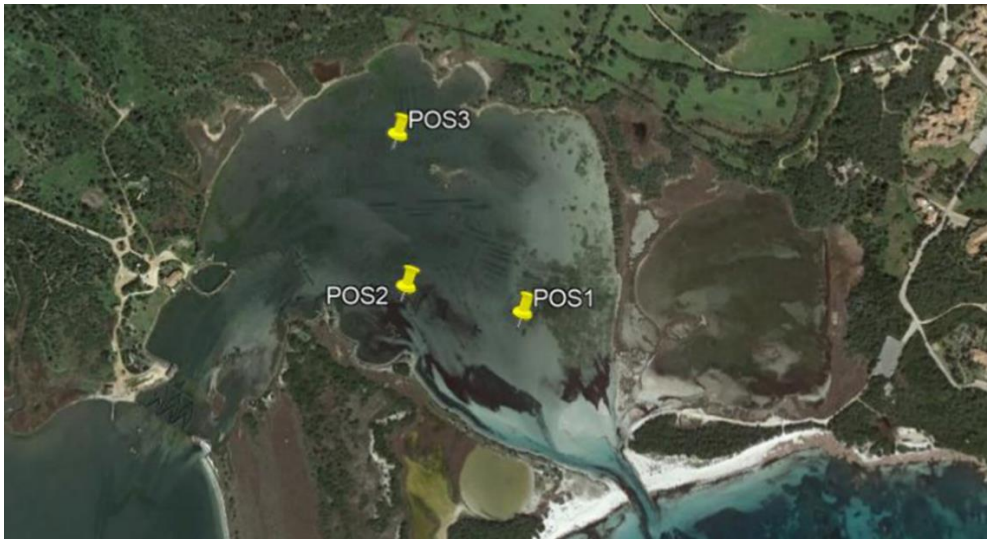



**Figure 3.1:** Different Farming and sampling position in the San Teodoro lagoon. POS1= Sampling point 1, POS2 = Sampling point 2 and POS3 = Sampling point 3 (Modified from: Google Earth, July 2019).

Within each of the selected experimental areas *C. gigas* were farmed following the standard procedure of the local Pacific oyster farm. Oyster biometrics were collected once per month for a total of five months. The biometrics were measured on three floating bags per sampling area, of which 80 individual unit$^{-1}$ were weighted and 30 of which were also measured in length, depth and width. Moreover other 10 individual's unit$^{-1}$ were collected for dry weights measurements.

The environmental data needed to run the growth model: temperature (T, °C), salinity (Sal, ‰), dissolved oxygen (DO, mg L$^{-1}$), total particulate matter (TPM, mg L$^{-1}$), particulate organic matter (POM, mg L$^{-1}$), particulate organic carbon (POC, mg m$^{-3}$) and chlorophyll-a

(Chl-a, µg $L^{-1}$) were collected in the immediate vicinity of the farming gears, at a depth of 10-15 cm once per month, except for temperature, salinity and dissolved oxygen that were collected in continuous using data loggers. Temperature data loggers were set-up to take measurements every 30 minutes, while the Sal and DO probes measured values every 2 hrs.

During the second production cycle the model performance was tested in POS2 (fig. 3.1) on Pacific oysters reared in Ortac units and floating bags. Oyster were farmed and biometrics, were collected as described previously in chapter 2 paragraph 2.2.1. The collections of all environmental parameters were conducted as described for the first production cycle.

In the Santa Gilla ShellSIM® validation trial, the experimental area used was inside the Pacific oyster farm “Lo Squalo”, this is located in the channel that connects the sea to the internal part of the lagoon (fig. 3.2). All the environmental and growth data needed to validate the growth model in this lagoon was collected by the University of Cagliari department of Life Science and Environment staff, using the same protocols used in the San Teodoro.

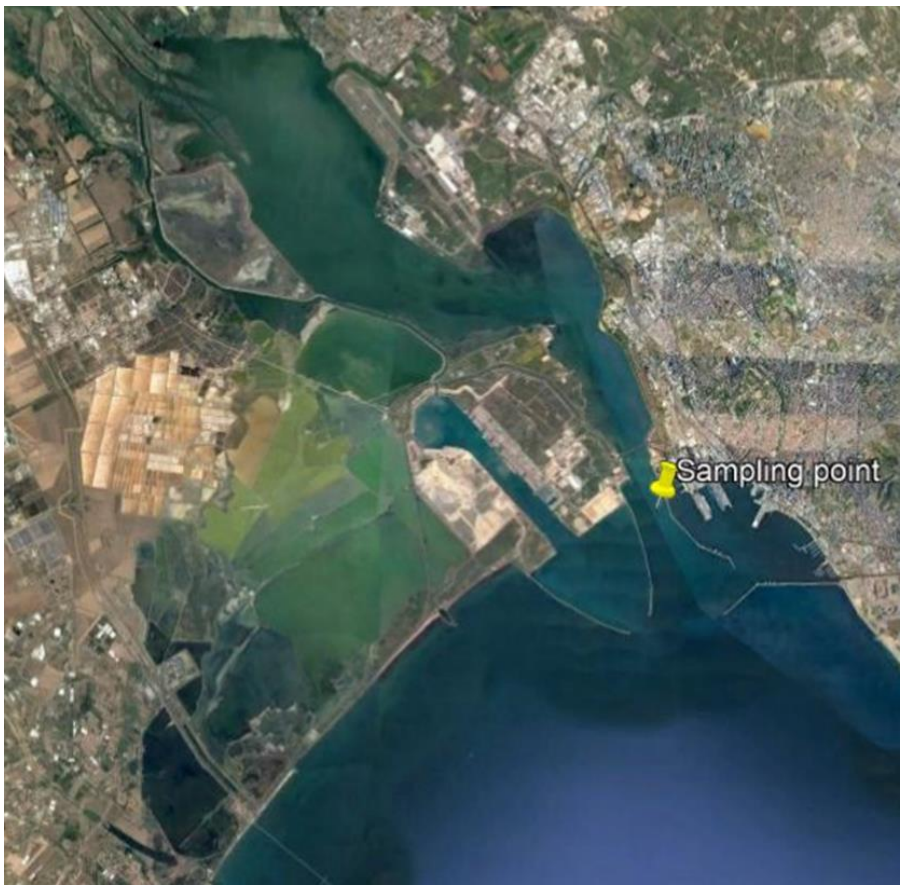



**Figure 3.2:** Sampling area in the Pacific oyster farm "Lo Squalo" located in the Santa Gilla lagoon (Modified from: Google Earth, July 2019).

The growth data needed to validate the model were collected on Pacific oyster reared into lanterns following the local production protocols. Three lanterns of 5 compartments each were stocked with 500 oysters' compartment$^{-1}$. Once per month, 70 individual lantern$^{-1}$ were randomly weighed and 30 of them were measured in length, depth and width. Furthermore, 10 individuals per lantern were collected for dry weight measurements.

Environmental data sampling was conducted as in the San Teodoro lagoon where the data needed to run the growth model: temperature, salinity, dissolved oxygen, total particulate matter, particulate organic matter, particulate organic carbon and chlorophyll-a were collected in the immediate vicinity of the farming gears, at a depth of 10-100 cm once per month, except for temperature, salinity and dissolved oxygen that were collected in continuous using data loggers. Temperature data loggers were set-up to take measurements every 30 minutes, while the Sal and DO probes measured values every 2 hrs.

### 3.2.2. Water quality assessment: Temperature, Salinity and Dissolved Oxygen

In this trials both in the San Teodoro and Santa Gilla lagoon, water parameters of temperature (T, °C), salinity (Sal, ‰), dissolved oxygen (DO, mg $L^{-1}$) were collected both using a multi-parametric probe (HACH HQ40d) and data loggers (HOBO: UTBI-001, U26-001 and U24-002-C respectively for T, DO and Sal).

The data loggers for T were setup to record data every 30 minutes while the Sal and DO, were setup to read values with 2 hrs intervals.

In the San Teodoro lagoon data loggers were kept in the water attach to a floating station (fig. 3.3), in order to read values at a depth of ~15 cm. In the Santa Gilla lagoon data logger were deployed at a depth of ~ 1 m inside a lantern net (fig. 3.4). Once the water quality parameters, measured by the data loggers, were downloaded these were calibrated and processed with the software HOBOware version 3.7.16.

**Figure 3.3:** Floating station with attach, temperature, salinity and dissolved oxygen data loggers.

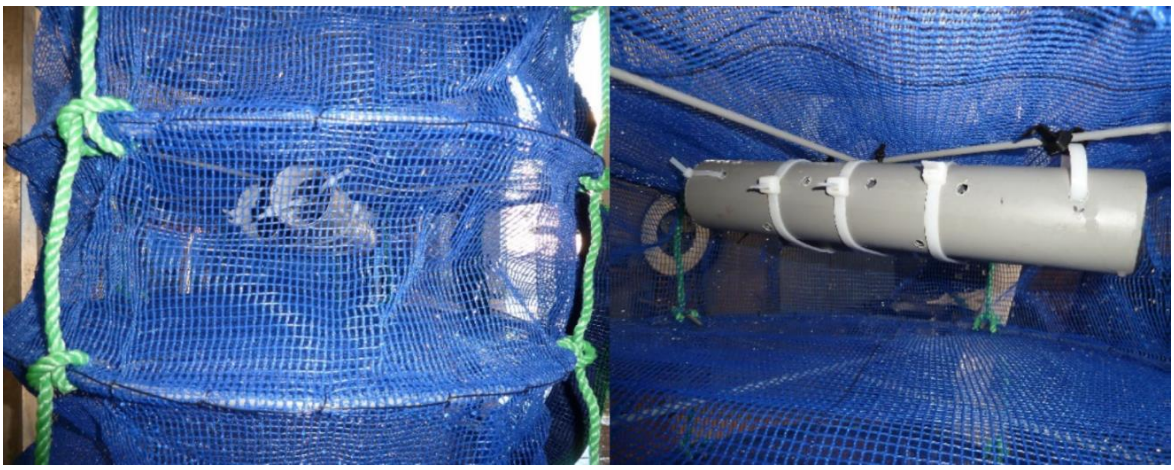


**Figure 3.4:** Data logger in the Santa Gilla lagoon. All of these were inserted in a pvc pipe and attached inside a lantern.

### 3.2.3. Water quality assessment: TPM, POM, POC and Chl-a

The total particulate matter (TPM, mg $L^{-1}$), particulate organic matter (POM mg $L^{-1}$) and particulate organic carbon (POC, mg $m^{-3}$) were collected using 1 L pre-rinsed, in sample water, plastics bottles. While 5 L pre-rinsed, in sample water, plastic bottles were used to collect water for chlorophyll-a (Chl-a, µg $L^{-1}$) analysis. All the samples were collected in triplicate at a depth of ~ 15 cm.

In all of these analysis 47 mm GF/F filter were used. These filters were previously prepared according to the following protocol: Filters were soaked in distilled water for > 1 hrs, and then rinsed for 3-4 times in distilled water and the excess of water was removed with a vacuum pump. Filters were placed into foil and oven dried at 105 °C overnight. After the oven, filters were individually numbered with a soft lead pencil (in the exposed margin) and placed into a foil tray with a lid, then were ashed in muffle furnace (GEFRAN 400) at 450 °C for 4 hrs. After the furnace, filters were placed in a desiccator for 30 minutes; all handling of filters was done using clean (acetone) forceps to avoid contamination. Finally, each filter was weighed on an analytical scale (Gibertini E50S) to the nearest milligram and stored in pre-labelled petri-slides (ICES, 2004; Hawkins *et al.*, 2013).

Laboratory analysis for TPM and POM were performed according to Hawkins *et al.*, (2013). In brief, this analysis was performed filtering the water with a vacuum pump into the pre-prepared 47 mm GF/F filters, these were then rinsed twice with 10 ml of 0.5 M ammonium formate solution, to remove salt, and then rinse with distilled water around the

margin of the filtration cup and removing the excess of water with a vacuum pump. Once the filters were dry, these were placed into labelled aluminium foils and oven dried at 60 °C for 2 days. Once ready, the samples were placed for 30 minutes in a desiccator and then weighted using the analytical scale (Gibertini E50S). TPM (mg $L^{-1}$) was calculated as:

$$((final\ weight\ of\ the\ filter - initial\ weight\ of\ the\ preprepared\ filter) * 1000)/volume\ (L)\ of\ filtered\ seawater$$

To calculate the POM, the TPM filters were ash at 450 °C in muffle furnace (GEFRAN 400) for > 4 hrs, then placed for 30 minutes in a desiccator and weighted with the analytical scale (Gibertini E50S). Particulate inorganic matter (PIM) was calculated as:

$$((final\ weight\ of\ the\ filter - initial\ weight\ of\ the\ preprepared\ filter) * 1000)/volume\ (L)\ of\ filtered\ seawater$$

Finally, the POM was calculated as:

$$TPM - (1.05 * PIM)$$

Where 1.05 is a constant (ICES, 2004; Hawkins *et al.*, 2013).

Chl-a analysis and calculation were performed according to Axler and Owen (1994) and Hawkins *et al.* (2013). The water collected to measure the Chl-a was immediately filtered using a vacuum pump into pre-prepared 47 mm GF/F filters. The filters were then placed individually in a centrifuge tube filled with 10 ml of 90 % acetone. These were then refrigerated (4 °C) for at least 16 hrs and analysis were performed within 24 hrs from filtration.

After extraction in 90 % acetone, the Chl-a analysis were performed using a spectrophotometer (Jasco, V-530). Before starting the wavelength reading, the samples were centrifuged for 5 mins at 4,000 rpm with cooling facility set at 5 °C. Afterwards, the centrifuge tubes were gently inverted several times and the samples were moved into the spectrophotometer cuvette. The first wavelength readings were made at 750 nm and 663 nm and immediately after the same readings were made on the same sample but adding 2 drops (~100 µL) of 1N HCL followed by 30 sec of mixing up (inversion). The Chl-a (µg $L^{-1}$) was calculated as:

$$Chlorophyll\ a = (26.7\ ((663b - 750b) - (663a - 750a))\ x\ Vol.ext)\ /\ (Vol.sample\ x\ L)$$

Where 26.7 is the absorbance correction and is equal to A × K where A is the absorbance coefficient for chlorophyll (11.0) and K is a ratio expressing the correction for acidification (2.43 = 1.7 × 0.7) (APHA *et al.*, 2005). The 663b and 750b were the readings at each wavelength before acidification and 663a and 750a are the readings after acidification. Vol.ext is the volume of 90 % acetone used in the extraction (ml), Vol sample is the volume of water filtered (liters) and L is the spectral path length (cm).

For POC measurements water was filtered using a vacuum pump and pre-weighed, and pre-prepared 47 mm GF/F filters. After filtration, filters were rinsed with distilled water and dried in an oven at 60 °C for 24 hrs. Once dried the filters were kept for 30 min in a desiccator and then weighed with an analytical scale. Before analysis, the filters were cut into 3-4 pieces around the centre, using a cylinder-cutters (6 mm). The resulted cylinders were weighed (with an analytical scale) and placed individually into aluminium cups, these were made into a ball and placed in a clean well-plate making note of the position for each sample and closing each row of the well-plate with parafilm. All the procedures were done without touching the filter and the aluminium cups. The sample were analysed with a CEI Flash smart elemental analyser (SOKI, 2009).

### 3.2.4. ShellSIM

ShellSIM® was originally written in STELLA, then translated in C# and delivered as a compiled dll (required interface). It has now been translated into FORTRAN adopting the framework for Aquatic Biogeochemical Models (FABM).

ShellSIM® allows for the consideration of 4 types of food as drivers: Chl-a, POC, POM and TPM and can work with any combination of these sources to simulate the differential food availabilities. This growth model takes into account different nutritional pools, the SELORG that is the chlorophyll-rich organic matter preferentially selected by oysters and the REMORG all the remaining organic matter (Hawkins *et al.*, 2013).

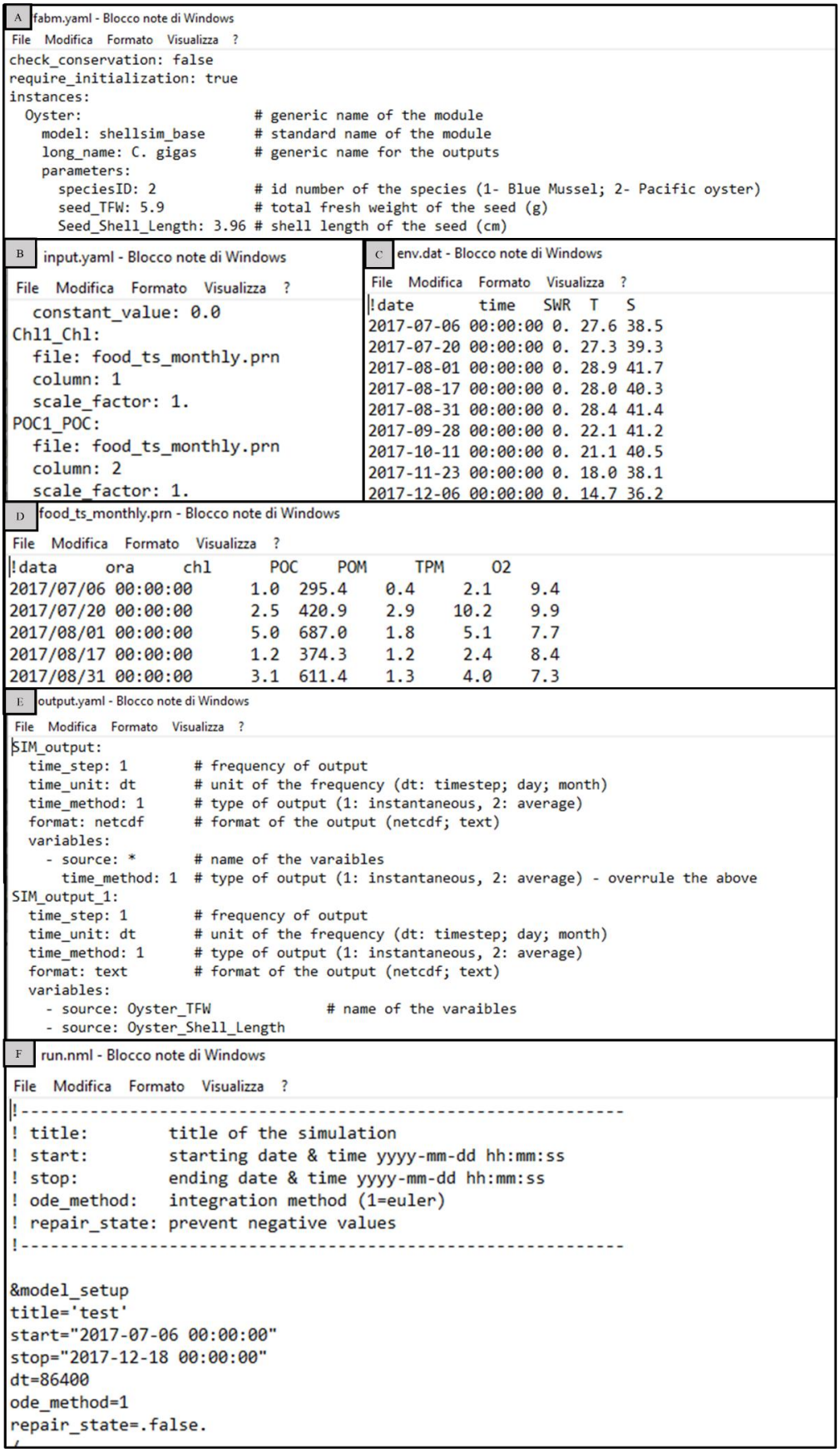
In this study to run the ShellSIM® growth model the environmental data of temperature (T, °C), salinity (Sal, ‰), dissolved oxygen (DO, mg $L^{-1}$), total particulate matter (TPM, mg $L^{-1}$), particulate organic matter (POM, mg $L^{-1}$), particulate organic carbon (POC, mg $m^{-3}$) and chlorophyll-a (Chl-a, µg $L^{-1}$) were collected in the immediate vicinity of the farming gears. These data were fed to the model to obtain the Pacific oyster growth prediction in weight and length.

The main folder that contains the ShellSIM® software is composed of the executable file.exe and the general configuration and data files. Moreover, there are two different ways to visualise the model outputs, a text file and a graphical file.

The first step using the ShellSIM® growth model was to setup the starting points and outputs to be visualized (fig. 3.5):

- Species: Blue Mussel or Pacific oyster (The version of ShellSIM® to which we had access only includes these two shellfish species)
- Initial seed weight and length
- Type of ploidy
- Number of food items
- Frequency at which outputs are required (as day or month)
- Format of the output (NetCDF and txt)
- Variables to be visualized in the output file (for example total fresh weight and shell length)

After this setting up process, the environmental data and the food values for each source was typed into the different data files (fig. 3.5). Then the model was ready to be run. The outputs different visualisations are shown in figure 3.6.



A fabm.yaml - Blocco note di Windows

File Modifica Formato Visualizza ?

```
check_conservation: false
require_initialization: true
instances:
  Oyster:                    # generic name of the module
    model: shellsim_base     # standard name of the module
    long_name: C. gigas      # generic name for the outputs
    parameters:
      speciesID: 2           # id number of the species (1- Blue Mussel; 2- Pacific oyster)
      seed_TFW: 5.9          # total fresh weight of the seed (g)
      Seed_Shell_Length: 3.96 # shell length of the seed (cm)
```

B input.yaml - Blocco note di Windows

File Modifica Formato Visualizza ?

```
  constant_value: 0.0
Chl1_Chl:
  file: food_ts_monthly.prn
  column: 1
  scale_factor: 1.
POC1_POC:
  file: food_ts_monthly.prn
  column: 2
  scale_factor: 1.
```

C env.dat - Blocco note di Windows

File Modifica Formato Visualizza ?

```
!date       time   SWR  T   S
2017-07-06 00:00:00 0. 27.6 38.5
2017-07-20 00:00:00 0. 27.3 39.3
2017-08-01 00:00:00 0. 28.9 41.7
2017-08-17 00:00:00 0. 28.0 40.3
2017-08-31 00:00:00 0. 28.4 41.4
2017-09-28 00:00:00 0. 22.1 41.2
2017-10-11 00:00:00 0. 21.1 40.5
2017-11-23 00:00:00 0. 18.0 38.1
2017-12-06 00:00:00 0. 14.7 36.2
```

D food_ts_monthly.prn - Blocco note di Windows

File Modifica Formato Visualizza ?

```
!data      ora      chl       POC     POM      TPM      O2
2017/07/06 00:00:00      1.0  295.4    0.4      2.1     9.4
2017/07/20 00:00:00      2.5  420.9    2.9     10.2     9.9
2017/08/01 00:00:00      5.0  687.0    1.8      5.1     7.7
2017/08/17 00:00:00      1.2  374.3    1.2      2.4     8.4
2017/08/31 00:00:00      3.1  611.4    1.3      4.0     7.3
```

E output.yaml - Blocco note di Windows

File Modifica Formato Visualizza ?

```
SIM_output:
  time_step: 1        # frequency of output
  time_unit: dt       # unit of the frequency (dt: timestep; day; month)
  time_method: 1      # type of output (1: instantaneous, 2: average)
  format: netcdf      # format of the output (netcdf; text)
  variables:
    - source: *       # name of the varaibles
      time_method: 1  # type of output (1: instantaneous, 2: average) - overrule the above
SIM_output_1:
  time_step: 1        # frequency of output
  time_unit: dt       # unit of the frequency (dt: timestep; day; month)
  time_method: 1      # type of output (1: instantaneous, 2: average)
  format: text        # format of the output (netcdf; text)
  variables:
    - source: Oyster_TFW             # name of the varaibles
    - source: Oyster_Shell_Length
```

F run.nml - Blocco note di Windows

File Modifica Formato Visualizza ?

```
!-----------------------------------------------------------------
! title:        title of the simulation
! start:        starting date & time yyyy-mm-dd hh:mm:ss
! stop:         ending date & time yyyy-mm-dd hh:mm:ss
! ode_method:   integration method (1=euler)
! repair_state: prevent negative values
!-----------------------------------------------------------------

&model_setup
title='test'
start="2017-07-06 00:00:00"
stop="2017-12-18 00:00:00"
dt=86400
ode_method=1
repair_state=.false.
/
```

**Figure 3.5:** Example of different compiled files to run the ShellSIM® growth model. **A,** General starting information; **B,** Input to be considered, for example food sources; **C,** Values of water temperature and salinity; **D,** Dissolved oxygen and different food source values; **E,** Outputs of the model; **F,** Run file.

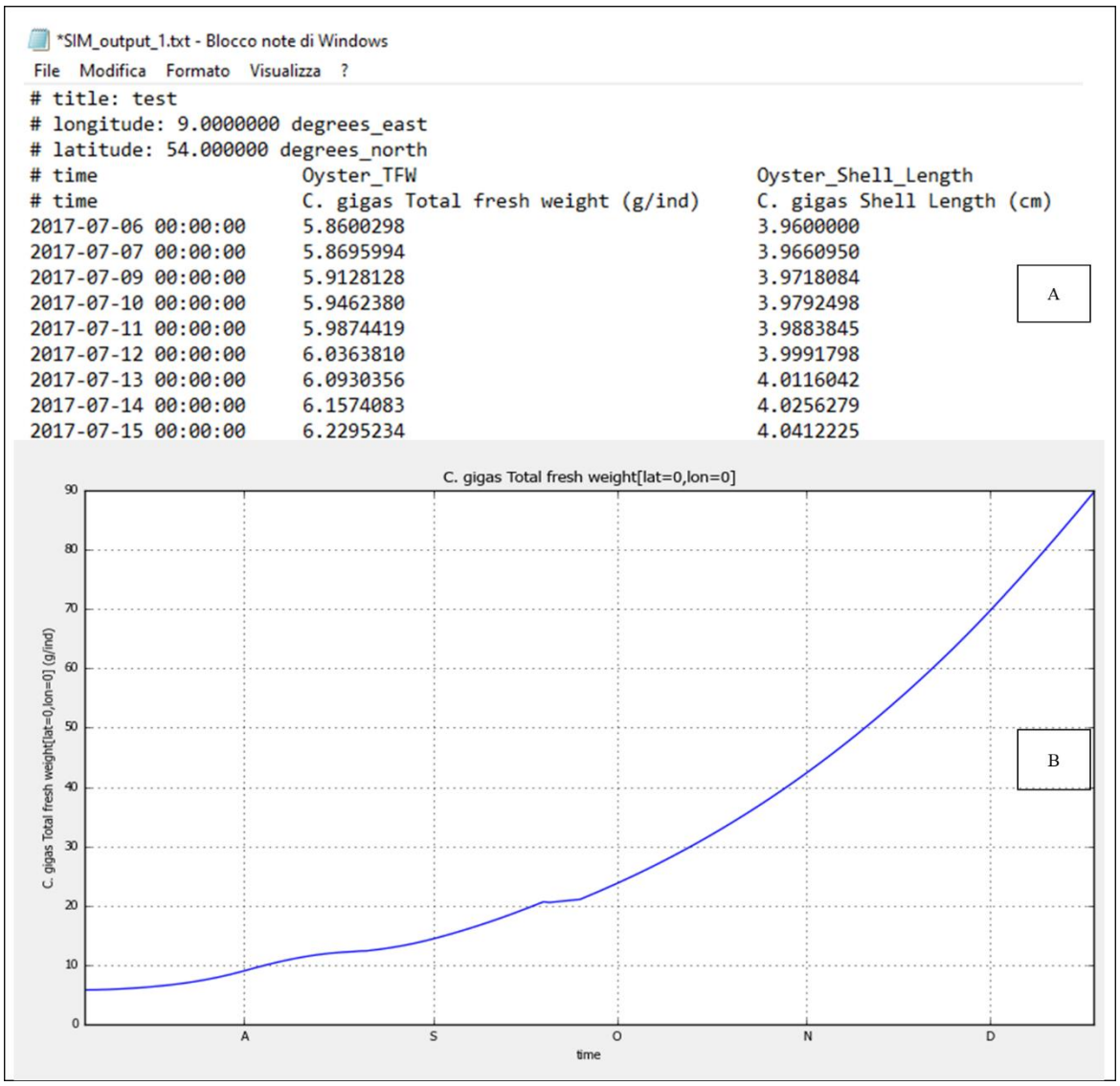



**Figure 3.6:** Different visualisation of the ShellSIM® outputs: **A,** Text visualisation; **B,** Graphical visualisation.

### 3.2.5. ShellSIM validation for floating bag units (San Teodoro Lagoon)

A survey of the dominant currents in the area was conducted during the neap (minimum) and spring (maximum) tides using drifters (drogues) (Cromey and Black, 2005). These preliminary investigations were conducted in order to understand the direction and speed of water movements within the lagoon. In both the spring and neap tides and during the high and low tide peaks three drifting buoys were deployed one hour before the tides' peaks until one hour after, simultaneously in 3 different zone of the Pacific oyster farming area in the San Teodoro lagoon (fig. 3.7). The buoys were followed by operators, who recorded the geographical coordinates on GPS every 20 minutes. During the survey the wind direction and speed was recorded by a fixed weather station (La Crosse WS3650). These data were

used to identify sectors of the "Compagnia Ostricola Mediterranea" farming area which had different currents speed, in order to be used as experimental position, and therefore testing the accuracy of the growth model under different hydrodynamic conditions.


**Figure 3.7:** Deployment area of the three drifters. Drifter were deployed simultaneously in the different points (Modified from: Google Earth, July 2019).

Three floating bags per each area were stocked with triploid *C. gigas* (838 ± 36.4 g, 811.5 ± 17.8 g and 709.8 ± 40.1 g total biomass) with a mean size in weight and length of 4.5 ± 0.3 g and 4.0 ± 0.2 cm (experimental position 1, POS 1), 4.5 ± 0.3 g and 3.9 ± 0.2 cm (experimental position 2, POS 2), 3.9 ± 0.2 g and 3.9 ± 0.2 cm (experimental position 3, POS 3). The oysters were cultured following the standard procedures of the local Pacific oyster farming company as described in the experimental chapter 2.

Sampling for oyster growth was performed monthly for 5 months (August 2016 – December 2016). Each month 80 individual's unit$^{-1}$ were randomly measured for wet weight, 30 of which were also measured for length, depth and width. Other 10 individual's unit$^{-1}$ were collected for dry weights measurements. Biometrics and CI measurements methods are described in the experimental chapter 2.

Environmental data: temperature (T, °C), salinity (Sal, ‰), dissolved oxygen (DO, mg $L^{-1}$), total particulate matter (TPM, mg $L^{-1}$), particulate organic matter (POM, mg $L^{-1}$), particulate organic carbon (POC, mg $m^{-3}$) and chlorophyll-a (Chl-a, µg $L^{-1}$) were collected in the immediate vicinity of the farming gears. The average values of each environmental parameter were used to run the model, excluding September 2017, when no data were collected due to farmers' activities and weather constraints.

### 3.2.6. ShellSIM® validation for lantern systems (Santa Gilla Lagoon)

In order to validate the growth model in a different location, a growth trial was performed between May 2017 and September 2017, in Santa Gilla lagoon (39°12'28.2''N 9°05'53.5''E). Three lanterns with five compartments each and a mesh size of 3.5 x 5 mm, were stocked with 500 triploids oysters per compartment (mean weight = 4.4 ± 0.1 g; mean length = 3.6 ± 0.6 cm). The oysters were farmed following the standard production protocols, grading and changing the mesh size according to oysters' size and biomass. Growth was measured monthly when 70 individuals per lantern were randomly sampled and weighted, 30 of which were also measured for shell length, depth and width. Furthermore, 10 individuals per lantern were collected for dry weight measurements. Environmental data sampling and analysis were conducted as described above. The monthly means of all the environmental data were used to run the growth model.

### 3.2.7. ShellSIM® validation for Ortac units and floating bags (San Teodoro Lagoon)

In order to validate the model for different gear types, a new experiment was set up in the lagoon of San Teodoro (July 2017 – December 2017) where the model performance was also tested on a different farming system, the Ortac units.

Farming methods and growth measurements, were conducted as described in experimental chapter 2 paragraph 2.2.1, while sample collection and analysis of all environmental parameters were conducted as described previously in this experimental Chapter. A bi-weekly mean of all the environmental data were used to run the ShellSIM®, except for November and December, when data were collected only once per month due to farmers' activities and weather constraints.

### 3.2.8. Statistical Analysis

To assess fitness between the prediction made by ShellSIM® and observed data, Taylor diagrams and skill scores (S) were used (Taylor, 2001). A Taylor diagram is a way to show graphically how well a model prediction fits the observed data, using correlation, centred root mean square difference (RMSD) and amplitude of their variation (standard deviations). The skill score proposed by Taylor (2001) quantifies model performance against observed data.

## 3.3. Results

### 3.3.1. ShellSIM® validation in San Teodoro Lagoon

The survey of the dominant currents allowed to estimate the speed and direction of the currents in the Pacific oyster farming area of the lagoon. From the data obtained, it appears that the farming area is most influenced by the currents in the zone bordering the south shore, where there is an artificial canal built with the aim of promoting the sea-lagoon water exchange and *vice versa*. On the contrary, the most internal and confined farming area with respect to the sea (north-western area) was the least affected by incoming and outgoing currents.

During spring high and low tide peak, the higher average speed values (respectively 0.159 m $s^{-1}$ and 0.104 m $s^{-1}$) were found along the channel, present in the farming area, that connects the sea to the inner part of the lagoon, while the lowest values (respectively 0.020 m $s^{-1}$ and 0.022 m $s^{-1}$) were found in the north-western area of the Pacific oysters farm.

During the high tide with new moon, the points showing the highest average speed values (0.068 m $s^{-1}$) were found along the channel, in the farming area, that connects the sea to the inner part of the lagoon, while the lower values (0.032 m $s^{-1}$) were found in the north-western area of the Pacific oysters farm. Even during low tide with new moon the highest average speed values were near the channel in the farming area (0.164 m $s^{-1}$), while the lowest values (0.063 m $s^{-1}$) have been calculated on the buoys deployed in the north-western area of the breeding area, i.e. the most confined area.

Three areas (POS1, POS2 and POS3 (fig. 3.1)) with different current speed were identified in San Teodoro. A decreasing speed gradient from the sea mouth to the internal part of the lagoon was identified. Consequently, these areas were used as experimental locations to monitor the oysters' growth and the environmental parameters required by the growth model.

Environmental data are illustrated in Table 3.1. ShellSIM® predicted, during a 5 months production cycle, a final weight and length of 19.7 g, 48.4 g and 121.6 g; 6.0 cm, 8.3 cm and 11.5 cm, respectively for POS1, POS2 and POS3.

**Table 3.1:** Summary of the environmental data collected to run ShellSIM®. These data were collected during the production cycles started in August 2016, in three different areas (POS1, POS2 and POS3) of the San Teodoro lagoon. Data are presented as mean ± SE.

| | ***T*** ***°C*** | ***Sal*** ***‰*** | ***DO*** ***mg/L*** | ***TPM*** ***mg/L*** | ***POM*** ***mg/L*** | ***POC*** ***mg/m$^3$*** | ***Chl-a*** ***µg/L*** |
|---|---|---|---|---|---|---|---|
| | | | | ***August 2016*** | | | |
| ***POS 1*** | *27.1 ± 0.1* | *39.3 ± 0.1* | *7.2 ± 0.2* | *31.5 ± 12.4* | *5.3 ± 1.9* | *848.2 ± 18.6* | *2.1 ± 0.2* |
| ***POS 2*** | *27.2 ± 0.1* | *39.7 ± 0.1* | *7.5 ± 0.1* | *15.5 ± 1.6* | *3.2 ± 0.3* | *1213.1 ± 67.9* | *4.3 ± 0.7* |
| ***POS 3*** | *27.2 ± 0.2* | *39.5 ± 0.1* | *6.8 ± 0.1* | *19.4 ± 1.1* | *3.6 ± 0.2* | *1421.9 ± 68.5* | *4.1 ± 0.1* |
| | | | | ***October 2016*** | | | |
| ***POS 1*** | *23.5 ± 0.1* | *39.2 ± 0.1* | *8.7 ± 0.1* | *5.2 ± 0.2* | *1 ± 0.1* | *206.9 ± 6* | *0.3 ± 0.1* |
| ***POS 2*** | *24.1 ± 0.1* | *38.8 ± 0.1* | *8.5 ± 0.1* | *5 ± 0.2* | *1 ± 0.1* | *211.3 ± 18.1* | *0.3 ± 0.1* |
| ***POS 3*** | *23.1 ± 0.3* | *38.8 ± 0.1* | *8.9 ± 0.3* | *21.1 ± 2.4* | *3.9 ± 0.2* | *1192.5 ± 55.8* | *3.7 ± 0.3* |
| | | | | ***November 2016*** | | | |
| ***POS 1*** | *17.1 ± 0.1* | *39.2 ± 0.1* | *9.3 ± 0.1* | *0.6 ± 0.03* | *0.5 ± 0.1* | *167.3 ± 9.1* | *0.4 ± 0.02* |
| ***POS 2*** | *15.8 ± 0.5* | *37.7 ± 0.5* | *8.8 ± 0.2* | *2.3 ± 0.1* | *1 ± 0.1* | *485.2 ± 33.9* | *2.8 ± 0.2* |
| ***POS 3*** | *14.5 ± 0.2* | *38.8 ± 0.1* | *9.6 ± 0.1* | *3.0 ± 0.04* | *1.1 ± 0.1* | *473.4 ± 20.4* | *2.9 ± 0.1* |
| | | | | ***December 2016*** | | | |
| ***POS 1*** | *16 ± 0.1* | *36.4 ± 0.2* | *10 ± 0.2* | *1.7 ± 0.1* | *0.6 ± 0.1* | *232.8 ± 21* | *0.7 ± 0.1* |
| ***POS 2*** | *17.5 ± 0.3* | *37 ± 0.3* | *10.7 ± 0.4* | *1.0 ± 0.1* | *0.6 ± 0.1* | *199.1 ± 28.4* | *0.4 ± 0.1* |
| ***POS 3*** | *15.1 ± 0.3* | *36.2 ± 0.2* | *9.2 ± 0.6* | *4.8 ± 0.2* | *1.2 ± 0.1* | *408.4 ± 24* | *0.9 ± 0.03* |

The measured weight and length at the end of this production cycle, was 16.4 ± 1.1 g, 46.9 ± 2.1 g, 48.9 ± 1.5 g and 5.4 ± 0.3 cm, 8.2 ± 0.3 cm, 9 ± 0.2 cm, respectively in POS1, POS2 and POS3.

Figure 3.8 shows that measured growth in weight and length, fitted the predicted growth curve in POS2, while in POS1 and POS3 ShellSIM® overestimate the final mean growth in weight and length respectively 20.5, 12.1, 148.8 and 27.9 %. The calculated skill score for the three different areas indicate the best fitting between observed and predicted measures of weight and length, respectively in POS2 (S=1; S=1), POS1 (S=0.87; S=0.81) and POS3 (S=0.42; S=0.79).

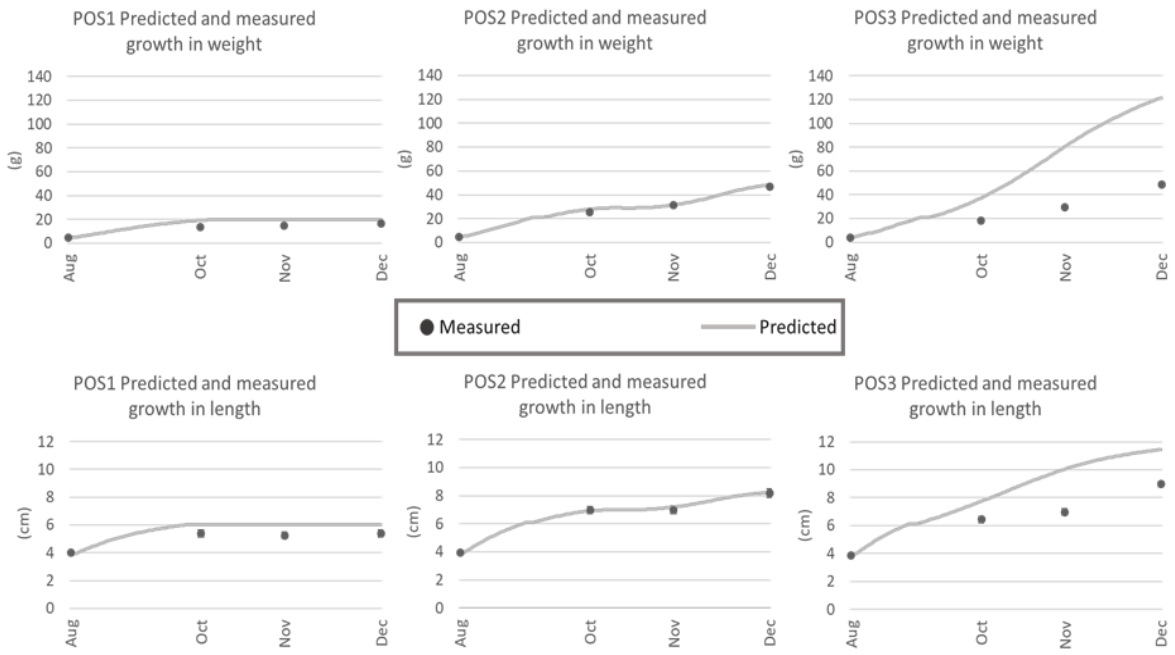



**Figure 3.8:** ShellSIM® growth prediction compared to the measured oyster growth in weight and length, during a production cycle performed in to three different areas (POS1, POS2 and POS3) of the San Teodoro lagoon. Measured growth data are presented as mean ± SE; n=3.

Standard deviation, Centred Root Mean square difference (RMSD), correlation and the overall skill score of the performance of the predicted growth curve to fit the observed data in the lagoon of San Teodoro are shown in Figure 3.9 and Table 3.2.

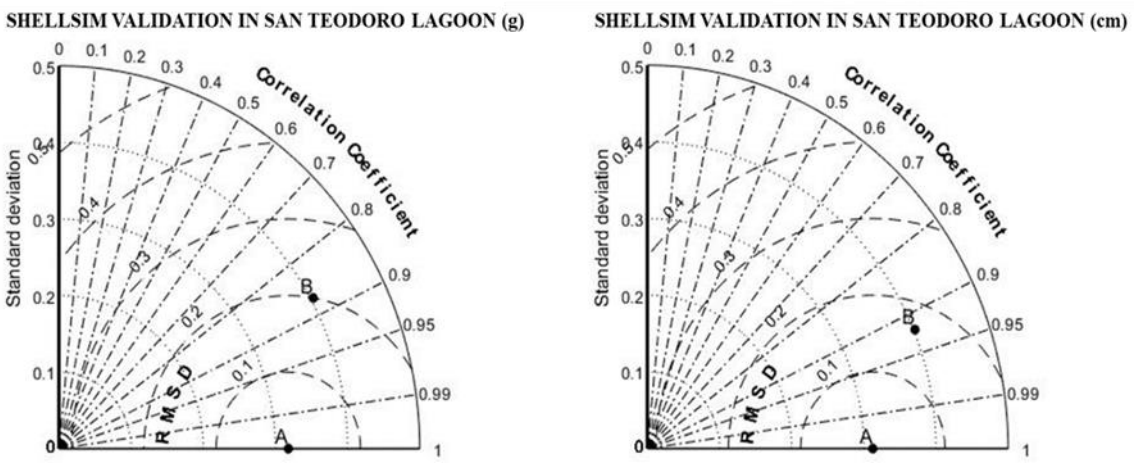



**Figure 3.9:** Taylor diagrams representing how closely model performance (B) match the observed data (A). The similarity between model prediction and observed data is quantified in terms of their correlation, the amplitude of their variation (normalised standard deviation) and their root mean square difference (RMSD) (dashed circular arcs). The left panel contain the results for the ShellSIM® validation in the San Teodoro lagoon in terms of predicting the overall growth in weight of the *C. gigas* farmed inside the floating bags. The right panel contain the results for the ShellSIM® validation in the San Teodoro lagoon in terms of predicting the overall growth in length of the *C. gigas* farmed inside the floating bags.

**Table 3.1:** Summary of how well observed data match predicted data by ShellSIM® in terms of their correlation, their root-mean square difference (RMSD), the ratio of their variances and skill score (Taylor, 2001).

***ShellSIM® Validation in San Teodoro lagoon (floating bags)***

| | ***St.dev Obs.*** | ***St.dev Pred.*** | ***RMSD*** | ***Correlation*** | ***Skill score*** |
|---|---|---|---|---|---|
| ***POS1 (g)*** | *0.3* | *0.43* | *0.14* | *0.98* | *0.87* |
| ***POS1 (cm)*** | *0.27* | *0.43* | *0.16* | *1* | *0.81* |
| ***POS2 (g)*** | *0.35* | *0.36* | *0.02* | *1* | *1* |
| ***POS2 (cm)*** | *0.36* | *0.37* | *0.02* | *1* | *1* |
| ***POS3 (g)*** | *0.14* | *0.38* | *0.24* | *0.99* | *0.42* |
| ***POS3 (cm)*** | *0.24* | *0.38* | *0.16* | *0.97* | *0.79* |
| ***OVERALL (g)*** | *0.32* | *0.4* | *0.2* | *0.87* | *0.83* |
| ***OVERALL (cm)*** | *0.31* | *0.4* | *0.17* | *0.92* | *0.87* |

| | | | | | |
|---|---|---|---|---|---|
| ***ShellSIM® Validation on Ortac and floating bags in San Teodoro lagoon*** | | | | | |
| ***ORTAC (g)*** | *0.32* | *0.30* | *0.1* | *0.95* | *0.95* |
| ***ORTAC (cm)*** | *0.31* | *0.28* | *0.11* | *0.93* | *0.93* |
| ***FLOATING BAGS (g)*** | *0.33* | *0.27* | *0.12* | *0.94* | *0.9* |
| ***FLOATING BAGS (cm)*** | *0.27* | *0.31* | *0.13* | *0.9* | *0.89* |
| ***OVERALL (g)*** | *0.32* | *0.29* | *0.11* | *0.94* | *0.93* |
| ***OVERALL (cm)*** | *0.29* | *0.30* | *0.14* | *0.89* | *0.9* |
| ***ShellSIM® Validation in Santa Gilla lagoon*** | | | | | |
| ***SANTA GILLA (g)*** | *0.01* | *0.4* | *0.39* | *0.97* | *0* |
| ***SANTA GILLA (cm)*** | *0.08* | *0.38* | *0.3* | *0.96* | *0.18* |

### 3.3.2. ShellSIM® validation in Santa Gilla Lagoon

Environmental data collected in the lagoon of Santa Gilla and their seasonal variations are illustrated in Table 3.3.

**Table 3.3:** Summary of the environmental data used to run ShellSIM®. These data were collected during the production cycles started in June 2017, in the Santa Gilla lagoon. Data are presented as mean ± SE.

| | ***T* °C** | ***Sal* ‰** | ***DO* mg/L** | ***TPM* mg/L** | ***POM* mg/L** | ***POC* mg/m³** | ***Chl-a* µg/L** |
|---|---|---|---|---|---|---|---|
| ***June 2017*** | *24.7 ± 0.02* | *37.6 ± 0.03* | *7 ± 0.03* | *2.3 ± 0.2* | *0.9 ± 0.1* | *413.1 ± 8.2* | *0.7 ± 0.1* |
| ***July 2017*** | *24.8 ± 0.02* | *43.3 ± 0.1* | *6.1 ± 0.04* | *4.6 ± 1.1* | *1 ± 0.1* | *349.5 ± 3.3* | *0.7 ± 0.1* |
| ***August 2017*** | *27 ± 0.01* | *35.4 ± 0.03* | *5.2 ± 0.04* | *6.6 ± 0.9* | *1.4 ± 0.1* | *451.4 ± 12.6* | *1.8 ± 0.1* |
| ***September 2017*** | *24.4 ± 0.02* | *36 ± 0.03* | *6 ± 0.03* | *8.1 ± 0.7* | *2.1 ± 0.3* | *561.6 ± 19.9* | *1.9 ± 0.1* |

The measured growth in weight and length (79.5 ± 1.8 g and 9.1 ± 0.1 cm) did not fit the predicted growth curve (fig. 3.10), and the calculated skill score indicates a very poor fit between observed and predicted measures of weight and length, respectively S = 0.003 and S = 0.17 (Table 3.2). Standard deviation, Centred Root Mean square difference (RMSD) and correlation are shown in Figure 3.11 and Table 3.2.

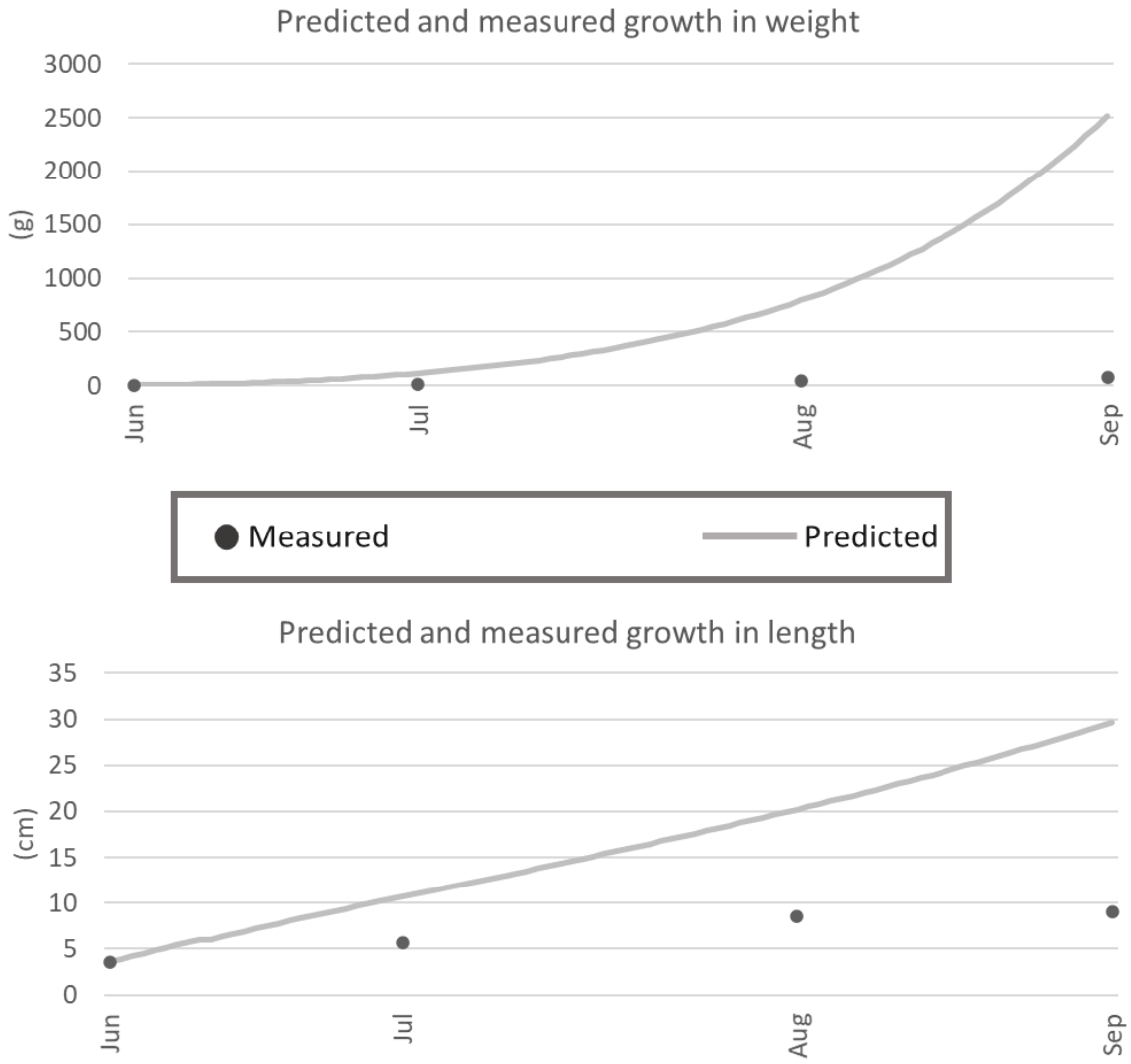



**Figure 3.10:** ShellSIM® growth prediction compared to the measured oyster growth in weight and length, during a production cycle performed in to the Santa Gilla lagoon. Measured growth data are presented as mean ± SE; n=3.

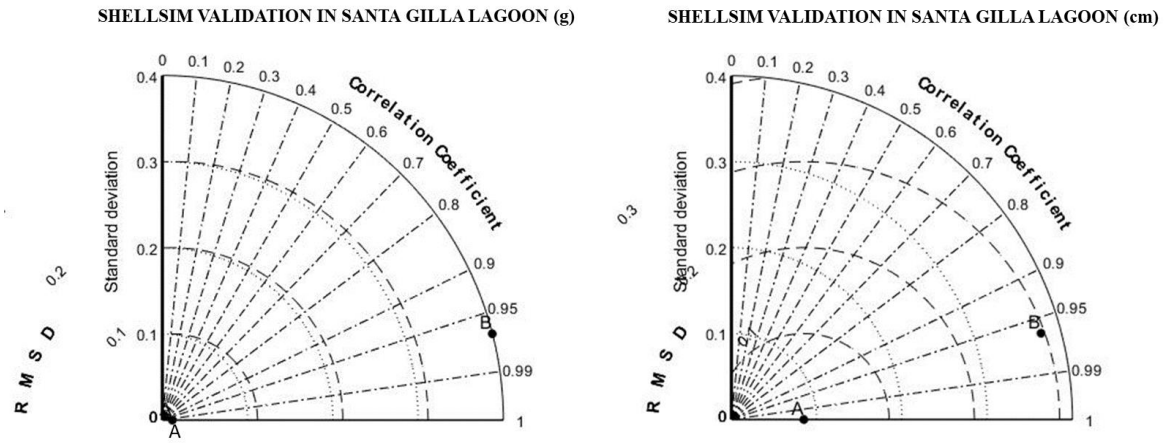



**Figure 3.11:** Taylor diagrams representing how closely model performance (B) match the observed data (A). The similarity between model prediction and observed data is quantified in terms of their correlation, the amplitude of their variation (normalised standard deviation) and their root mean square difference (RMSD) (dashed circular arcs). The left panel contain the results for the ShellSIM® validation in the Santa Gilla lagoon in terms of predicting the growth in weight of the *C. gigas*. The right panel contain the results for the ShellSIM® validation in the Santa Gilla lagoon in terms of predicting the growth in length of the *C. gigas*.

### 3.3.3. ShellSIM® Validation on Ortac *vs* floating bags

Environmental data collected to run ShellSIM® and their changes are shown in Table 3.4.

**Table 3.4:** Summary of the environmental data used to run ShellSIM®. These data were collected during the production cycles started in July 2017, in the San Teodoro lagoon. Data are presented as mean ± SE.

| | ***T*** ***°C*** | ***Sal*** ***‰*** | ***DO*** ***mg/L*** | ***TPM*** ***mg/L*** | ***POM*** ***mg/L*** | ***POC*** ***mg/m³*** | ***Chl-a*** ***µg/L*** |
|---|---|---|---|---|---|---|---|
| ***July 2017*** | *27.5 ± 0.2* | *38.9 ± 0.4* | *9.6 ± 0.3* | *6.1 ± 4.0* | *1.7 ± 1.2* | *358.2 ± 62.7* | *1.7 ± 0.7* |
| ***August 2017*** | *28.4 ± 0.3* | *41.1 ± 0.4* | *7.8 ± 0.3* | *3.9 ± 0.8* | *1.5 ± 0.2* | *557.6 ± 94.2* | *3.1 ± 1.1* |
| ***September 2017*** | *21.5 ± 0.1* | *40.2 ± 1.1* | *9.2 ± 0.1* | *5.4 ± 0.3* | *1.5 ± 0.2* | *491.1 ± 30.1* | *1.7 ± 0.6* |
| ***October 2017*** | *18 ± 2.1* | *40.7 ± 0.1* | *8.3 ± 0.3* | *5.2 ± 0.3* | *1.4 ± 0.1* | *769.2 ± 99* | *2.8 ± 0.6* |
| ***November 2017*** | *18.3 ± 0.3* | *38.4 ± 0.3* | *9.4 ± 0.1* | *23.2 ± 3.3* | *3 ± 0.4* | *151.3 ± 23.9* | *0.5 ± 0.03* |
| ***December 2017*** | *14.7 ± 0.1* | *36.7 ± 0.5* | *10 ± 0.1* | *12.3 ± 9.5* | *1.9 ± 1.3* | *222.4 ± 67.4* | *0.8 ± 0.3* |

In this trial, ShellSIM® was run in POS2 for two different farming systems. It predicted a growth of 48.6 g and 8.3 cm in weight and length respectively for the Ortac system, and a growth of 49.1 g and 8.2 cm for the floating bags over a 6 months production cycle. At the end of this production cycle, the measured weight and length were 50.1 ± 1.3 g and 8.7 ± 0.1 cm for the Ortac and 55.8 ± 0.9 g and 7.5 ± 0.1 cm for the floating bags. Figure 3.12 shows that during the production cycle, the measured mean weight and length in the Ortac units and floating bags were underestimated by ShellSIM®, except for the final length farmed in the floating bags which was accurate. Indeed, in November there was a change in trend of the model prediction, from underestimation to overestimation (the model overestimated the final mean length of 8.2 %), while the final weight was still overestimated by 11.9 %.

Moreover, Figure 3.12 shows that ShellSIM® at the end point of the production cycle of the oysters reared inside the Ortac units, unlike the rest of the predictions, slightly underestimated growth in weight and length by 3 % and 4.4 % respectively.

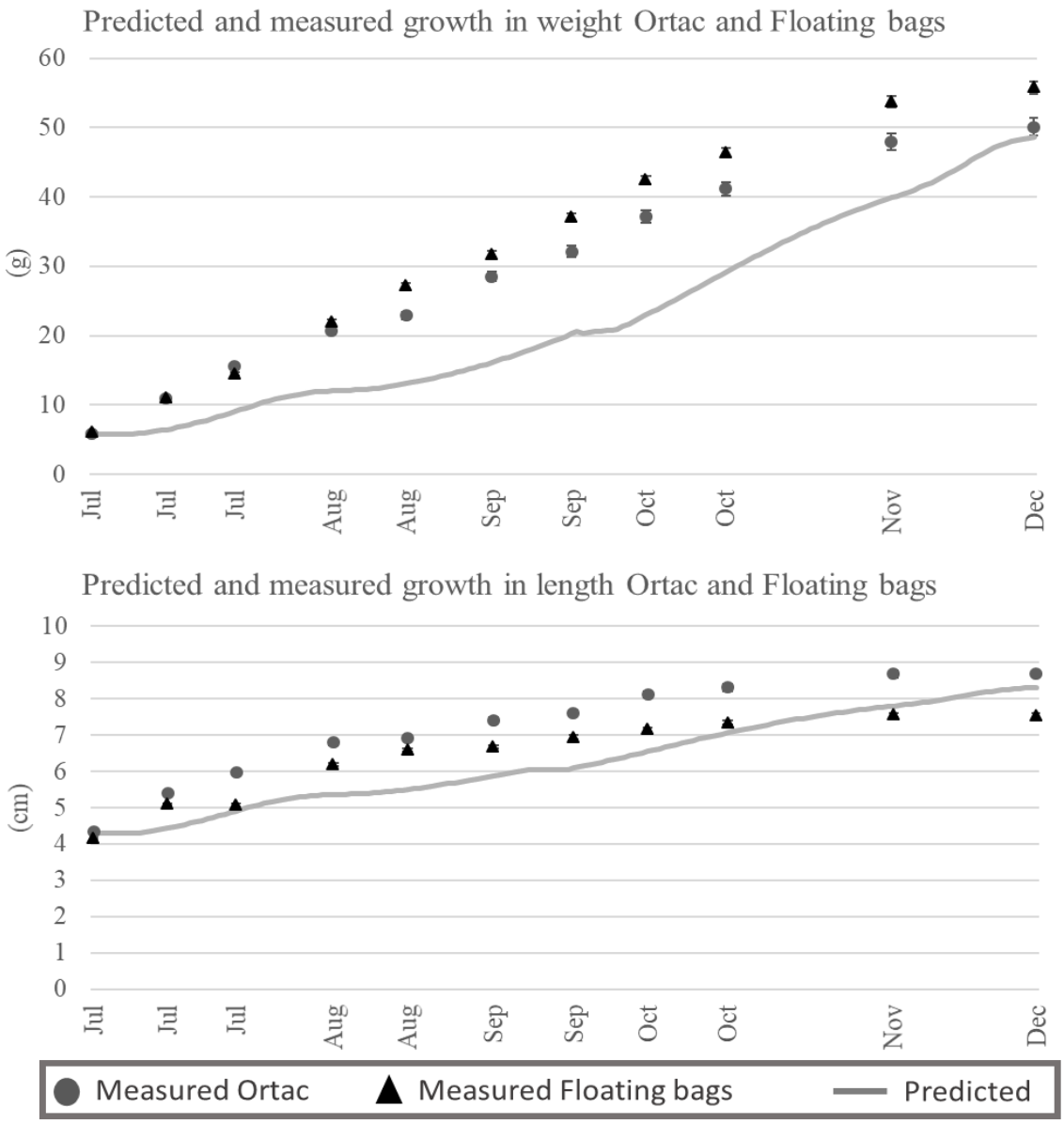



**Figure 3.12:** ShellSIM® growth prediction compared to the measured oyster growth in weight and length, during a production cycle performed in to two different farming systems (Ortac units and floating bags) in the San Teodoro lagoon (July 2017 – December 2017). Measured growth data are presented as mean ± SE; n=6.

The calculated skill score indicates that the best fitting between observed and predicted measures of weight and length was respectively obtained in Ortac (S=0.95, S=0.93) compared to floating bags (S=0.90, S=0.89). Standard deviation, Centred Root Mean square difference (RMSD), correlation and the overall skill score of the performance of the predicted growth curve to fit the observed data in this trial in the San Teodoro lagoon are shown in Figure 3.13 and Table 3.2.

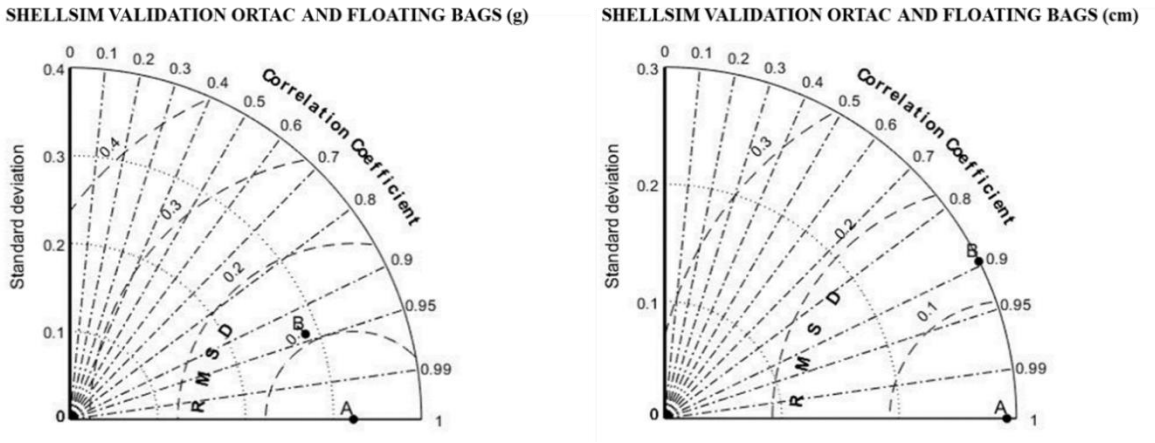



**Figure 3.13*:*** Taylor diagrams representing how closely modelled performances (B) matched the observed data (A). The similarity between model prediction and observed data is quantified in terms of their correlation, the amplitude of their variation (normalised standard deviation) and their root mean square difference (RMSD) (dashed circular arcs). The left panel contain the results for the ShellSIM® validation in the San Teodoro lagoon on Ortac and floating bags in terms of predicting the growth in weight of the *C. gigas*. The right panel contain the results for the ShellSIM® validation in the San Teodoro lagoon on Ortac and floating bags in terms of predicting the growth in length of the *C. gigas*.

### 3.4. Discussion and conclusions

The results of this study provide new information to improve *C. gigas* growth prediction tool in Mediterranean coastal lagoons.

Results of this study indicate that the predicted growth by ShellSIM® fitted well with field measurements in the lagoon of San Teodoro. However, results from the growth trial in Santa Gilla lagoon demonstrate that the model would require further tailoring to local conditions to produce realistic growth projections. In particular, we tested the hypothesis that ShellSIM® assumptions on the conversion of food concentration into available/digestible energy for the oysters, may not apply to Santa Gilla Lagoon. In order to do this, we run the model reducing the amount of POC available to one quarter of the measured POC and the model prediction was more accurate (S = 0.97 and S = 0.95 respectively for weight and length). Indeed, POC can be considered as a very heterogeneous nutrient source composed by different materials with large variations in digestible energy content (Lawacz, 1977; Mazzola and Sarà, 2001; Watanabe and Kuwae, 2015).

Further studies to identify the real digestible energy content of the Particulate Organic Carbon in Santa Gilla area is required to modify the model assumption and improve its performances. Our data also suggest that seasonality and farming system used can influence the accuracy of ShellSIM® providing scope for further tailoring of the model to reflect gear types and local environmental conditions.

During the first-year trial in the lagoon of San Teodoro the measured growth closely fitted the predicted growth in POS2, while in POS1 ShellSIM® slightly overestimated and in POS3 considerably overestimated the growth, both in weight and length. Similar results in POS2 were observed in the second-year validation trial. The growth in weight and length of the oyster was different between the two farming tools, with a higher growth in weight recorded for oysters reared in the floating bags and a higher growth in length for oysters reared in the Ortac units. In this trial, ShellSIM® underestimated the weight and length during the production cycle except at the end point where it only slightly underestimated weight and length in the Ortac units providing a better accuracy at harvest time. While in the floating bags the final mean weight was underestimated and the length was overestimated.

These overestimation and underestimation can be potentially associated with a less than optimal rearing method (the Ortac), combined with the potential different production capacity of each farming areas within the lagoon. Furthermore, as reported by several authors, the grow-out methods employed could affect oyster growth (Sheridan *et al*., 1996; Bayne, 2000). ShellSIM® does not consider different grow-out methods in its variables, possibly generating the discrepancy between observed and predicted growth measured in this study. Overall, ShellSIM® predictions correspond with the growth trends observed by the farmers over the years (POS3 with higher growth rates and POS1 with lower growth rates) suggesting the good accuracy of the model with the general growth dynamics in the different areas of San Teodoro lagoon. This is reflected in the calculated skill scores, for both validation trials in the fore mentioned lagoon.

Taken together, the results of this study provide information to improve bivalve growth prediction tools for Mediterranean lagoons. They could be applied to study the productivity of different sites to potentiate the oyster's aquaculture industry and for coastal spatial planning.

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# 4. A modelling approach to classify the suitability of shallow Mediterranean lagoons for Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) farming.

**Abstract**

In this study, we have developed an approach to classify the suitability of shallow coastal lagoons for Pacific oyster aquaculture as the first step in a site selection process. Historical bio-physical data and local knowledge were combined to produce overall scores for biological and logistical criteria relevant for oyster farming which were then combined using Multi-Criteria Analysis (MCA) for an overall lagoon suitability score. A Dynamic Energy Budget growth model was also used to identify and rank suitability of shallow coastal lagoons to host Pacific oysters farming sites. Furthermore, modelled growth data were used to estimate the production cycle length and the potential productivity of the newly identified sites. The results indicated that biological and logistic factors were suitable for Pacific oyster farming in eleven out of twelve of the lagoons considered. However, acquiring water classification for shellfish farming and maintaining high water quality standards will be critical for any sustainable development of culture areas. Potential production figures and logistic scores, clearly indicates in which lagoons investments should be focused and what output could be realised from these very productive ecosystems. The results can be used to indicate where more detailed assessment should take place. As remote-sensing technologies continue to develop and algorithms for the interpretation of ocean colour in coastal areas keep improving, this multidisciplinary approach will increase our ability to estimate aquaculture production in complex aquatic systems. This approach will provide stakeholders, policy makers and regulators with a new and powerful decision-making tool for site selection of sustainable oyster farming activities and the management of the surrounding coastal areas.

## 4.1. Introduction

Coastal lagoons are shallow, semi-enclosed, aquatic systems that are largely isolated from the open sea due to barriers or land features, with inlets and channels acting as the connection (Newton *et al.,* 2014; Pérez-Ruzafa *et al.,* 2019). These water bodies are

amongst the most productive ecosystems in the world (Pérez-Ruzafa *et al.,* 2019), and have an important role in providing ecosystem services, including food provision through fish and shellfish culture (Newton *et al.,* 2014; Newton *et al.,* 2018). There are over 100 coastal lagoons in the Mediterranean (Pérez-Ruzafa *et al.,* 2011), many of which are underutilised and could potentially be used for aquaculture. However, conditions vary and often activities such as agriculture, urban development, recreation and transport, change the biological and ecological dynamics of the systems (Pérez-Ruzafa *et al.,* 2011). Consequently, there is a need to plan and manage these activities, including aquaculture, to optimise the benefits from lagoon systems whilst minimising potential negative impacts on ecosystem health and other activities.

In Italy there is a high demand for seafood products, with 64 % of national commercial aquaculture production coming from shellfish farming. Farmed bivalve species include the Mediterranean mussel, *Mytilus galloprovincialis* (Lamarck, 1819), grooved carpet shell, *Ruditapes decussatus* (Linnaeus, 1758), Manila clam, *Ruditapes philippinarum* (Adams & Reeve, 1850) and Pacific oyster, *Crassostrea gigas* (Thunberg, 1793). However, demand is greater than supply, and in 2017 over 1.3 million tonnes of seafood were imported to the country. In particular, demand for Pacific oysters cannot be met by domestic production alone, consequently over 65,000 tonnes per year are imported from other countries to fulfil requirements (FAO, 2018). This suggests there is a considerable market for higher production of Pacific oyster in Italy if suitable locations can be identified. One such case are the highly productive coastal lagoons, which should be explored for this purpose.

Spatial models, developed using Geographic Information Systems (GIS), are often used for aquaculture site selection as they can provide an assessment based on factors which influence the suitability of a site (Falconer *et al.,* 2019). The use of Multi-Criteria Analysis (MCA)/Multi-Criteria Evaluation (MCE) within GIS models is particularly effective as it allows the combination of environmental, socio-economic and logistical parameters, providing a more holistic overview of multiple criteria, rather than considering those criteria separately (Falconer *et al.,* 2018). This supports the decision-making process by using factors, which indicate suitability of an area or production constraints, to show the limits of a given location for aquaculture development. Not all factors will be of equal importance, as some will have more influence over production than others, affecting the overall suitability. Within the MCE approach, factors are weighted based on their importance, with analytical hierarchy process (AHP) (Saaty, 1988) being the most commonly and increasingly used method for determining these weights (Nath *et al*., 2000;

Buitrago *et al*., 2005; Longdill *et al*., 2008; Radiarta *et al*., 2008; Silva *et al*., 2011; Micael *et al*., 2015; Falconer *et al.*, 2016).

The ability to develop and apply a GIS-based site selection model is dependent on the availability and quality of data (Falconer *et al.*, 2018; Falconer *et al.*, 2019). As data collection can be time consuming and expensive it is efficient to use data readily available for an initial large-scale assessment, before more detailed site-specific assessment are conducted. Many spatial models rely on gridded raster data (Falconer *et al.*, 2018); however, when this is not available, alternative methodologies such as those presented in this study, are required to incorporate the available data in the most appropriate manner.

For a shellfish site, stock growth potential is one of the most important characteristics as this directly translates into economic performances of the venture. A range of modelling approaches have been developed to simulate the growth of shellfish (Pouverau *et al*., 2006; Bourlès *et al.*, 2009; Barillé *et al.*, 2011; Filgueira *et al.*, 2011; Hawkins *et al.*, 2013), among them, models based on dynamic energy budget (DEB) theory (Kooijman, 2009) are becoming increasingly popular. DEB models can use data on temperature and food availability at a location to simulate shellfish growth; this can then be used to compare multiple locations to discover which has the most suitable stock growth potential.

The aim of this study was to develop, through a case study in the east coast of Sardinia, a methodology to classify the suitability of coastal Mediterranean lagoons for Pacific oyster culture. This used existing environmental data, collected by government and private agencies, and logistic information collected by stakeholder interviews and satellite imagery. The use of approaches such as those presented here, could assist decision-makers and industry stakeholders with the site selection process, by prioritising the lagoons with the most potential for production and for more detailed assessment, to ultimately boost the growth and sustainability of Pacific oyster farming in the region.

## 4.2. Study area

Sardinia is the second largest island in the Mediterranean Sea and, with a coastline of 1,850 km, it offers ample opportunity for sustainable exploitation of marine resources. In particular, the coastline is dotted with approximately 10,000 ha of biologically productive lagoons which for centuries have provided employment to local communities (Bazzoni *et al.*, 2013). Most lagoons are still utilized for extensive fish farming (valliculture), but could also be potential sites for Pacific oyster farming. Pacific oyster requires shallow and relatively sheltered sites, productive waters and can withstand relatively high salinity and temperature variability. All of these conditions can be found in Sardinian lagoons and therefore many of the Italian oyster farms are already located there. Nonetheless, only 3 % of the island Gross Domestic Product (GDP) is now produced by primary activities (farming and fishing) and youth unemployment has risen to 46.8 % in 2017 (http://www.sardegnastatistiche.it/argomenti/istruzionelavoro/). Against this backdrop, it would appear that sustainable aquaculture of a product in high demand, such as Pacific oysters, could provide significant development opportunities. On the other hand, over 25 % of the GDP is due to tourism and related services, highlighting the critical importance of properly managing coastal land use, via appropriate site selection and decision-making processes for primary industries, to assess the conflicts and opportunities arising from competing interests (Cho *et al*., 2012).

Twelve Sardinian lagoons where chosen for this case study, after a detailed survey on their historical environmental parameters. The chosen lagoons are: San Giovanni, Tortolì, Feraxi, Sa Praia, San Teodoro, Tartanelle, Gravile, Stagno Longo, Colostrai, Petrosu, Sa Curcurica and Su Graneri, all located in the east coast of Sardinia (fig. 4.1).These lagoons cover an area of 1,145 ha which correspond to more than 10 % of the total coastal lagoon area in Sardinia (regione.sardegna.it, 2019a). All these key transitional waters are already used for extensive valliculture of grey mullet (*Mugil cephalus* and *Chelon auratus*), sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) with the exception of Stagno Longo, Su Graneri, Tartanelle and Gravile where no fish or shellfish farming takes place. Small scale Pacific oyster production is already taking place in Tortolì, San Giovanni, Feraxi, and San Teodoro (Sardegnaagricoltura.it, 2019).

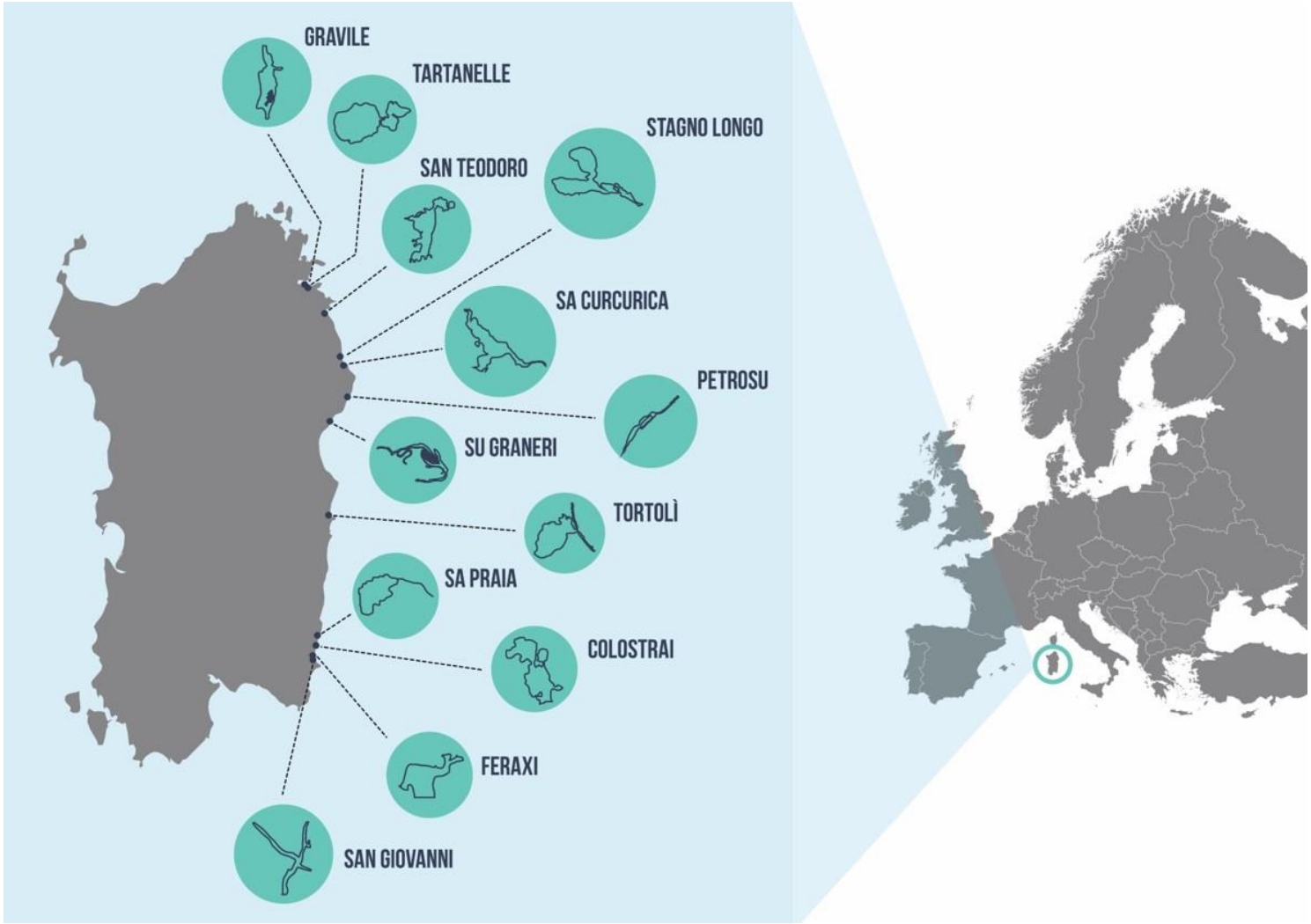


**Figure 4.1:** Study area: twelve lagoons chosen for this case study and their locations in the East coast of Sardinia.

## 4.3. Materials and Methods

### 4.3.1. Study Design and Overview of modelling approach

The modelling approach for this study is shown in Figure 4.2. The overall model has two main components; lagoon suitability assessment – based on biological and logistical criteria - and growth modelling – based on DEB models over production time. In combination these were used to give the potential productivity of most suitable lagoons.

The lagoon suitability assessment was performed combining, through using multi criteria analysis (MCA), biological criteria and logistic criteria. For these two criteria, local knowledge, shellfish farming expert focus groups and data from published literature were used to identify the factors that influence site suitability for Pacific oysters farming. The biological criteria were scored and weights for importance were established by expert focus groups using analytical hierarchy process AHP, while logistic criteria were also scored but were considered to be of equal importance.

The growth modelling was based on Dynamic Energy Budget (DEB) model, this was used to predict Pacific oyster culture cycle length and therefore to give the annual potential productivity of the lagoons.

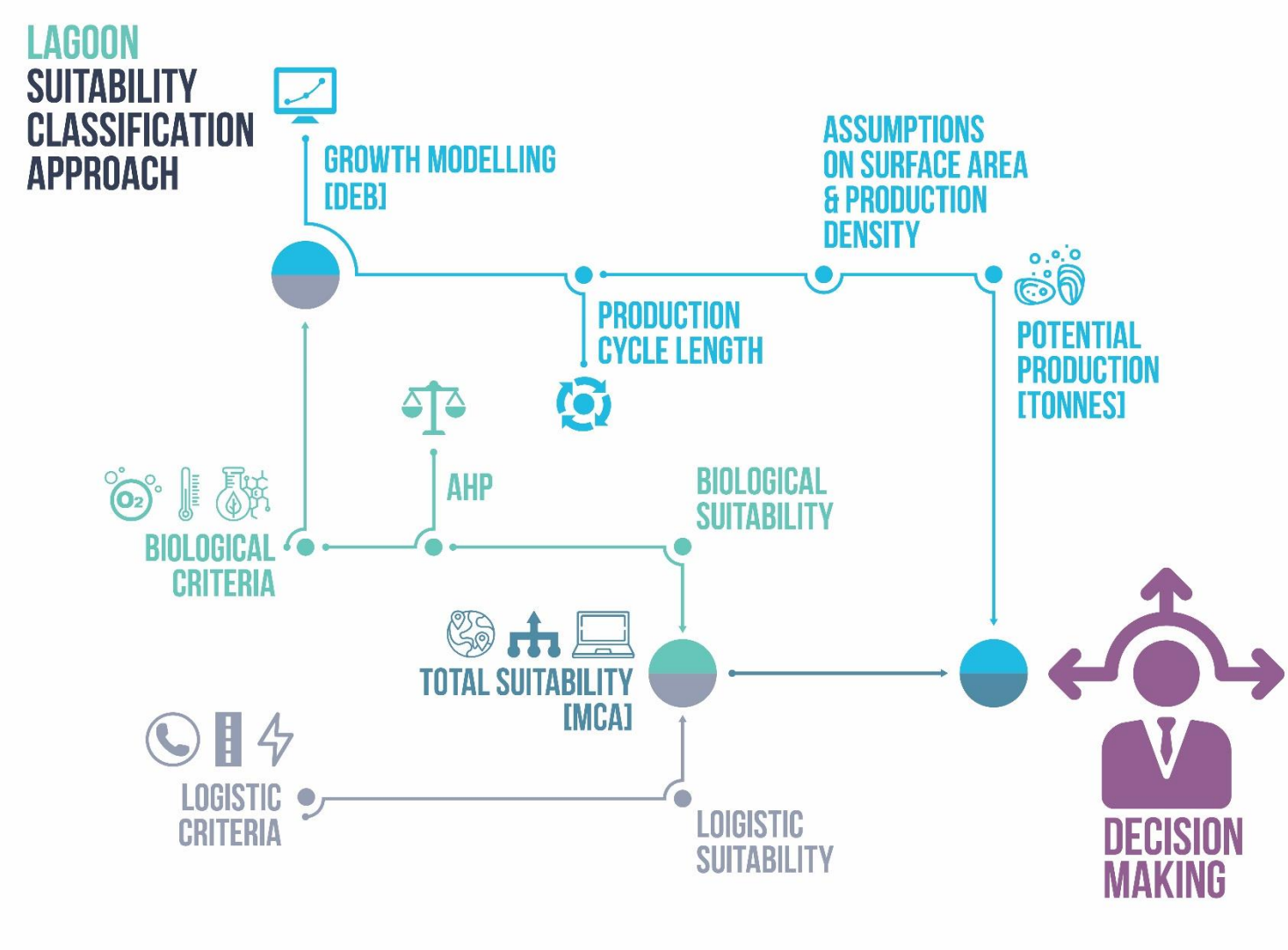


**Figure 4.2:** Diagram of the lagoons suitability classification approach.

### 4.3.2. Data collection and database generation

In the Site selection procedure, Biological factors and logistic factors were used to investigate the suitability for Pacific oyster farming of different Sardinian lagoon.

Once the biological factors to be used for the biological suitability were established, their values were extracted from the Sardinian Government Regional Environmental Information System (portal.sardegnasira.it, 2019; Sardegnaambiente.it, 2019). In this database, it was only possible to find usable data for twelve lagoons and for a period between 2002 and 2009.

From the SIRA database, data was extracted producing mean seasonal values and annual mean of each parameter, in each sampling point within each lagoon (fig. 4.3). These were then used to force the DEB model and establish biological suitability of the considered lagoons.

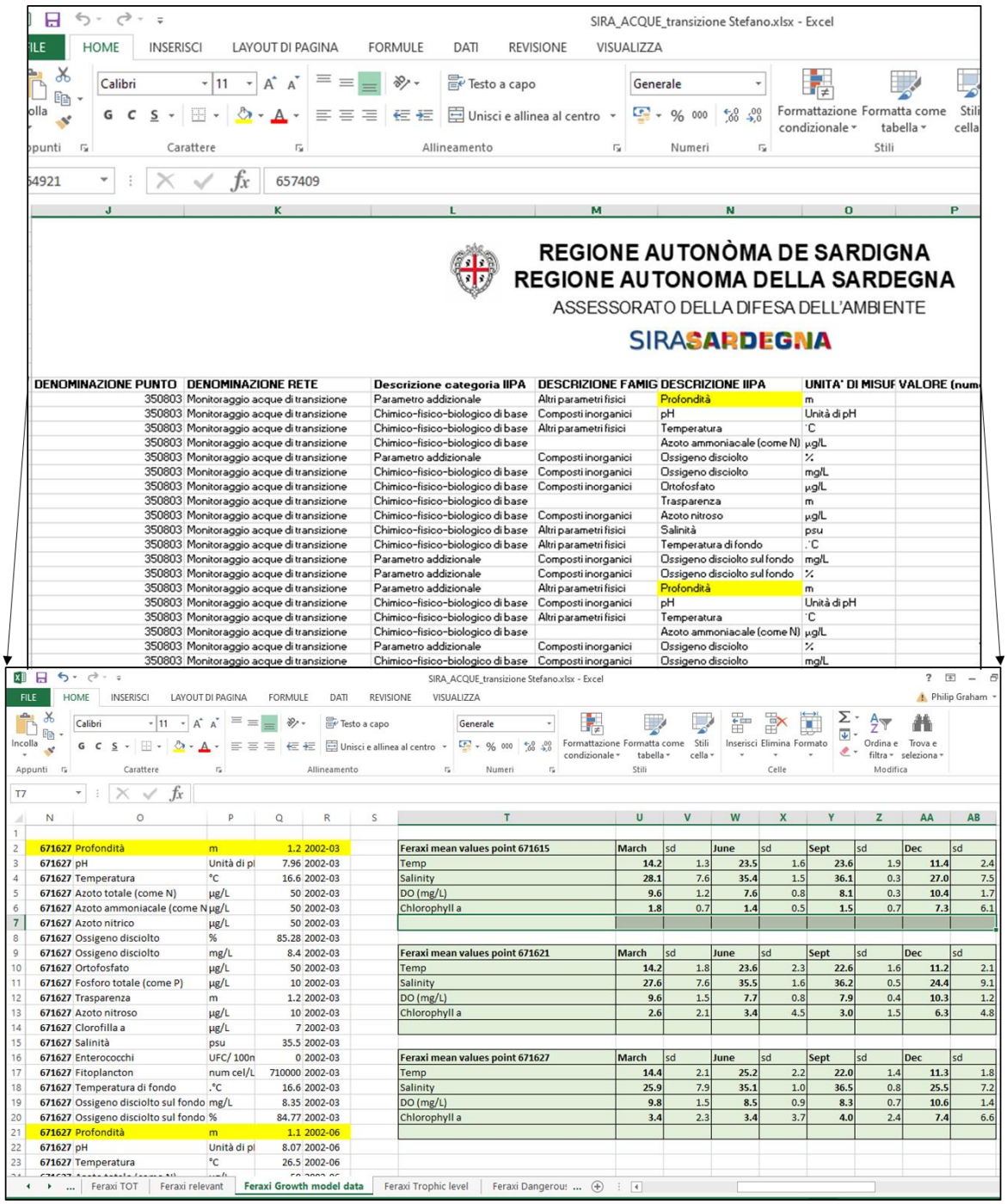


**Figure 4.3:** SIRA database (in the top of the figure). Temperature, salinity, dissolved oxygen and chlorophyll-a values were extracted to make new database with seasonal means values of each parameter, in each sampling point within each lagoon.

Logistical factors were chosen via expert focus group discussions and data were collected asking local stakeholders and farmers to fill a form (fig. 4.4), and by visualisation of freely available satellite images (Google Earth). Moreover, water classification for shellfish farming (as defined by Regulation (EC) No 854/2004, Regulation (EC) No 853/2004), was obtained from the Aquaculture and Fishery Service Office of the Sardinian Regional Government.

**Lagoon:**

| Roads | Score | |
|---|---|---|
| Wide asphalt road | 1 | |
| Wide gravel road/narrow asphalt road | 0.75 | |
| Gravel road | 0.5 | |
| No road | 0.25 | |

| Facilities | Score | |
|---|---|---|
| Fresh water | 0.25 | |
| Electricity | 0.25 | |
| Suitable building | 0.25 | |
| Unsuitable building | 0.15 | |
| Phone line/GSM | 0.10 | |

**Figure 4.4:** Logistic factors form. This was distributed to local stakeholders and farmers.

### 4.3.3. Lagoon suitability assessment

Local knowledge, shellfish farming expert focus groups and data from published literature were used to identify criteria that influence site suitability for Pacific oysters farming. These were divided into biological criteria, comprising water quality data that would directly influence oyster growth, and logistic criteria which would affect site development and farm operations. A common scoring system was established, ranging from 0 (constraint to farming) to 1 (optimal), and used to classify each criterion. Absolute constraints to farming, such as environmental parameters outside species tolerance ranges and adaptation abilities, or water microbiological classification non compatible with bivalve farming, were scored as 0. Some criteria are more important than others, as there will be greater influence on growth and farming operations, therefore weights were determined and assigned using the Analytical Hierarchical Process (AHP) first developed by Saaty (1988). Multi-Criteria Analysis (MCA) in a GIS environment was then used to

combine the Biological and Logistic criteria to produce the Total Suitability layer, for each lagoon, as outlined in Figure 4.2.

#### 4.3.3.1. Biological criteria

Bio-physical parameters (Temp ℃, Sal ‰, Chl-a µg $L^{-1}$, DO mg $L^{-1}$) spanning from 2002 to 2009 were extracted from the Sardinian Government Regional Environmental Information System (SIRA; Sardegnaambiente, 2019) and used to define the environment of each lagoon and establish how suitable each site was in satisfying Pacific oysters' biological requirements. Environmental data were available for three different locations in each lagoon; with the exception of Sa Praia lagoon where only one data point was available. Each parameter was considered as a mean per season and per sampling point for the data from 2002 to 2009. By averaging the values per season, we ensured that short-lived stochastic events that could perturb the local environment, such as a flash flood or a particularly cold week, that oyster would be able to withstand, would not affect our modelling outputs. Each bio-physical parameter was then assigned a suitability score between 0 (Constraint) and 1 (Optimum) as described below. These scores (for each season and sampling point within each lagoon) were averaged in order to generate an overall biological score for each lagoon.

In brief, each bio-physical parameter was considered independently and established the species tolerance boundary (maximum and minimum), intermediate and optimal values as illustrated in Table 4.1 and according to previous studies (Pagou *et al.*, 2002; Wiltshire, 2007; Patterson, 2018; Le Moullac *et al.*, 2007). For instance, it was considered that optimal growth would be achieved at a mean temperature between 20 and 25 °C, acceptable growth would still be achieved at temperatures between 7 °C and 29 °C, whilst temperatures above 30 °C and below and 6 °C would not be appropriate for Pacific oyster farming and would be considered constraints.

**Table 4.1:** Weight and score for each biological factor. The factor weight (%) is given in brackets as result of the Analytical Hierarchy Process. These were established considering Pacific oyster's biological needs.

| | **Temp °C** (21.17%) | | | **Sal ‰** (21.17%) | |
|---|---|---|---|---|---|
| | Value | Score | | Value | Score |
| Maximum | >30 | 0 | Maximum | >42 | 0.1 |
| Intermediate High | 26 - 29 | 0.5 | Intermediate High | 35 - 41 | 0.5 |
| Optimum | 20 - 25 | 1 | Optimum | 25 - 35 | 1 |
| Intermediate Low | 7 - 19 | 0.5 | Intermediate Low | 14 - 24 | 0.5 |
| Minimum | <6 | 0 | Minimum | <13 | 0.1 |
| | **DO mg L $^{-1}$** (5.30%) | | | **Chl-a µg L$^{-1}$** (52.40%) | |
| | Value | Score | | Value | Score |
| Optimum | >6 | 1 | Optimum | >2.21 | 1 |
| Intermediate | 3 - 6 | 0.5 | Intermediate High | 0.6 - 2.21 | 0.5 |
| Minimum | <2 | 0 | Intermediate Low | 0.1 – 0.6 | 0.25 |
| | | | Minimum | < 0.1 | 0 |

#### 4.3.3.2. Logistic criteria

Logistic criteria were also taken into account (tab. 4.2) in the model. These included accessibility to the sites (presence and type of roads), presence and type of ancillary facilities (fresh water, electricity, office/storage buildings, phone line) and presence/absence and type of microbiological water classification for shellfish farming (A, B, C, Not classified). In a similar manner to the biological criteria, the logistic criteria were selected and individually scored (between 0 and 1, where 0 represented a constraint) via expert focus group discussions, consultation with local stakeholders and farmers, and by visualisation of freely available satellite images (Google Earth).

Water classification for shellfish farming (as defined by Regulation (EC) No 854/2004, Regulation (EC) No 853/2004), was obtained from the Aquaculture and Fishery Service Office of the Sardinian Regional Government and was used to identify sites where farming could already take place (Class A scored as 1, and B scored as 0.5) and sites where farming could not take place (Class C scored as 0), as illustrated in Table 4.2. Importantly, because our objective was to identify potentials new sites for Pacific oyster development, which by definition do not necessarily have water classification, we decided to give a score of 0.25 to sites for which water classification was unavailable in order not to *a priori* exclude potentially suitable sites. Nonetheless, we also considered absence of water classification as a partial constraint with a value of 0.5 (tab. 4.2) when assessing total suitability scores (Equation 3) for each lagoon. The reason for this choice lies on the administrative burden and time involved in obtaining water classification from the relevant authorities.

**Table 4.2:** Logistics Factors and Constraints scores. The maximum score for each of the logistics factors is 1. In the site accessibility and in the water classification only one of the options could be chosen, while in the facilities, the score is given by the sum of the different options.

| Logistic Factors | | | | | |
|---|---|---|---|---|---|
| **Site Accessibility** | **Score** | **Facilities** | **Score** | **Water classification for shellfish farming** | **Score** |
| Wide asphalt road | 1 | Fresh Water | 0.25 | A | 1 |
| Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | 0.5 |
| Gravel road | 0.5 | Suitable building | 0.25 | Absent | 0.25 |
| No road | 0.25 | Unsuitable Building | 0.15 | C | 0 |
| | | Phone line/GSM | 0.10 | | |

| Constraints | |
|---|---|
| **Water classification for shellfish farming** | **Score** |
| Absent | 0.5 |
| C | 0 |
| **Biological constraints** | |
| Chl-a (µg $L^{-1}$) < 0.1 | 0 |
| T (°C) < 6 / > 30 | 0 |
| Salt (‰) < 13 / > 42 | 0 |
| DO (mg $L^{-1}$) < 2 | 0 |

#### 4.3.3.3. Analytical Hierarchy Process & Multi-Criteria Analysis

Once the biological criterion had been scored, they were assigned weights established by expert focus groups using analytical hierarchy process AHP (Saaty, 1988) (tab. 4.1). The logistic criteria were considered to be of equal importance. The AHP is a method that allows assigning priority to a series of decision-making alternatives. The method is based on a series of pairwise comparisons between the criteria, giving them a score of relative importance and ends with the assignment of a percentage weight.

The scores to be used for each comparison are arbitrary and generally correspond to the number of qualitative levels to be considered during pairwise comparison. Generally, score are given using an evaluation scale that varies from 1 to 9 (tab. 4.3).

**Table 4.3:** Saaty's scale for pairwise comparison.

| **Intensity of importance** | **Definition** |
|---|---|
| 1 | Equal importance |
| 3 | Moderate importance |
| 5 | Strong importance |
| 7 | Very strong or demonstrated importance |
| 9 | Extreme importance |
| 2,4,6,8 | For interpolation between the above values, intermediary values |

Next, the overall Biological and Logistic suitability scores of each lagoon were calculated using MCA. Multi criteria analysis is a type of decision-making approach which allows for the evaluation of different scenarios across a range of different criteria and indicators, creating a ranking of the performance of each scenario (Stephen *et al.*, 2005).

In this PhD study, the MCA in a GIS environment was used to combine the Biological and Logistic criteria to produce a Total Suitability layer, for each lagoon using QGIS 3.14

Biological Suitability (Sb) of each site was calculated using equation 1:

Eq. 1: $$Sb = \sum(W * P)$$

Where W is the weight and P is the parameter.

The logistical suitability (Sl) was calculated using equation 2:

Eq. 2: $$Sl = \sum(P)$$

Total suitability scores for each lagoon were then calculated as the mean between biological and logistic scores multiplied by any constraint (0) in such a way that if a biological or logistic constraint to farming is present the overall suitability score becomes 0.

Total suitability (St) was calculated using equation 3:

Eq. 3: $$St = ((Sb + Sl)/2) * C$$

Where C is a constraint.

The Geo-referencing process and overall lagoon score classification was completed using the GIS software QGIS 3.14 [QGIS Development Team]. GIS outputs have then been converted into the figures using Adobe CC Illustrator®, 2019

### 4.3.4. Growth modelling and sites potential productivity

Once total suitability was established and the growth model was validated, the length of the production cycle (from seed to market size) for each lagoon and in all sampling points of each lagoon (tab. 4.6) was investigated to establish the potential annual productivity of all lagoons object of this study.

Dynamic Energy Budget (DEB) theory is a mathematical modelling approach which describes how an organism assimilates and uses energy for key physiological processes such as growth and reproduction (Kooijman, 2009). The theory is fully described in Kooijman (2009), but as a general summary, it uses three differential equations, which describe growth of structural volume, dynamics of energy reserves and storage and use of energy allocated for reproduction and environmental drivers such as temperature are used as input (Kooijman, 2009; Pouvreau *et al*., 2006). The model can be set up for frequent time-steps (e.g. daily or weekly) so can be a useful way of exploring the potential physiological impact of variable environmental conditions, and this makes it useful for assessing production potential at different farm locations. The DEB modelling approach has been used to simulate the life cycle of many different animals including sea cucumbers (Ren *et al.,* 2017), sea urchins (Yeruham *et al.,* 2019 ), Sea Bass (Stavrakidis-Zachou *et al*., 2019) , and is an established approach for modelling bivalve growth and shellfish production potential (Bacher and Gangnery, 2006; Pouvreau *et al.,* 2006; Hatzonikolakis *et al*., 2017; Palmer *et al*., 2020; Saraiva *et al*., 2020).

A DEB model for Pacific oysters was developed using R software (R Core Team, 2018), based on the modelling approach originally established by Pouvreau *et al.* (2006) (fig. 4.5), and calibrated to local conditions. The model was validated using growth data from Pacific oyster farming sites in San Teodoro and Santa Gilla lagoons (Experimental Chapter 2 and 3) to ensure it represented conditions in Sardinia. Knowledge of local oyster farming practices was used to set up the model: where the production cycle started in March, the initial oyster size was 8 mm, and the modelled oysters were assumed to be sterile triploids. For each location, interpolated daily values of temperature and Chl-a concentrations (used as proxy to food availability), were used to force the model. The model simulated the increase in shell length, which was then converted to weight using equation 4, which was empirically derived from morphometric data collected *in situ* (Experimental Chapter 2 and 3):

Eq. 4: $$W = 0.1496 * (L^{2.6681})$$

The endpoint of the simulation was a harvest weight of 80 g per individual.

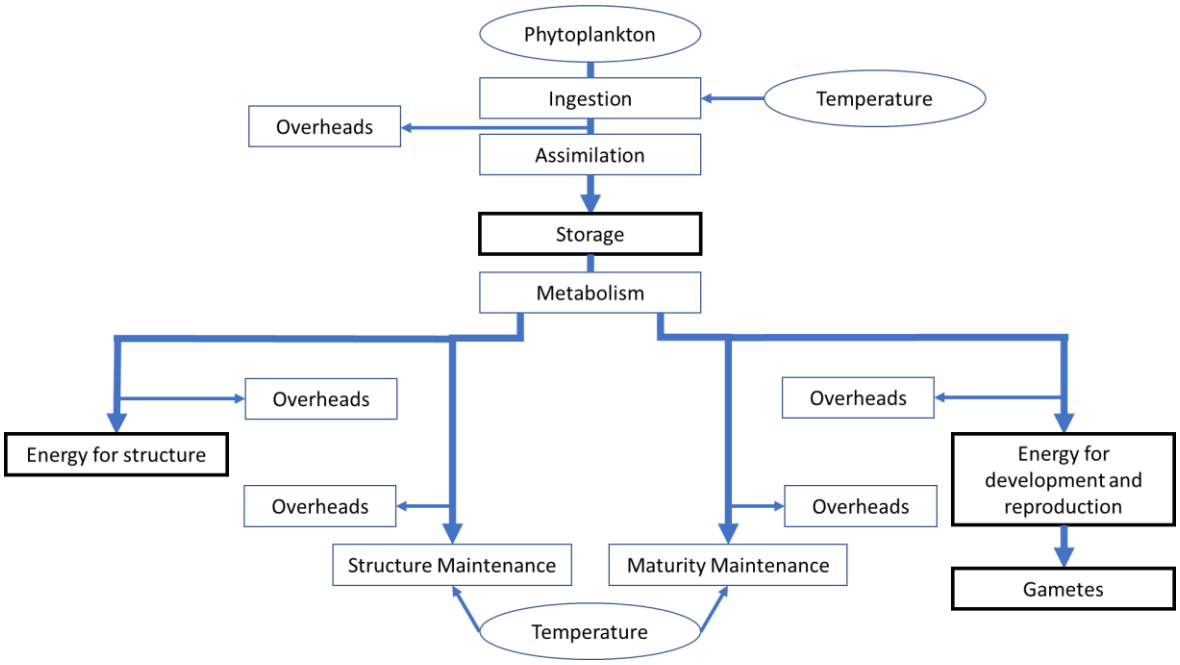


**Figure 4.5:** Structure and flow of the DEB modelling approach used in the study of Pouvreau *et al*. (2006) on which the DEB model developed in this study is based on. The rectangular boxes show the state variables (in which the black ones represent the different equations that describe the dynamics of growth of structural volume, energy reserves and storage and use of energy allocated for reproduction) while oval boxes are the forcing variables. Small rectangular boxes are the overheads (i.e. heat fluxes). (Modified from Pouvreau *et al*., 2006)

To run the DEB model the value of water temperature (°C) and amount of chlorophyll-a (µg $L^{-1}$), were typed in a .csv file. This file is the database that the R script uses to run the model (fig. 4.6). Before running the model, the initial oyster size and the time (growth period) to be explored were entered. The outputs come as a .csv file with daily weight and length values.

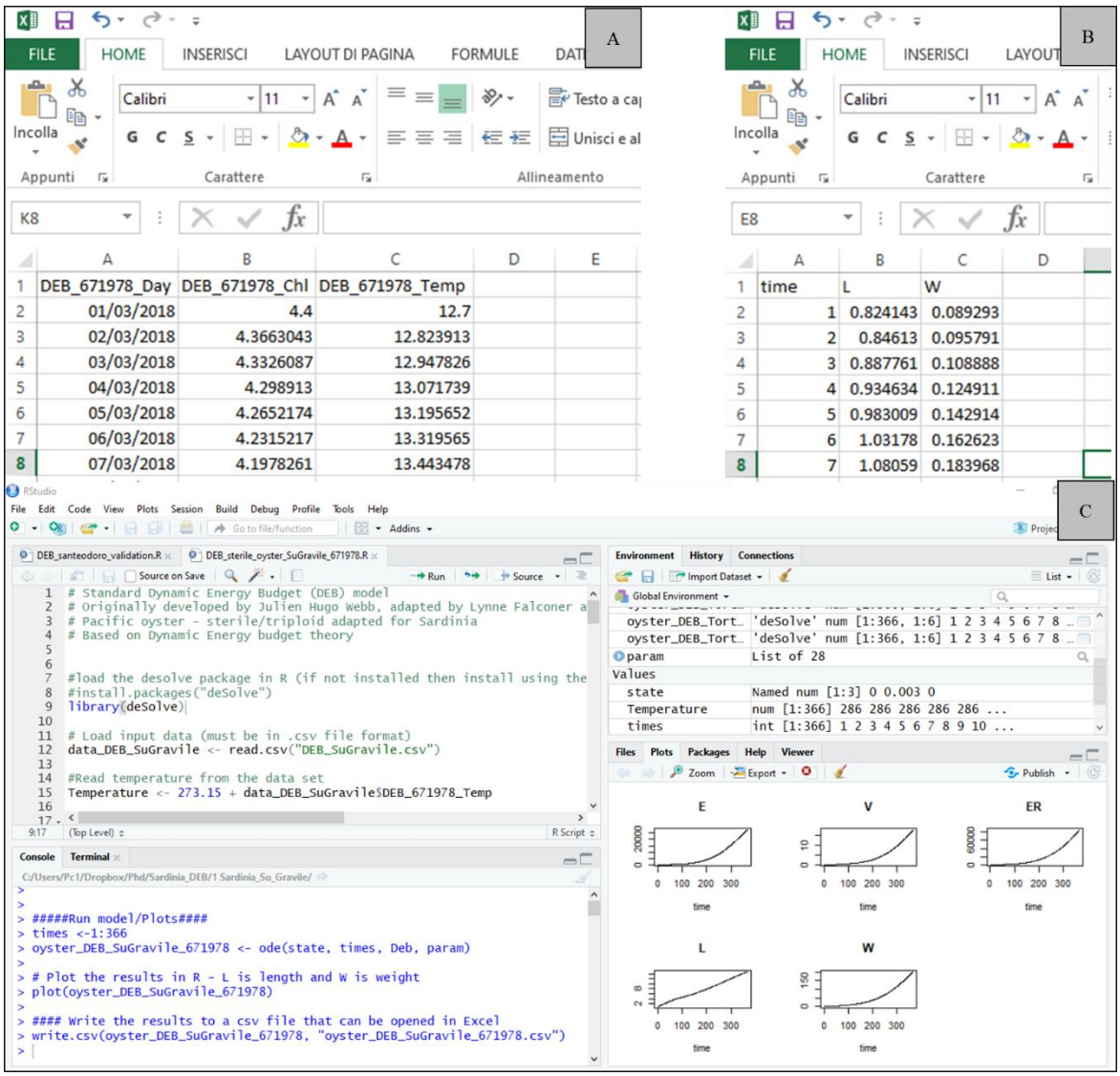


**Figure 4.6:** Example of: **A,** Database to run the DEB model; **B,** Output of the DEB growth model; **C,** DEB model script in R studio.

Using the average of temperature and Chl-a values of each sampling point, the production cycle length for each lagoon was calculated. In order to calculate the potential productivity per production cycle and per year of each lagoon, an arbitrary 25 % of the surface area of each lagoon, acquired as secondary data from the Sardinian government website (regione.sardegna.it, 2019b), was assumed as usable for Pacific oyster farming. Productivity (Oysters Biomass) per unit area was also considered to be 1 kg $m^{-2}$ in accordance with local farming practices.

Potential production per year was then calculated using equation 5:

Eq.5: $$PP = \lfloor (Surface\ area\ x\ 0.25)\ x\ (1kg\ /\ m^2) \rfloor\ x\ (\%\ of\ production\ cycle\ per\ year)$$

## 4.4. Results

### 4.4.1. Lagoon suitability

In general, all bio-physical parameters (T, Sal, DO and Chl-a) were highly suitable for Pacific oyster farming (tab. 4.4), however, there were four exceptions: salinity in Sa Curcurica, Su Graneri and Stagno Longo, and chlorophyll-a in Colostrai. In Sa Curcurica salinity was higher than optimal in spring, summer and autumn (score 0.46) due to low freshwater inputs from the catchment and high evaporation during the warmer months. Su Graneri and Stagno Longo lagoons had lower than optimal salinity, particularly in winter due to high fresh water inputs (scores 0.21 and 0.46 respectively). Chlorophyll-a concentrations were lower than optimal, but still suitable, throughout the year in Colostrai (score 0.5), possibly due to high water exchange rate with the Mediterranean Sea resulting in lower nutrient waters. However, all lagoons resulted in an overall score higher than 0.6, as calculated using the weights in Table 4.1, indicating that from a strictly biological point of view all examined lagoons could potentially host Pacific oyster farming activities. These are shown in Figure 4.7.

The overall picture of Sardinian lagoons from a logistic view point (tab. 4.5) is one of suitable overall conditions for most categories (site accessibility, utilities and building). However, only three out of twelve lagoons (Feraxi, San Giovanni and Tortolì) were serviced by a wide asphalt road which, according with local farmers, would allow for large equipment and harvest to be easily moved in and out of the farming sites (score 1). Seven lagoons had wide gravel or narrow asphalt road that could limit farming operations particularly when scope for expansion is considered (score 0.75). The remaining two lagoons (Su Graneri and Tartanelle) only had access through narrow gravel roads (score 0.5). Suitable buildings were present in all lagoons with the exception of Tartanelle (score 0.25). Only five lagoons held water classification for bivalve farming and all five were classed as A waters and scored as 1. The other sites were given a score of 0.25 as being only newly considered for bivalve culture they had no classification. As classification is depended on constant monitoring and can change, these scores should be re-evaluated at for all lagoons when new information becomes available. The overall Logistic suitability score for each lagoon is shown in Figure 4.8. As neither biological nor logistic considerations on their own would be enough to determine lagoon suitability and they have to be combined to generate a Total Suitability Score presented in Figure 4.9. This clearly indicates that although all lagoons were biologically suitable (scores from 0.63 to 0.95),

and their logistic suitability was also acceptable (scores from 0.45 to 0.95) the combination of both sets of parameters creates a divide between the top five lagoons (Scores from 0.74 to 0.95) and the remaining seven (scores from 0.30 to 0.36). The difference is due to the absence of water classification for bivalve farming in the lower scoring lagoons.

**Table 4.4:** Mean ± Standard Error of seasonal biological factors within each lagoon and total score for each factor within each lagoon.

| ***Lagoon*** | ***Season*** | ***Temp °C*** | ***Total Score*** | ***Sal ‰*** | ***Total Score*** | ***DO mg $L^{-1}$*** | ***Total Score*** | ***Chl-a µg $L^{-1}$*** | ***Total Score*** |
|---|---|---|---|---|---|---|---|---|---|
| *Feraxi* | Spring | 14.3 ± 0.0 | 0.75 | 27.2 ± 0.7 | 0.71 | 9.6 ± 0.1 | 1 | 2.6 ± 0.5 | 0.88 |
| | Summer | 24.1 ± 0.1 | | 35.3 ± 0.1 | | 7.9 ± 0.3 | | 2.7 ± 0.7 | |
| | Autumn | 22.7 ± 0.1 | | 36.3 ± 0.1 | | 8.1 ± 0.1 | | 2.8 ± 0.7 | |
| | Winter | 11.3 ± 0.1 | | 25.6 ± 0.8 | | 10.4 ± 0.1 | | 7.0 ± 0.3 | |
| *Colostrai* | Spring | 13.9 ± 0.1 | 0.75 | 31.8 ± 1.3 | 0.83 | 9.4 ± 0.1 | 1 | 1.0 ± 0.0 | 0.5 |
| | Summer | 22.7 ± 0.1 | | 35.5 ± 0.2 | | 7.9 ± 0.1 | | 1.1 ± 0.0 | |
| | Autumn | 22.8 ± 0.3 | | 36.0 ± 0.1 | | 7.7 ± 0.2 | | 1.2 ± 0.1 | |
| | Winter | 14.3 ± 0.2 | | 31.6 ± 0.5 | | 9.9 ± 0.1 | | 1.3 ± 0.0 | |
| *San Giovanni* | Spring | 14.5 ± 0.4 | 0.75 | 30.3 ± 1.6 | 1 | 10.3 ± 0.4 | 1 | 6.1 ± 2.6 | 1 |
| | Summer | 24.2 ± 0.4 | | 33.6 ± 0.8 | | 7.9 ± 0.2 | | 6.8 ± 2.2 | |
| | Autumn | 22.3 ± 0.1 | | 33.0 ± 0.8 | | 8.1 ± 0.1 | | 7.2 ± 1.6 | |
| | Winter | 12.4 ± 0.1 | | 30.8 ± 1.0 | | 10 ± 0.4 | | 4.5 ± 1.9 | |
| *Sa Praia* | Spring | 14.7 ± 0.7 | 0.75 | 23.9 ± 4.0 | 0.75 | 9.6 ± 0.6 | 1 | 1.7 ± 0.3 | 0.75 |
| | Summer | 25.1 ± 1.5 | | 31.7 ± 2.6 | | 8.2 ± 0.4 | | 1.8 ± 0.3 | |
| | Autumn | 23.8 ± 0.5 | | 35.4 ± 1.2 | | 7.4 ± 0.4 | | 6.1 ± 2.3 | |
| | Winter | 11.7 ± 1.1 | | 25.2 ± 2.6 | | 9.4 ± 0.4 | | 7.5 ± 3.0 | |
| *Tortolì* | Spring | 13.9 ± 0.1 | 0.75 | 29.3 ± 1.4 | 0.83 | 9.6 ± 0.1 | 0.92 | 2.7 ± 0.6 | 0.96 |
| | Summer | 24.5 ± 0.4 | | 33.6 ± 0.7 | | 7.2 ± 0.2 | | 5.0 ± 1.1 | |
| | Autumn | 24.5 ± 0.5 | | 36.8 ± 0.9 | | 6.1 ± 0.2 | | 4.0 ± 0.7 | |
| | Winter | 15.2 ± 0.6 | | 35.7 ± 3.4 | | 8.5 ± 0.2 | | 2.2 ± 0.0 | |
| *Petrosu* | Spring | 13.6 ± 0.2 | 0.75 | 24.4 ± 2.9 | 0.79 | 9.9 ± 0.2 | 0.79 | 1.6 ± 0.1 | 0.63 |
| | Summer | 24.4 ± 0.3 | | 32.6 ± 1.4 | | 5.8 ± 0.2 | | 1.7 ± 0.1 | |
| | Autumn | 24.4 ± 0.1 | | 27.7 ± 3.6 | | 5.4 ± 0.5 | | 2.8 ± 0.1 | |
| | Winter | 12.4 ± 0.1 | | 27.3 ± 1.8 | | 8.0 ± 0.3 | | 1.3 ± 0.1 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Sa Curcurica* | Spring | 13.7 ± 0.2 | 0.71 | 39 ± 0.1 | 0.46 | 8.3 ± 0.3 | 0.88 | 0.9 ± 0.2 | 0.65 |
| | Summer | 23.9 ± 0.5 | | 41.7 ± 0.2 | | 6.6 ± 0.1 | | 2.1 ± 0.7 | |
| | Autumn | 25.6 ± 0.4 | | 43.0 ± 0.1 | | 5.4 ± 0.2 | | 2.8 ± 0.8 | |
| | Winter | 12.4 ± 0.2 | | 33.5 ± 0.8 | | 7.5 ± 0.2 | | 1.2 ± 0.1 | |
| *Su Graneri* | Spring | 14.3 ± 0.5 | 0.67 | 18.1 ± 10.6 | 0.21 | 8.7 ± 0.3 | 0.88 | 4.7 ± 2.5 | 0.81 |
| | Summer | 25.6 ± 0.7 | | 23.1 ± 9.7 | | 6.7 ± 0.6 | | 4.2 ± 1.2 | |
| | Autumn | 24.3 ± 1.0 | | 23.8 ± 9.9 | | 5.9 ± 0.5 | | 8.7 ± 2.5 | |
| | Winter | 12.4 ± 0.4 | | 13.8 ± 9.2 | | 9.0 ± 0.6 | | 1.8 ± 0.2 | |
| *Stagno Longo* | Spring | 14.4 ± 0.3 | 0.67 | 17.6 ± 3.9 | 0.46 | 9.4 ± 0.4 | 1 | 4.4 ± 1.0 | 1 |
| | Summer | 26.2 ± 0.7 | | 32.6 ± 6.1 | | 7.2 ± 0.1 | | 7.6 ± 1.8 | |
| | Autumn | 25.1 ± 0.1 | | 34.8 ± 5.7 | | 8.0 ± 0.5 | | 12.4 ± 2.3 | |
| | Winter | 12.9 ± 0.1 | | 18.7 ± 4.5 | | 7.8 ± 0.6 | | 3.9 ± 0.9 | |
| *San Teodoro* | Spring | 12.4 ± 0.4 | 0.71 | 21.1 ± 3.6 | 0.67 | 9.4 ± 0.1 | 0.88 | 4.1 ± 1.8 | 0.96 |
| | Summer | 24.1 ± 1.0 | | 37.3 ± 1.2 | | 7.7 ± 0.1 | | 13.1 ± 3.2 | |
| | Autumn | 23.8 ± 0.7 | | 31.3 ± 3.0 | | 5.8 ± 0.0 | | 17.7 ± 3.9 | |
| | Winter | 11.1 ± 0.1 | | 16.3 ± 2.6 | | 9.5 ± 0.1 | | 4.8 ± 1.6 | |
| *Tartanelle* | Spring | 12.4 ± 0.2 | 0.75 | 30.9 ± 0.3 | 0.71 | 9.8 ± 0.2 | 1 | 1.8 ± 0.5 | 0.75 |
| | Summer | 25.0 ± 0.2 | | 40.6 ± 0.7 | | 8.0 ± 0.1 | | 4.2 ± 2 | |
| | Autumn | 23.4 ± 0.2 | | 36.4 ± 0.2 | | 9.3 ± 1.3 | | 2.4 ± 0.8 | |
| | Winter | 12.8 ± 0.2 | | 35 ± 0.7 | | 9.3 ± 0.2 | | 2.9 ± 1.1 | |
| *Gravile* | Spring | 11.2 ± 0.8 | 0.75 | 28.8 ± 1.2 | 0.67 | 10.2 ± 0.4 | 0.96 | 2.3 ± 1.1 | 0.79 |
| | Summer | 24.3 ± 0.1 | | 40.2 ± 1.4 | | 7.9 ± 0.2 | | 3.2 ± 1.5 | |
| | Autumn | 23.1 ± 0.8 | | 41.4 ± 1.5 | | 6.8 ± 0.4 | | 7.7 ± 2.0 | |
| | Winter | 12.7 ± 0.6 | | 31.2 ± 1.2 | | 9.3 ± 0.1 | | 3.9 ± 0.8 | |

**Table 4.5:** Logistic factors and scores within each lagoon.

| ***Lagoon*** | ***Site Accessibility*** | ***Score*** | ***Facilities*** | ***Score*** | ***Water classification for shellfish farming*** | ***Score*** |
|---|---|---|---|---|---|---|
| *Feraxi* | Wide asphalt road | 1 | Fresh Water | 0.25 | A | 1 |
| | Wide gravel road/ narrow asphalt road | | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Colostrai* | Wide asphalt road | | Fresh Water | 0.25 | A | 1 |
| | Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | | Absent | |
| | No road | | Unsuitable Building | 0.15 | C | |
| | | | Phone line/GSM | 0.10 | | |
| *San Giovanni* | Wide asphalt road | 1 | Fresh Water | 0.25 | A | 1 |
| | Wide gravel road/ narrow asphalt road | | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Sa_Praia* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Tortolì* | Wide asphalt road | 1 | Fresh Water | 0.25 | A | 1 |
| | Wide gravel road/ narrow asphalt road | | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Petrosu* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Sa Curcurica* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Su Graneri* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | | Electricity | 0.25 | B | |
| | Gravel road | 0.5 | Suitable building | 0.25 | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Stagno Longo* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *San Teodoro* | Wide asphalt road | | Fresh Water | 0.25 | A | 1 |
| | Wide gravel road/ narrow asphalt road | 0.75 | Electricity | 0.25 | B | |
| | Gravel road | | Suitable building | 0.25 | Absent | |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Tartanelle* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | | Electricity | 0.25 | B | |
| | Gravel road | 0.5 | Suitable building | | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |
| *Gravile* | Wide asphalt road | | Fresh Water | 0.25 | A | |
| | Wide gravel road/ narrow asphalt road | | Electricity | 0.25 | B | |
| | Gravel road | 0.75 | Suitable building | 0.25 | Absent | 0.25 |
| | No road | | Unsuitable Building | | C | |
| | | | Phone line/GSM | 0.10 | | |

**Table 4.6:** Geographical coordinates of all sampling points in each lagoon and their relative production cycle length as predicted by the DEB model.

| ***Lagoon*** | ***latitude WGS84*** | ***longitude WGS84*** | ***DEB prediction: number of days to reach 80g*** |
|---|---|---|---|
| *Su Gravile* | 40.91168286 | 9.558594584 | 265 |
| | 40.91505424 | 9.555378475 | 224 |
| | 40.91315258 | 9.555698495 | 253 |
| *Tratanelle* | 40.89834924 | 9.570562881 | 236 |
| | 40.90083179 | 9.575988909 | 375 |
| | 40.90072233 | 9.581805449 | 282 |
| *San Teodoro* | 40.79899461 | 9.666547992 | 194 |
| | 40.81161023 | 9.674499871 | 217 |
| | 40.78725778 | 9.662628092 | 186 |
| *Stagno Longo* | 40.62763766 | 9.737587361 | 168 |
| | 40.62361909 | 9.734107696 | 212 |
| | 40.62267731 | 9.74298532 | 228 |
| *Su Graneri* | 40.59119242 | 9.756449649 | 317 |
| | 40.5904792 | 9.752048762 | 212 |
| | 40.58789851 | 9.760191327 | 212 |
| *Sa Curcurica* | 40.45530492 | 9.788701076 | 317 |
| | 40.4525334 | 9.793746304 | 540 |
| | 40.45879218 | 9.784133041 | 306 |
| *Petrosu* | 40.35028709 | 9.692103586 | 340 |
| | 40.34480307 | 9.688707973 | 320 |
| | 40.35597254 | 9.696397058 | 339 |
| *Tortolì* | 39.9438354 | 9.671366161 | 236 |
| | 39.94902027 | 9.677137788 | 296 |
| | 39.93858202 | 9.670602606 | 231 |
| *Sa Praia* | 39.44155745 | 9.620803052 | 261 |
| *San Giovanni* | 39.40089714 | 9.612894816 | 253 |
| | 39.39990496 | 9.612145863 | 195 |
| | 39.40173876 | 9.612255155 | 215 |

| | | | |
|---|---|---|---|
| *Colostrai* | 39.34954034 | 9.590548198 | 500 |
| | 39.34619926 | 9.598277912 | 495 |
| | 39.3464525 | 9.592457119 | 449 |
| *Feraxi* | 39.33853389 | 9.596256987 | 652 |
| | 39.33649056 | 9.593185264 | 257 |
| | 39.33551025 | 9.588629203 | 246 |

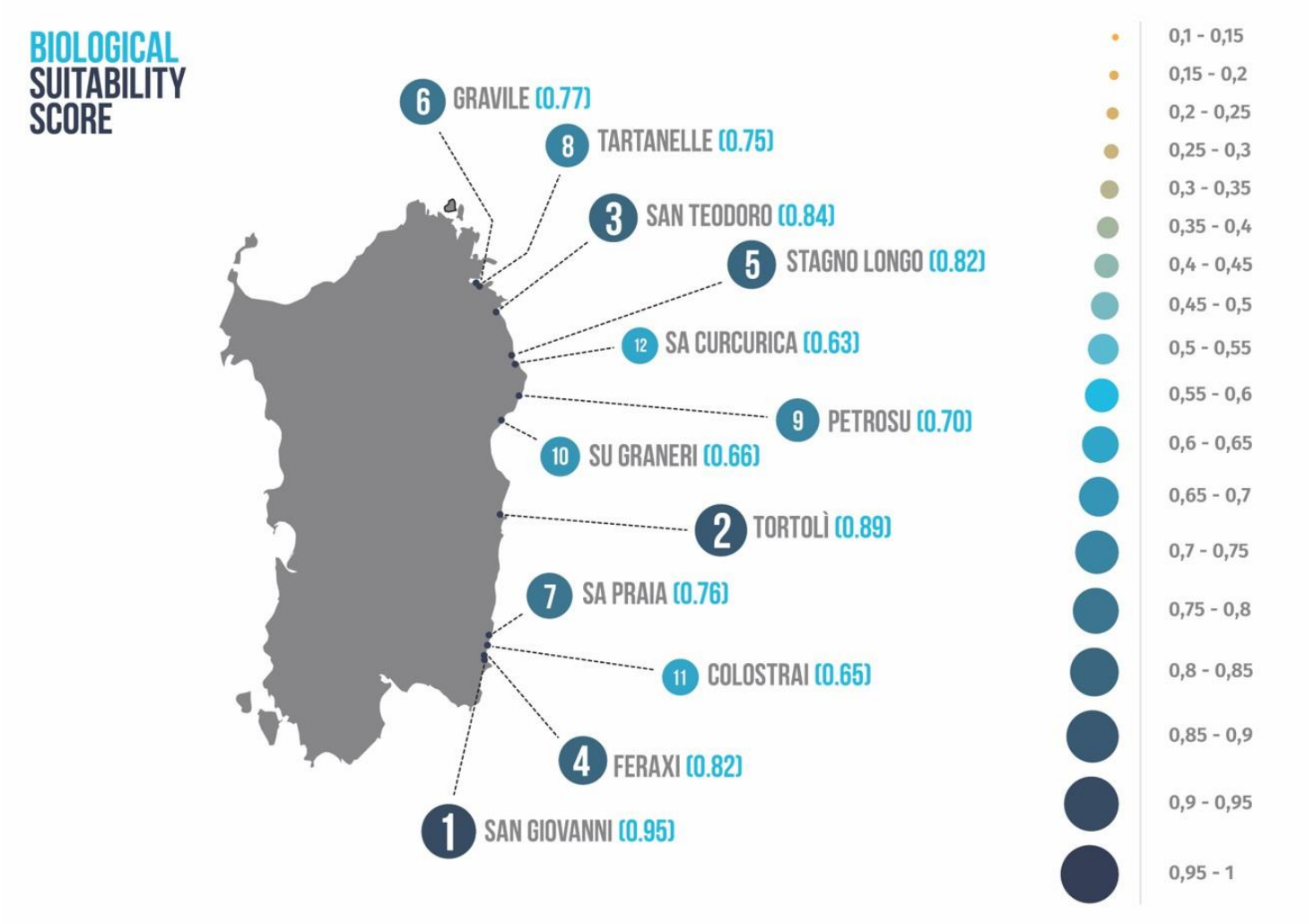


**Figure 4.7:** Biological suitability as calculated by AHP. Size of the circles and numbers are indicative of suitability scores and ranking.

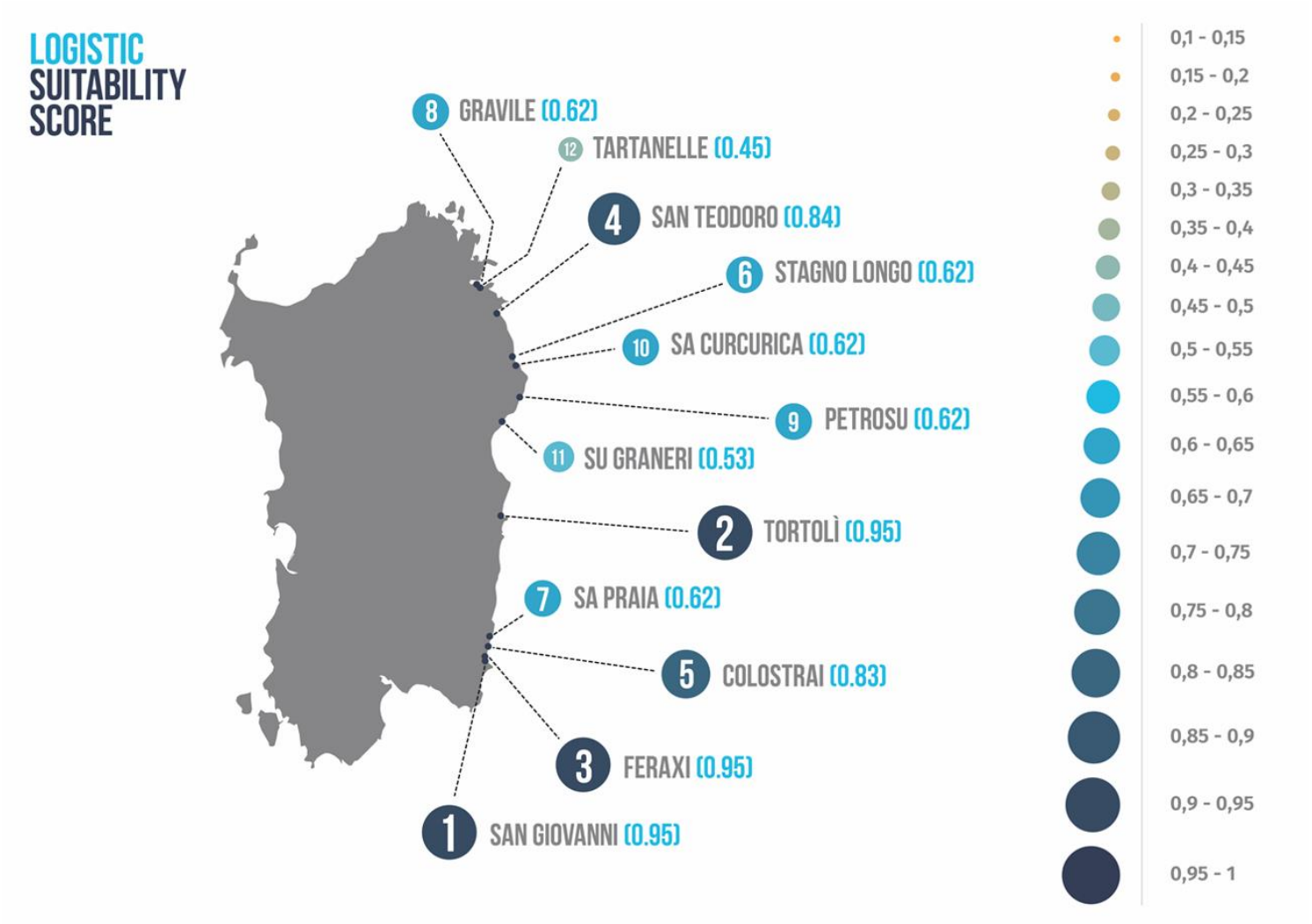


**Figure 4.8:** Logistic suitability. Size of the circles and numbers are indicative of suitability scores and ranking.

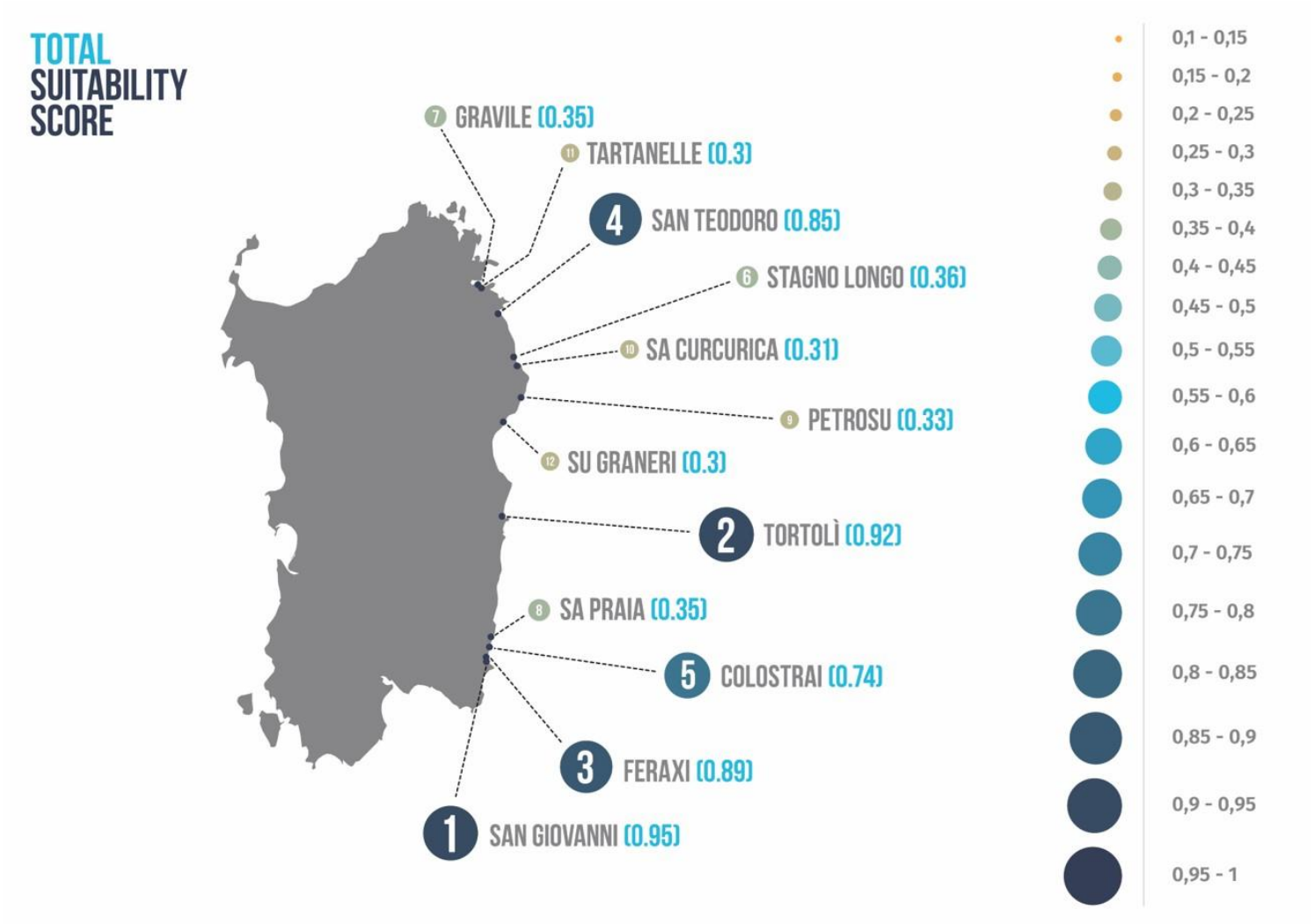


**Figure 4.9:** Total suitability as calculated by MCA. Size of the circles and numbers are indicative of suitability scores and ranking.

### 4.4.2. Oysters growth and sites potential productivity

The outputs from the DEB growth model for all available sampling points showed that the time to reach market size (80 g) ranged from 168 to 652 days (tab. 4.6). This is due to the variability in temperature and Chl-a concentrations (main drivers of the growth model) between lagoons but also between areas within each lagoon. Indeed, distance of the sampling points from fresh water inputs, the lagoon opening to the sea, the specific bathymetry, and the position of the sampling point within the overall lagoon circulation are all critical parameters able to influence the model's main drivers. The ability to distinguish which area within the lagoon offer the best opportunity for growth is obviously of great importance during the site selection process. This is clearly exemplified by one location in Sa Curcurica and one location in Feraxi where growth prediction is significantly longer than the other sampling points considered, within the same lagoons. Moving from a comparison between sampling points within lagoons to wider comparison between lagoons, Figure 4.10 showed that the time to reach 80 g ranged from 177 days in Stagno Longo to 481 days in Colostrai lagoons. Though this suggested there was growth potential for all lagoons, there were significant variations in production length between each lagoon.

The potential productivity per production cycle was then calculated and predictions generated using the outputs from the DEB model and the assumptions on available area for cultivation and production density are given in Table 4.7. The annual potential production of each lagoon was then calculated based on the number of production cycles that could be theoretically performed within one year based on equation 5. These results are shown in Figure 4.11 and show that two lagoons have considerably more production potential that the rest: Tortoli (1063.4 tonnes) and San Teodoro (1025.4 tonnes). The results also highlight that the size of the lagoon is not necessarily related to production capacity, as there could be more suitable environmental conditions in smaller lagoons. For example, San Giovanni lagoon (assumed cultivation area of 27.5 ha) is smaller than Colostrai (assumed cultivation area of 34.25 ha) but has significantly higher annual potential production (475.7 tonnes *vs* 259.9 tonnes) due to the lower chlorophyll levels in the latter. The total annual combined production within the twelve lagoons was calculated to be 4113.5 tonnes/year, equal to 6.25 % of the total Pacific oyster annual imports to Italy. However, more detailed lagoon-specific assessment and site selection analysis would be required to enable more robust estimates of potential production.

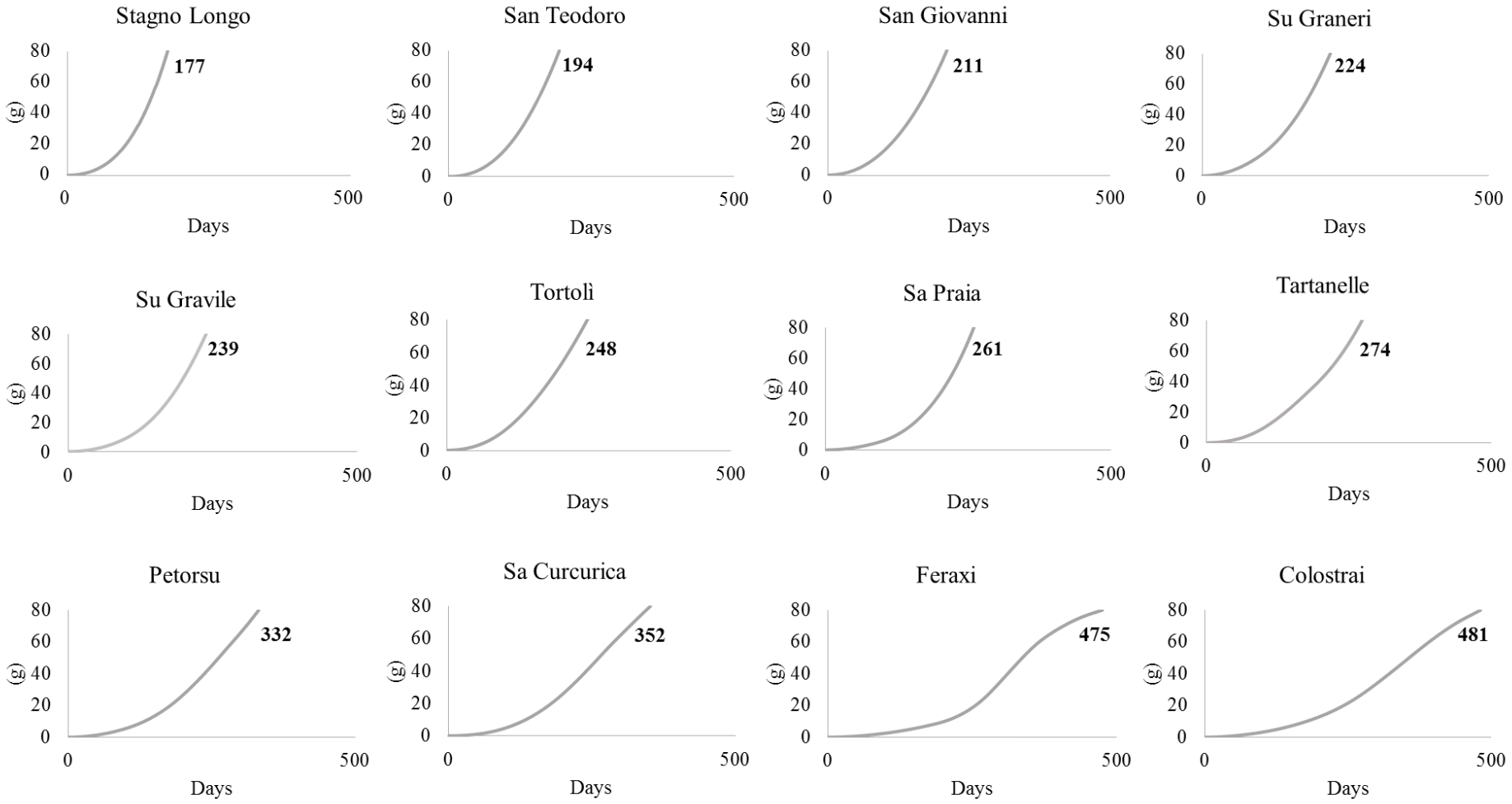


**Figure 4.10:** Production cycle length expressed in number of days to reach market size of 80 g for each lagoon as predicted by the DEB model.

**Table 4.7:** Potential area available to Pacific oyster farming in each lagoon (an arbitrary 25 % of the whole lagoon was assumed to be usable for oysters farming) with their relative production potential based on the assumed density of 1kg $m^{-2}$.

| ***Lagoons*** | ***Potential surface area for Pacific oyster farming (ha)*** | ***Potential productivity for each production cycle (Tonnes)*** |
|---|---|---|
| *Tortolì* | 72.25 | 722.5 |
| *San Teodoro* | 54.50 | 545.0 |
| *Colostrai* | 34.25 | 342.5 |
| *San Giovanni* | 27.50 | 275.0 |
| *Sa Praia* | 21.50 | 215.0 |
| *Tartanelle* | 19.38 | 193.7 |
| *Feraxi* | 15.00 | 150.0 |
| *Gravile* | 12.50 | 125.0 |
| *Sa Curcurica* | 10.00 | 100.0 |
| *Stagno Longo* | 9.50 | 95.0 |
| *Petrosu* | 7.50 | 75.0 |
| *Su Graneri* | 2.57 | 25.7 |
| *TOTAL* | 286.45 | 2864.4 |

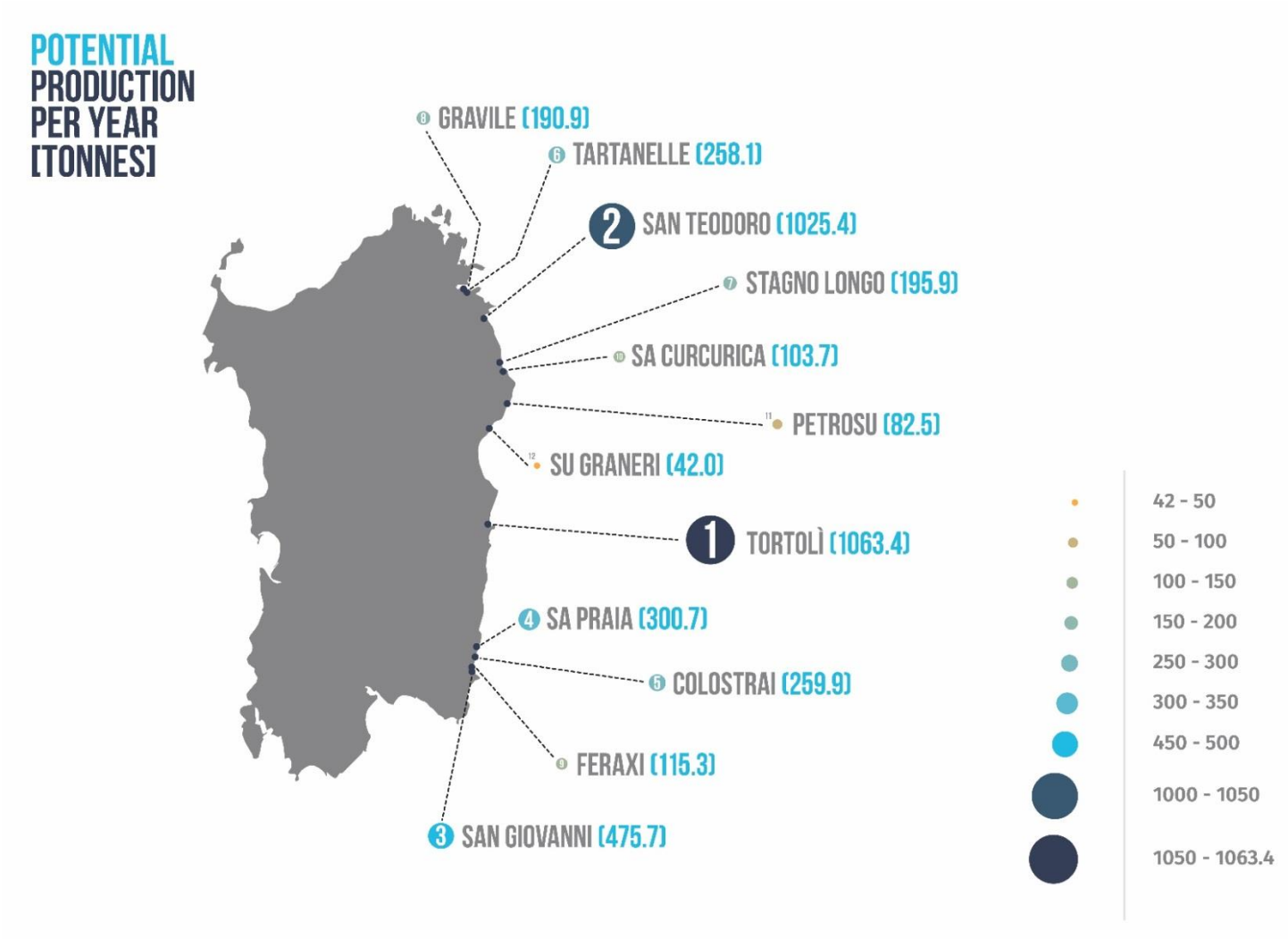


**Figure 4.11:** Total potential annual production (Tonnes). Size of the circles and numbers are indicative of production volumes.

## 4.5. Discussion

This study, focused on the selection of the most suitable coastal lagoons for Pacific oyster farming in Sardinia, and demonstrated an approach that decision makers can use to prioritise areas with potential for development and where to target resources. The approach described here is composed of two complementary processes, each providing a separate piece in the decision making system: 1) Classification of lagoon suitability based on biological and logistical criteria, combined using an MCA approach and 2) Biological data through the DEB (to give production cycles per year) and size of the lagoon to give potential productivity for each lagoon.

The analysis of biological factors allows for clear identification of potential constraints to farming linked to unsuitable bio-physical parameters, which would exclude any such site from further consideration on development of farming activities. The analysis of logistic factors and constraints, allows for detailed consideration of limiting factors for economic sustainable development, highlighting where investment may be needed and where these would be more effective to achieve production potentials. This approach, therefore, allows for the combination of multiple criteria and, using historical environmental data, generates predictions on potential productivities even where oyster farming activities have never taken place. It is interesting to note that despite the use of historical environmental data, the results presented here are consistent with the current landscape in Sardinia and the most suitable lagoons identified via the process presented here are already involved in Pacific oyster farming. Furthermore, lagoons where oysters had never been farmed, such as Sa Praia and Tartanelle, and with a relatively low logistic score (0.62 and 0.45), would appear to show annual production potentials (300 and 258 tonnes respectively) comparable or higher than other lagoons where farming already takes place and with higher suitability scores, such as such as Feraxi (115 tonnes annual potential production and 0.89 total suitability score). These data clearly indicate that potential investments and further investigation would be very valuable in those locations.

The combined modelling approach presented here can be used by industry and policymakers to identify the most suitable lagoons and resources needed to support development within them. For example, improving site accessibility in Tartanelle and Sa Praia lagoons would improve their logistic suitability and allow for easier scale up of future production. Also, granting building consent or upgrade, would improve logistic suitability and help achieve their potential annual productions. Importantly, however, the

combination of lagoon size, logistic and biological factor and ultimately production potential, would indicate that investment in some lagoons may not be appropriate. For example, due to the limited scope for production output in Petrosu and Su Graneri lagoon, despite their relatively high biological suitability scores (0.70 and 0.66 respectively) would indicate that investment may not be appropriate. Consequently, combination of lagoon suitability and growth modelling approaches can be used to highlight the most important challenges and the trade-offs to be considered for the effective use of public investment to maximise production outputs.

An important consideration in this study was the water classification for shellfish farming and the consequent critical importance of keeping a class A or B status. Indeed, the most effective way to improve logistic suitability of most lagoons would be to streamline the administrative process required for the acquisition of water classification. On the other hand, if microbiological quality of the farming water was to decline this would have immediate and severe repercussions on the overall suitability of any lagoon. Once again, this combined modelling approach helps with the prioritisation of investment towards the lagoons with the highest production potential. For instance, if all lower production potential lagoons such as: Su Graneri, Petrosu, Sa Curcurica, Feraxi, Gravile, Stagno Longo and Tartanelle (988 tonnes of combined potential production) were to be classed as C waters, the loss in potential production would be lower than 50 % of the loss that would be expected if San Teodoro and Tortolì (2,088 tonnes combined annual potential production) were to be downgraded to Class C. Once again, this consideration would urge policymakers to invest in water quality protection initiatives particularly for the most productive sites.

It is tempting to look at the potential production figures presented here and simply scale them up to include the reminder 90 % of lagoon surface area in Sardinia, and the other lagoons on the Italian national territory. By doing so, it would appear that Italy has the potential to meet the demand for Pacific oyster through domestic production, rather than relying on imports. However, not all lagoons will be suitable and differences within lagoons will also impact potential production which further highlights the need to employ methodologies such as those presented here. Equally, it would be tempting to use spatial analysis of shellfish aquaculture suitability based on its contribution to pollution mitigation (Theuerkauf *et al.*, 2019), however the approach presented here highlights the important fact that aquaculture is a food production industry and an important economic activity. Therefore, environmental services provided by this activity needs to be counterbalanced by

the requirement for the main output of this food production sector to find its place on the market, consequently prioritising pollution mitigation might limit the possibility for the product to be sold.

The strength of the combined modelling approach presented here is that it is a cost-effective and efficient way of prioritising the lagoons that are most likely to be suitable for production, and to estimate what that production could be. However, within an area such as a lagoon, there can be spatial variation in suitability and production potential (Barillé *et al.*, submitted; Gernez *et al.*, 2017). In this study, useful information on what areas within each lagoon are likely to provide better growth have been identified, however this output has been generated but using point data source and to investigate this further would require more detailed spatial datasets (grid data). Therefore, once the most appropriate lagoons have been identified as potential for Pacific oyster culture, further analysis can take place. Earth observation and remote sensing technology are becoming increasingly used and can provide data on environmental parameters relevant for oyster production at coastal (Barillé *et al.*, submitted) or farm scale (Gernez *et al.*, 2017). Additional data on other factors may have to be collected, although the development and implementation of marine spatial plans in many areas is a good source of information. To assess the long-term production potential of the sector, it may also be important to consider potential implications of climate change on the suitability of production areas for oysters.

Even when data collection and modelling is optimised it is important to consider the potential consequences of any future increase in production. Coastal lagoons are one of the most sensitive environments to biological perturbation and examples of bivalve farming contributing to dystrophic events are mostly located in coastal lagoons (e.g. Sacca di Goro lagoon, Italy; Vincenzi *et al.*, 2006). Therefore, careful monitoring of environmental impact from oyster farming, aimed at keeping stocking densities within sustainable ranges, must be integral component of any future development. Furthermore, our data did not take into account the potential for persistent pollutants or other toxic discharges from other anthropic activities into the lagoons. These would severely limit marketability of the product and suitability of the sites and potentially drastically impact on the island's production potential. Therefore, data on any toxic compounds present, their concentrations and on future risks associated with their discharge remain to be gathered and analysed.

Any increase in production will also need to be sustained by the strengthening of the entire supply chain, from seed to farming equipment availability, development of modern and

large-scale depuration units to products distribution to retailers and seafood operators. For the most part seed is currently sourced from French hatcheries; however, increased demand for seed may put unforeseen pressure on current seed suppliers. Furthermore, increased production may result in disease outbreaks, particularly of the Oyster Herpes Virus (OsHV-1µvar). It will therefore be critical that seed sourced from hatcheries possess disease free-status or will originate from selectively bred lines for disease resistance. The development of a local commercial hatchery may become a requirement and, in that case, investments towards triploids seed production to ensure sterility and the development of in-house selective breeding may be required.

Market demand and consumer acceptance is also a major factor in the economic viability of oyster production. At present, most imported oysters are sourced from France and consumers are familiar with this product so there may be a market penetration issue for locally produced oysters. Importantly, oyster farming is not formulaic and farmers' expertise is critical in the delivery of a high-quality product. Mechanisms by which the already available local and international knowledge and experience can be made available to new entries in the industry will have to be strengthened or developed from scratch to ensure that local products could compete with the currently perceived better quality of imported product. This in turn will involve branding development via specific marketing intervention. Finally, increased production and competition between local farming companies may contribute to product depreciation that could only partially be compensated by economy of scale. This would potentially have a negative impact on product value and, as a consequence, affect the profitability of the businesses involved.

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# 5. Microplastics Uptake and Egestion Dynamics in Pacific oysters, *Crassostrea gigas* (Thunberg, 1793), Under Controlled Conditions


## Abstract

Microplastics debris (< 5 mm) are increasingly abundant in the marine environment, therefore, potentially becoming a growing threat for different marine organisms. Through aquatic animals, these can enter in the human food chain, and can be perceived as a risk for consumers' health.

Different studies report the presence of particles in marketable shellfish including the world wide commercially grown Pacific oyster, *Crassostrea gigas* (Thunberg, 1793). The aim of this study is to examine the potential risk of microplastics entering in the human food chain through this shellfish species, investigating the dynamics of the uptake, egestion (faeces) and rejection (pseudo faeces) of microplastics in Pacific oysters under controlled conditions.

*C. gigas* collected from a farm in the San Teodoro lagoon (Italy), were exposed for 24 hrs to 60 fluorescent orange polystyrene particles $L^{-1}$ of known sizes (100, 250 and 500 µm). The uptake of each particle size was 19.4 ± 1.1 %, 19.4 ± 2 % and 12.9 ± 2 % respectively. After exposure *C. gigas* were left to depurate for 72 hrs, during which 84.6 ± 2 % of the particles taken up were released whilst 15.4 ± 2 % were retained inside the shell cavity. No microplastic particles were found in the animals' soft tissues.

The results of this study, suggest that depuration is an effective method to reduce presence of large microplastic particles, in the size range 100 to 500 µm, in *C. gigas*. Importantly, the data suggests that the burden that could theoretically be up taken by consumers from these shellfish is negligible when compared to other routes.


## 5.1. Introduction

Plastics are ubiquitously present throughout the world's oceans. In 2016 it was estimated that the production of plastics reached 335 million metric tonnes (Mt) globally (PlasticsEurope, 2018). In 2015, 6300 Mt of plastic waste was generated and, if plastic production trends and waste management will remain similar, it is expected that 12,000 Mt

of plastic waste will be released to the environment by 2050 (Jambeck *et al.,* 2015; Gündoğdu *et al.,* 2018).Plastics are believed to be one of the main contributors to ocean pollution with some areas of the ocean presenting very high concentrations, as a result in 2013 it was estimated that a minimum of 268,940 tons of plastics were present in the oceans (Eriksen *et al.,* 2014).

Microplastics are becoming ever more present in the marine environments due to human population growth and peoples' reliance on plastic materials. Therefore, an increase in this type of pollution is expected over the coming years and decades. Plastics and micro-plastics (particles < 5 mm in size) are part of everyday life and can be found in many products used daily such as packaging for food and drinks, shopping bags, toothbrushes and cosmetics (Cole *et al.,* 2011; Browne *et al.,* 2011; Hidalgo-Ruz *et al.,* 2012). Microplastics can be classified into primary microplastics which are intentionally produced at a microscopic scale (Costa *et al.,* 2010; Browne, 2015) and secondary microplastics resulting from the degradation of larger plastics into smaller pieces by environmental processes such as weathering and photo-oxidation (Mathalon and Hill, 2014; Gewert *et al.,* 2015).

Because primary microplastics are present in cosmetics and medical applications, a major source in the sea and fresh water bodies is waste water from depuration plants (Browne *et al.,* 2011; Cole *et al.,* 2011; Duis and Coors, 2016; Carr *et al.,* 2016).

Microplastics have been considered to be dangerous for aquatic organisms' health (Alomar, 2017). Indeed, their accumulation by ingestion can lead to increased exposure to pollutants and pathogens, and effects on physiological activities linked to nutrient uptake, growth and survival (Fendall and Sewell, 2009; Browne *et al.,* 2011; Cauwenberghe and Janssen, 2014; Sussarellu *et al.,* 2016).

Nonetheless, when environmental toxicity tests were performed in different marine invertebrates, for example in larvae of *Tripneustes gratilla* (Linnaeus, 1758) exposed to 10 - 45 µm microspheres and *Mytilus edulis* (Linnaeus, 1758) exposed to microspheres with diameters between 3 and 90 µm, it became apparent that only very high concentrations of microplastics (10,000 times higher than the maximum concentration of microplastic particles currently found in the sea water) generated significant adverse physiological effects (Duis and Coors, 2016). Still, some considerations would warrant caution since very high concentrations of microplastics have already been observed at some sites; plastics are extremely persistent in the environment and, due to further fragmentation, their presence is expected to further increase (Auta, 2017).

Von Moos *et al.* (2012) studied the effect of exposure and ingestion of microplastics (≤ 80 µm) in Blue mussel, *Mytilus edulis* (Linnaeus, 1758). These authors reported that the smallest particle sizes were accumulated in gills and digestive gland with a consequent strong inflammatory response and a lysosomal membrane destabilization. Unfortunately, no information on excretion was provided by these authors and conclusions on the fate of the larger particles cannot be made. Cole *et al.* (2011) investigated the presence of microplastics (between 1 and 10 µm) and their effect on food intake and growth of Pacific oyster larvae. They found that microplastics were ingested with only limited impact on feed intake and no consequences on growth rates being observed. Van Cauwenberghe and Janessen (2014), investigated the presence of different microplastics particles (size class 5-10, 11-15, 16-20, 21-25, > 25 µm) in farmed Blue mussel and Pacific oyster, showing that these were present in both species at concentration of 0.36 ± 0.07 particles $g^{-1}$ and 0.47 ± 0.16 particles $g^{-1}$ soft tissue, respectively. The same authors also depurated animals from the same batches for 72 hrs observing a significant reduction in the abundance of microplastics, concluding that although depuration was an effective procedure, the consumption of farmed bivalves could potentially represent a risk to consumers' health. Nonetheless, Wright and Kelly (2017), in their review of the current literature, about plastic and human health, reports that there is still no evidence that the absorption of microplastics impact on human health, but that the accumulation of these if inhaled or ingested can exert (dose-dependent) toxicity, due to factors such as the leaching of components, additives and environmental pollutants, therefore the assessment of exposure levels is fundamental.

Still, the concomitant evidence of microplastics being accumulated in bivalve soft tissue and the presence of wastewater effluent (one of the major sources of microplastics in the environment) in the same water catchment areas as shellfish farming activities deserves further studies (Rochman *et al.*, 2015). Indeed, Sussarellu *et al.* (2016) studied possible influence of microplastics (2 and 6 µm) on the physiology of the Pacific oysters, finding that oysters exposed to microplastics showed lower fecundity possibly linked to the substances leached by the microplastics during digestion process. Nonetheless, this study also indicated that although microplastics were observed in the digestive system, no tissue accumulation was observed, therefore suggesting an efficient egestion process. This may indicate that reproductive pathways could potentially be disrupted by ingestion of microplastics but this may not necessarily be linked with their physical presence and accumulation in the digestive tissues.

The presence of microplastics in commercially relevant bivalves, including Pacific oysters, has been reported by different studies (Von Moos *et al.*, 2012; Van Cauwenberghe and Janessen, 2014; Li *et al.*, 2015; Cole and Galloway, 2015; Pont *et al.*, 2016; Silva *et al.*, 2016; Sussarellu *et al.*, 2016; Phuong *et al.*, 2018; Fernández *et al.*, 2018). Two main objectives have been pursued by previous investigations: 1) determination of the presence and the abundance of microplastics in individuals collected from the wild, farms and retailers to establish potential risks for consumers; 2) the determination of the potential adverse effects to animals' physiology caused by the exposure to plastics under controlled conditions.

However to date, there is still limited knowledge on the relationship between plastics uptake and egestion (Van Cauwenberghe and Janessen, 2014). Therefore, the first aim of this present study was to investigate the adult oysters' egestion dynamics after exposure to known concentration of microplastics under controlled conditions. Moreover, previous studies have so far used microplastics of sizes comparable to phytoplankton cells. However, in the marine environment, microplastics are present in sizes often larger than microalgae cells and there are evidence suggesting that bivalves could potentially up-take particles as large as 500 µm (O'Donohe and McDeromtt, 2014). Still, no information on the ability of oysters to uptake, retain and egest larger particles is currently available. Consequently, the second aim of this study was to determine whether larger particles had the potential to remain in the marketable product post depuration by employing sizes larger than those commonly used in previous microplastics absorption studies. The size classes of 100 ± 7.42, 250 ± 23.2 and 500 ± 52,34 µm were chosen because Van Cauwenberghe and Janssen (2014), found that *Crassostrea gigas* reared in the Atlantic Ocean (average shell length of 9.0 ± 5.0 cm), showed a prevalence of microplastics size > 25 µm, and because studies on mussels and Pacific oysters so far were focused only on microplastics of a size comparable to phytoplankton or in general at size between 0.5 and 90 µm (Browne *et al.*, 2008; Von Moos *et al.*, 2012; Van Farrell and Nelson, 2013; Cole and Galloway, 2015; Cauwenberghe *et al.*, 2015; Sussarellu *et al.*, 2016), without taking in to account that in the marine environment microplastics are present in different sizes and adults' Pacific oysters can uptake larger size microplastics from the environment.

## 5.2. Materials and Methods

### 5.2.1. Pacific oyster source and experimental set-up

Pacific oysters (20 oysters 85 ± 2.3 g ind.$^{-1}$) were collected from a farm in the San Teodoro Lagoon (Italy) (40°48'39.18''N, 9°40'24.42''E), and kept in a cold box until arrival to the laboratory. Oysters were then transferred to an aerated rectangular tank and left to acclimatize for 48 hrs at 22 °C and 36 ppm (Choi *et al.,* 2008). For the purpose of this study, oysters were individually deployed in 20 x 1.5 L glass spherical aquariums filled with filtered sea water.

With the aim to keep the water in movement each aquarium was supplied with an air-stone connected to a valve and an air pump. Water temperature, salinity and dissolved oxygen were monitored and maintained (by daily water exchange) respectively at 22 °C, 36 ppm and 8.5 mg L$^{-1}$.

Preliminary trials were performed to determine both the level of aeration required and the most suitable type of microplastics polymer. For this purpose, three polymers of the following densities were tested: polystyrene 1.04-1.1 g cm$^{-3}$; polyamide 1.12-1.15 g cm$^{-3}$; polycarbonate 1.20-1.22 g cm$^{-3}$ (Enders *et al.,* 2015; Avio *et al.,* 2016). With the aim to keep the microplastics beads suspended in the water column to maximise their chances to be filtered by the oysters, batches of 30 microplastics per polymer were deployed to an experimental tank and aeration was adjusted by a valve. Once the appropriate aeration was identified by observing the microplastics distribution on the water column, the ability of the chosen polymer to withstand the tissue digestion procedure (Li *et al.,* 2015) was tested. This was conducted using a sterile container containing soft tissues of 3 Pacific oysters (80 ± 3.5 g ind.$^{-1}$) plus 9 plastic beds per size class (100 ± 7.42, 250 ± 23.2 and 500 ± 52,34 µm) of the microplastics chosen for the study (3 replicates). The soft tissue was covered with hydrogen peroxide 15 %, this was added until the oyster was completely digested (Avio *et al.,* 2015).

Once the oysters were digested the remaining solution was filtered using 47 mm Whatman GF/F filters (0.6 – 0.8 µm) and then analysed under the dissecting microscope (Leica Mz8).

### 5.2.2. Microplastics

The selected microplastics were fluorescent polystyrene microspheres purchased from Degradex Hopkinton (MA 01748). These particular beads were selected because of their colour (fluorescent orange with Excitation/Emission 530/582 nm) and because their density was similar to seawater (UNESCO,1981, Capolupo *et al.,* 2018).

Three microplastics sizes were used: 100 ± 7.42, 250 ± 23.2 and 500 ± 52,34 µm (fig. 5.1A) and 600 microplastics of each size, were individually counted under a stereo microscope (Leica MZ8), using an UV lamp (Surenhap 100 LED) to enhance fluorescence (fig. 5.1B), and micro-dissecting tweezers (World Precision Instruments, FL 34240-9258 USA).

Beads were then allocated (thirty beads per size) to twenty 1.5 ml Eppendorf tubes, (fig. 5.1C).

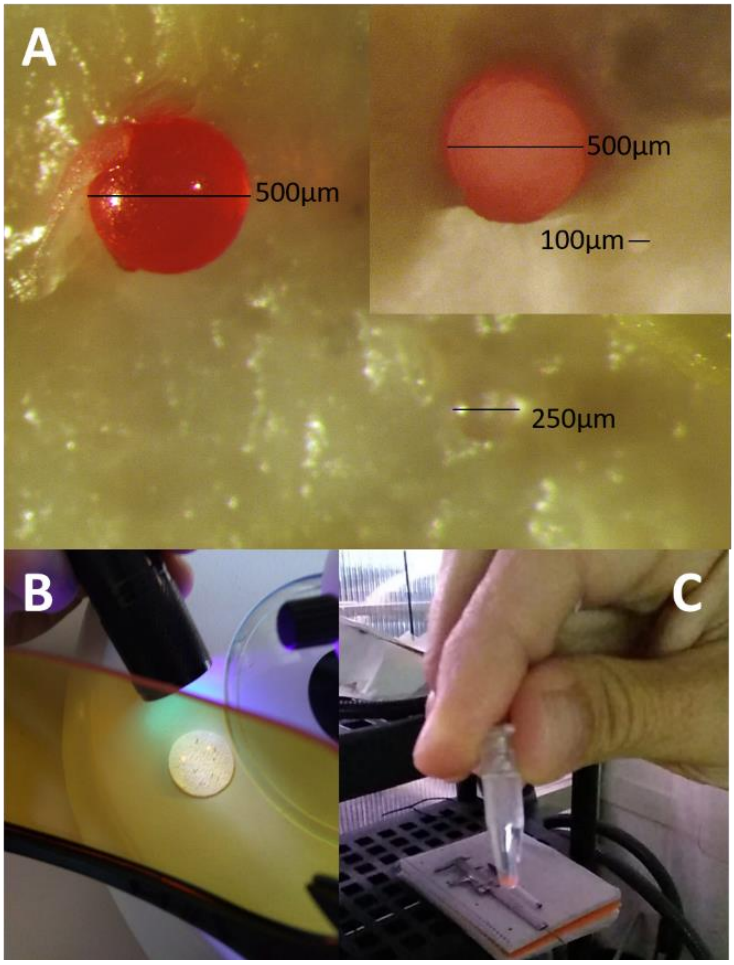


**Figure 5.1:** A. Different microplastic particle sizes used during this study. Picture was taken on a 47 mm GF/F filter; B. 500 µm microplastics on a 25 mm GF/F filter with fluorescence enhanced by a UV light; C. Microplastics mix composed by 30 microplastics per size class (100, 250 and 500 µm) ready to be deployed for the exposure trial.

### 5.2.3. Exposure and Microplastics uptake

The experiment was carried out in 2 parts: 24 hrs exposure (Cole and Galloway, 2015) and 72 hrs depuration (Van Cauwenberghe and Janessen, 2014). During the first 24 hrs experimental individuals (n=20) were individually exposed to 30 microplastic particles of each size (100, 250 and 500 µm) with a density of 60 particles per litre. This particles density despite being higher than the ones commonly reported in sea water (De Lucia *et al*., 2014) was chosen for analytical and practical reasons.

At the end of the exposure period the aeration was stopped and each oyster was collected using long tweezers, oysters and tools were carefully observed using a UV lamp to increase beads fluorescence and washed taking care that no microplastics adhered to the oysters' shell and to the tools used. The water used for the exposure was, at this point, filtered through a 47 mm GF/F filter using a filtration unit Millipore and a vacuum pump. Again, all filtration equipment was checked for the presence of adhered beads. Post filtration each filter was individually stored inside labelled 50 mm petri dishes. Uptake was measured subtracting the final number of beads recovered onto the filters from the initial number used for exposure.

### 5.2.4. Depuration and egestion

The oysters collected after exposure were transferred to a new tank, again filled with 1.5 L of filtered sea water. Aeration was not supplied in order to avoid faeces and pseudo-faeces mixing.

At 24 hrs intervals over a total of 72 hrs, each oyster was removed from each tank using the same procedure described earlier, and transferred to a new tank under the same environmental conditions.

The water left in the original tank during the 24, 48 and 72 hrs after exposition, was filtered and beads counted using the same procedure described before.

Finally, at the end of the trial (72 hrs after exposure) oysters were collected from the experimental tanks and externally washed and dissected taking care that the water contained in the shell cavity was stored in a plastic tray.

The Digestive gland, gills and mantle of each oyster were dissected, washed and placed in labelled sterile containers. The water contained in the shell and the water used to wash the tissues was collected and filtered as described previously.

All dissected tissues of each individual were digested using hydrogen peroxide 15 %, at room temperature of 22 °C for 7 days, and the resulting digestate was filtered as described previously.

### 5.2.5. Statistical Analysis

Prior to analyses, percentage data were arc-sin transformed, and all data were checked for normality (Shapiro's test using Minitab v.18) and homogeneity of variance (Lavene's test using Minitab v.18). Uptake and residual microplastics post depuration data were analysed by one-way ANOVA followed by post-hoc Tukey's Multiple Comparison tests where significant differences occurred. Egestion over time for particles of all sizes was analysed by general linear model followed by a Tukey post-hoc test where significant differences occurred.

Statistical analyses were performed using Minitab v.18 with a significance level of 5 % ($p$ value $< 0.05$). All results are presented as mean ± SE.

## 5.3. Results

### 5.3.1. Microplastics uptake

At the end of the 24 hrs exposure, the uptake (% of missing beads) of the different sizes (100, 250 and 500 µm), was 19.4 ± 1.1 %, 19.4 ± 2 % and 12.9 ± 2 % respectively. No significant difference in uptake between the microplastics of 100 and 250 µm was observed, however beads of 500 µm in size had a significant lower uptake when compared with the others sizes (*p* value = 0.009; DF = 2; F = 5.13) (fig. 5.2).

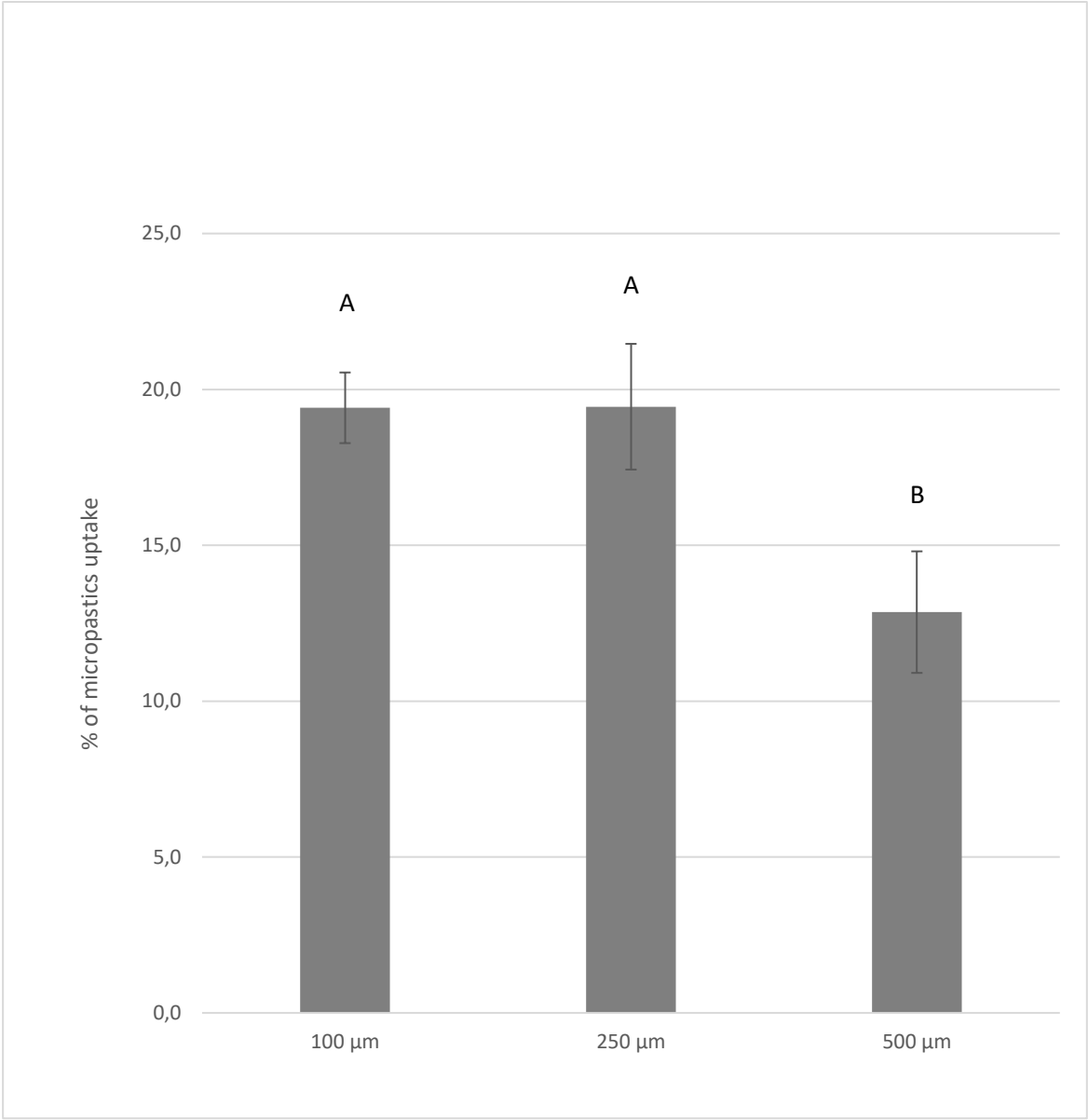


**Figure 5.2:** Uptake of the different microplastic particle size classes from ambient water. Significant differences (*p* value < 0.05) are showed by different letters, results are presented as mean ± SE; n=20.

### 5.3.2. Depuration and egestion

Table 5.1 illustrates the percentage of microplastics recovered from the depuration water, and tissues at the different time points over the depuration period. A significant effect of time (*p* value < 0.001; DF = 2; F = 178.67) and a significant interaction between time and treatment (*p* value = 0.001; DF = 4; F = 4.9) was observed. The excretion of microplastics beads of all sizes was significantly higher during the first 24 hrs in comparison with the later time points. Furthermore, no significant difference was recorded in the excretion of microplastic particles of 100 µm and 500 µm between 48 and 72 hrs of depuration, whilst significantly more beads of 250 were released after 48 hrs in comparison to 72 hrs of exposure (fig. 5.3).

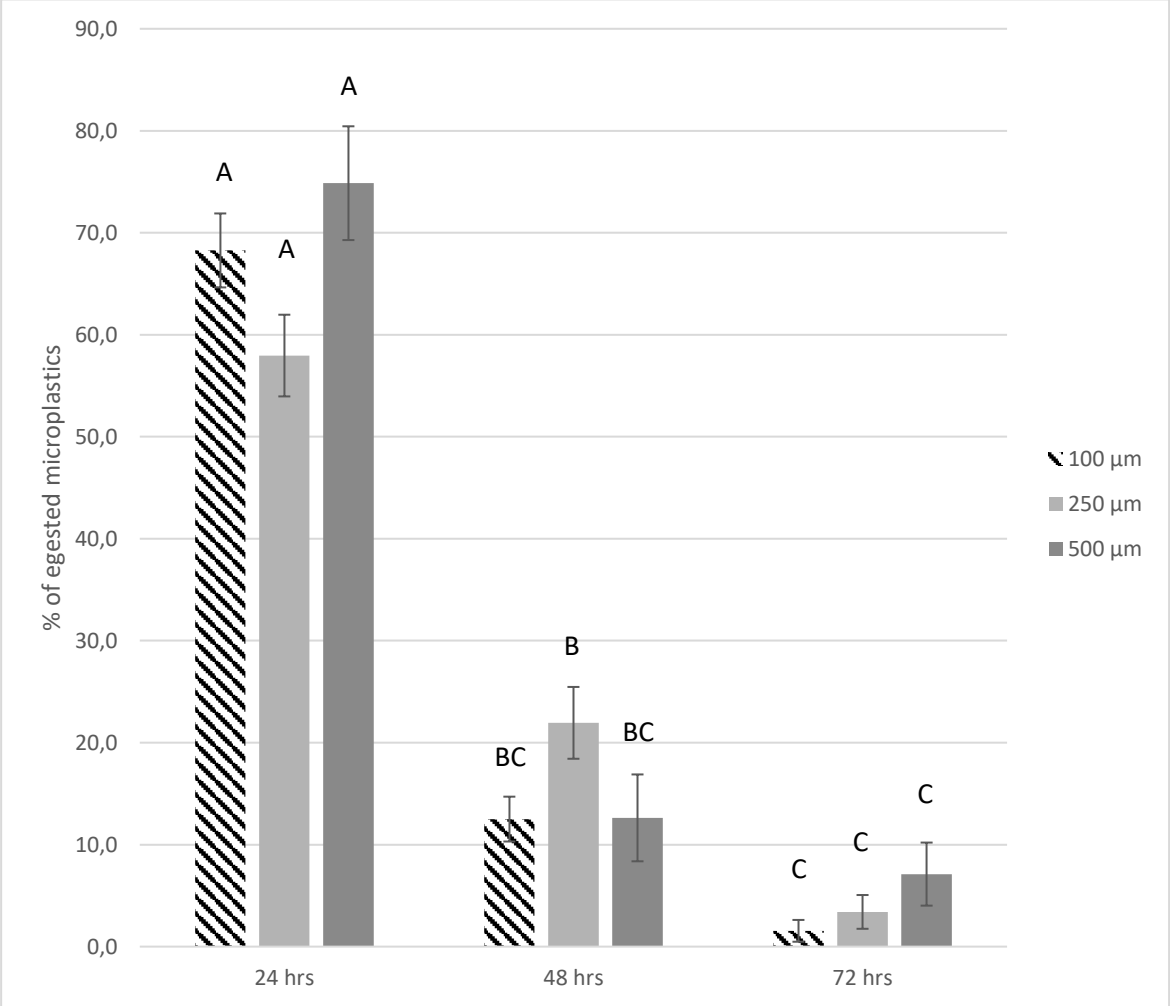


**Figure 5.3:** Egestion dynamics of the different microplastic particle sizes. Significant differences (*p* value > 0.05) are showed by different letters, results are presented as mean ± SE; n=20.

Although the vast majority of ingested microplastic particles were released during the 72 hrs of depuration, 17.7 ± 3.8, 16.7 ± 2.4 and 5.4 ± 2.7 % of microplastic particles of 100,

250 and 500 µm respectively were still present in the water contained inside the shell cavity. At this location a significant difference in the abundance of each particle size class was observed, with the largest size class being significantly less abundant than the other two (*p* value = 0.007; DF = 2; F = 5.68) (fig. 5.4). Importantly, no microplastic particles were found in the digestive gland and in the other tissues post digestion.

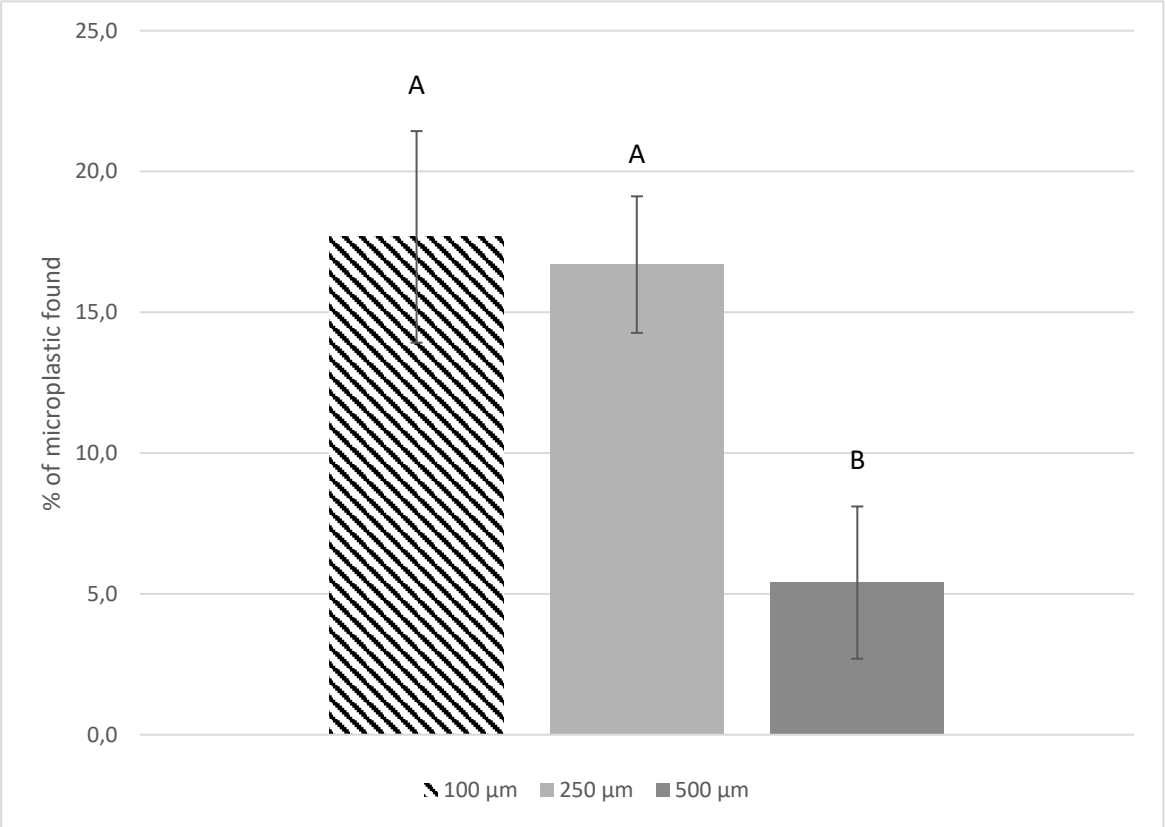


**Figure 5.4:** Residual microplastic particles of the different sizes post depuration. Significant differences (*p* value > 0.05) are showed by different letters, results are presented as mean ± SE; n=20.

Taking into account each time step there was a decreasing egestion of microplastic particles during the depuration time: 63.9 ± 3 %, 17 ± 2.2 % and 3.7 ± 0.9 % in 24, 48 and 72 hrs, respectively. Only 15.4 ± 2 % of the microplastic particles were retained within the oysters after 72 hrs of depuration (Tab. 5.1).

**Table 5.1:** Summary of the percentages of egested beads during 72 hrs depuration, and percentage of beads retained in the internal cavity post depuration. divided by sizes.

| *Microplastics beads egested and non-egested in:* | *100µm* *%* | *250µm* *%* | *500µm* *%* | *Mix* *%* | *Mix* *%* |
|---|---|---|---|---|---|
| *24 hrs* | *68.3 ± 3.6* | *58 ± 4.0* | *74.9 ± 5.6* | *63.9 ± 3.0* | |
| *48 hrs* | *12.5 ± 2.2* | *21.9 ± 3.5* | *12.6 ± 4.3* | *17 ± 2.2* | *84.6 ± 2* |
| *72 hrs* | *1.5 ± 1.1* | *3.4 ± 1.7* | *7.1 ± 3.1* | *3.7 ± 0.9* | |
| *Internal cavity* | *17.7 ± 3.8* | *16.7 ± 2.4* | *5.4 ± 2.7* | *15.4 ± 2* | *15.4 ± 2* |
| *Digestive gland* | *0* | *0* | *0* | *0* | |
| *Other soft tissues* | *0* | *0* | *0* | *0* | |

## 5.4. Discussion

The aim of this study was to investigate the uptake and egestion dynamics of known sizes (100, 250 and 500 µm diameter) of microplastic particles in Pacific oysters, during a 24 hrs exposure and a subsequent 72 hrs depuration period. Depuration is a common practice in bivalve aquaculture whereby bacteria are egested to comply with European food safety legislation (regulation 853/2004, 852/2004 and 2073/2005) (Doré and Lees, 1995; Martínez *et al.,* 2009; Who, 2019). In this study, Pacific oysters showed an efficient egestion rate, egesting 84.6 ± 2 % of the microplastic particles taken up, while only the 15.4 ± 2 % of beads taken up were retained within the shell cavity, post depuration.

To date, studies on microplastic uptake have been conducted mainly to investigate their potential negative physiological effects on marine live, including bivalves, or to establish whether animals entering the human food chain could be a carrier of these particles and therefore represent a risk for consumers (Von Moos *et al.,* 2012; Van Cauwenberghe and Janessen, 2014; Sussarellu *et al.,* 2016; Pont *et al.,* 2016; Silva *et al.,* 2016; Fernández *et al.,* 2018). The main difference between these approaches has been the controlled nature of the studies. The former employed controlled conditions (known density, type and size of the microplastics employed), whilst the latter focused on the abundance of plastics in marketable products without considering levels of exposure, uptake or the nature of the polymers.

In contrast, our study investigated both the uptake and egestion dynamics under controlled conditions to more robustly describe the fate of microplastic particles of 100 to 500 µm diameters during exposure and depuration therefore contributing to the collective knowledge on these dynamics in shellfish produced for human consumption.

Amongst the studies focused on the risks for consumers, the one conducted by Van Cauwenberghe and Janessen (2014) provides the only comparable platform for the interpretation of the results presented here. Comparison of the studies shows a slight difference in egestion rate post-depuration (74.5 % *vs* 84.6 ± 2 %), this can be attributed to the difference in materials and diameters of the particle used and by the food sorting mechanisms of the Pacific oysters which discriminates not only based on size but also based on chemical cues present on the surface of the particles (Ward *et al.,* 1997; Kiørboe, *et al.,* 2012).

In this study no microplastic particles were observed within the oysters' tissues, while in the Sussarellu *et al.* (2016) study, microplastic particles were found in the stomach and the intestine of Pacific oysters. This can be attributed to the difference in the particle size used (100, 250 and 500 *vs* 2-6 µm), and it is possible that the *C. gigas* food sorting mechanisms recognise only the smaller size as a food source due to similarity in size with phytoplankton (Ward and Shumway, 2004). However, different studies point out that bivalve can ingest larger particle size. For instance, blue mussels can ingest early larval stages of sea lice, *Lepeoptheirus salmonis* (Kröyer 1837), with an average size of roughly 500 µm. Furthermore, during a microplastics survey conducted in the Dutch North Sea, the presence of large plastics (up to 5 mm in size) was also observed in Pacific oysters (Molloy *et al*., 2011; Leslie *et al.,* 2013; O'Donohe and McDeromtt, 2014). Our results suggest that these larger particles could probably be filtered by the oysters but, instead of being ingested, they are retained within the shell cavity by adhesion. Therefore, with the assumption that in the marine environment microplastics of different size have the potential to be accumulated in marketable bivalves (Andardy, 2011; Koelmans *et al.,* 2015), the present study further clarifies the uptake and egestion dynamics of larger particles and the associated potential risks for consumers.

Importantly, during the depuration period, microplastic particles were observed in faeces and pseudo faeces, but it is not possible to conclude here that the beads have been ingested, because these were not observed within the digestive system. Further work focused on the ingestion and excretion of microplastic particles of different sizes class, including particles larger than microalgae cells, should be conducted to estimate gut transit time of these particles.

In conclusion our data, taken together with results from other studies, strongly indicate that *C. gigas* could be a carrier of different microplastic sizes in the human food chain, not only through the absorption and inclusion in tissues (Bricker *et al.,* 2014; Van Cauwenberghe and Janessen, 2014; Li *et al.,* 2015; Bouwmeester *et al.,* 2015; Rochman, *et al.,* 2015; Wright and Kelly, 2017), but also through the adhesion of these particles in different parts of the internal cavity of the oysters shell. Nonetheless, the exposure density of 60 microplastics $L^{-1}$ used in this study, is higher than the density of microplastic particles (< 5 mm) commonly reported in coastal Mediterranean Sea areas 5 $*10^{-4}$ microplastic particles $L^{-1}$ (De Lucia *et al*., 2014). Assuming that the uptake for all sizes observed in this study (16.2 ± 1.2 %) is applicable to the wider farming context, the number of particles filtered by each individual would be 1.2 $*10^{-4}$, which would become 4.3 $*10^{-5}$ per individual after

24 hrs depuration. This final microplastic burden can be considered lower if compared with the number of microplastic particles found by Schymanski *et al.* (2018) contained in drinking water (from 11 ± 8 to 118 ± 8 particles $L^{-1}$ depending on the type of package). Therefore, the risks for consumers can be considered negligible for the particle size tested if compared to the amount of microplastic particles that can be uptaken in everyday life.

Moreover, it is important to consider that this study was made under laboratory controlled conditions and when taking into account the wider context of a marine environment other factor must to be taken into account. Different studies, reported that microplastics size and shape and surface proprieties can affect the ingestion and egestion of plastic particles in oysters (Ward and Shumway 2004; Rosa *et al.*, 2018; Ward *et al.*, 2019a). In a recent study, of Ward *et al.* (2019b) oysters were exposed to polystyrene microspheres (19-1000 μm) and to nylon microfibers (length 75-1075 μm x diameter 30 μm). The results of the experiment show that 10 to 30 % of the smallest and 98 % of the larger microspheres were rejected. Despite, the similar proportion of large microfiber and microspheres ingested, there was a lower number of large microfibers rejected (~ 60 %) compared to the large microspheres rejected (98 %), demonstrating that microplastics size and shape can affect the ingestion and egestion of plastic particles in oysters. Other factors that can affect microplastics uptake in the environment are microplastic concentration and the presence of natural particles (i.e. food, suspended sediment), these can reduce the microplastic uptake (Kaposi *et al.*, 2014; Scherer *et al.*, 2017; Capolupo *et al*., 2018), moreover microplastics in marine environments can be colonise by micro and macro organism (Lobelle and Cunliffe, 2011) creating biofilms that could improve the palatability of these and therefore increasing the ingestion by some marine species (Hodgson *et al*., 2018).

Pacific oysters are farmed world-wide for human consumption, and microplastic particles are widely distributed in the environment and therefore available to filter feeders. However, after depuration the number of microplastic particles decreased significantly suggesting that this standard procedure is an effective method to reduce the presence of larger microplastic particles in marketable Pacific oysters even when no depuration would be compulsory due to sanitary reasons such in the case of class A waters.

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# 6. General Discussion

## 6.1. Thesis Summary

The results of this PhD study provide new information on how to improve *C. gigas* sustainable aquaculture industry in Mediterranean coastal lagoons, with particular focus on the Italian territory where Pacific oyster culture is underdeveloped compared to other European countries, but with great growth potential.

Italy has the potential for increasing the size of this industry, mostly due to the fact that many coastal areas and lagoons present suitable environmental conditions that allow for good growth rates and optimal market quality (shell shape, flesh colour and Condition Index). Moreover, Italy and in particular Sardinia, features many coastal lagoons currently not utilized for shellfish farming. Therefore, the current production of Pacific oyster does not meet market demand. Consequently, Italy imports most of the oysters consumed locally from nearby producing countries.

In order to improve shellfish farming activities and in particular Pacific oyster production, the focus of this study was defined taking into account local aquaculture industry's ambitions and challenges. These are related to the relatively small outputs linked to traditional farming protocols and the limited availability of new spaces for industry expansion. Indeed, lack of specific knowledge on the suitability of coastal areas and lagoons and the potential conflicts between farming and other anthropic activities and their impacts (including microplastic release to the coastal environment) has so far limited investments into initiatives aimed at expanding production. Therefore, in this PhD study different Pacific oyster culture aspects were investigated with the aim to fill the current knowledge gaps limiting expansion of this sector:

- Novel farming tools and their benefits and implications on animals' growth, survival and production planning were investigated (Experimental chapter 4).
- Validations and applications of growth models able to reliably predict animals' performances in a new environment was carried out (Experimental chapter 5).
- Data generated in chapter 5 were used to develop site selection approaches for Pacific oyster farming in the local context (Experimental chapter 6).
- Finally, potential risks to consumers linked to microplastics uptake in Pacific oyster were investigated (Experimental chapter 7).

Importantly, the local industry had never before experienced close collaboration with academic institutions and the methodologies adopted in these studies had never before been applied in the local context. This has provided an opportunity to bolster local industry and academia collaboration and ensured that outputs were directly relevant to the local users. Through field work, laboratory work and collection of existing data, this study represents an important step to promote the sustainable intensification of Pacific oyster production in Sardinia. It provides new tools and knowledge to local stakeholders and the wider scientific community. Furthermore, the results obtained during this project are not only applicable to the local environment but also more widely to other contexts.

This PhD study is part of a Sardinian Government funded project on Pacific oyster farming (Ostrinnova), and the majority of the outcomes have already been shared through scientific publications, technical reports, and during stakeholders workshops and meetings with policy makers, raising awareness on the potential opportunities offered by the sustainable development of the local industry and the challenges that this industry and its regulators will be facing.

Following this thesis summary, the main outcomes, limitations and implications for industry and stakeholders will be summarized in paragraph 8.2 and 8.3 respectively. In the last part of the conclusion sections, future work will be discussed in paragraph 8.4 followed by concluding remarks (8.5).

## 6.2. Summary of findings

This PhD study was performed with the aim of improving C. *gigas* farming in Mediterranean coastal lagoons. This was done by investigating the effect of novel farming gear on the growth and survival of this species; by validating the ShellSIM® growth model in two different lagoons and when different farming tools were employed; by the development of a lagoon suitability assessment methodology; and, finally, by investigating the microplastics uptake and egestion dynamics in marketable size oyster.

### 6.2.1. Effect of Ortac units on the growth and survival of Pacific oysters

The effects of Ortac units on the *C. gigas* growth and survival were investigated during a production cycle performed simultaneously on Ortac units and floating bags. These last tools are the traditional farming tools used in shallow Sardinian coastal lagoons.

It was decided to compare the growth performance of both these tools, in order to obtain information to establish if the Ortac unit could have significant benefits on oyster farming in Mediterranean lagoons that could justify their use instead of or in parallel with the floating bags.

The main findings of this first study showed that at the end of the trial, there was a significant higher growth in weight and condition index on the animals reared inside the floating bags (55.8 ± 0.9 g, 50.1 ± 1.3 g; 4.6 ± 0.1 %, 3.9 ± 0.1 % respectively on floating bags and Ortac units), while there was a higher survival rate (in the first two months of the production cycle) on the animals farmed inside the Ortac units (95.8 ± 0.9 %, 82.1 ± 3.4 % respectively on floating bags and Ortac units).

This suggests that in order to improve the farming protocols in this type of environment, both tools can be employed during the production cycle, Ortac in the initial stage to boost survival and floating bags thereafter to improve growth and market acceptance. This combined approach would result in reduced mortality during the early production stages without compromising growth rate and oyster quality.

### 6.2.2. ShellSIM® growth model validation

This study represents the first attempt to employ growth modelling tools in the local context and provided new and useful insight into spatial and production planning. The predictions made by ShellSIM® growth model, run to predict growth in two different coastal lagoons on different farming tools (Santa Gilla and San Teodoro lagoons on Ortac, floating bags and lanterns), fitted well the observed data in the San Teodoro lagoon, while in the Santa Gilla lagoon the growth was widely overestimated by the growth model. Overall, this study provides new information to improve bivalve growth prediction tools for Mediterranean lagoons useful to study the productivity of different sites. However, some discrepancies between predicted and observed data were recoded, providing opportunities for additional studies aimed at improving our understanding of the nutrient sources available to the growing animals, how these nutrients are utilised (digestible energy content), and finally how energy derived by these nutrients is modelled.

### 6.2.3. Site selection process

The developed methodology for site selection in this study showed that biological factors were suitable for oyster farming in all of the lagoons where this procedure was applied, while the logistic suitability scores were acceptable. The combination of these factors with the potential constraints, suggested that the water quality it is an important factor to improve the suitability of the different sites, and an appropriate monitoring plan for water quality will be fundamental to improve this industry.

Moreover, the site suitability and the potential productivity of the investigated lagoons, give a powerful tool to assist the site selection process and this is a key factor for improving the Pacific oyster farming industry.

### 6.2.4. Microplastics uptake and egestion dynamics in Pacific oysters

Microplastics are pollutants expected to increase in the coming years. These can be perceived as a risk for human health, due to the fact that shellfish farms are often close to wastewater treatment plants that discharge directly in to the coastal lagoons where these activities are carried out.

The results of this study, performed under laboratory condition, to investigate for the first time the microplastics uptake and egestion dynamics in marketable size Pacific oysters, showed that depuration is an effective procedure to reduce the amount of large microplastics that could be theoretically uptake in the environment by this shellfish. Moreover, through these animals microplastics theoretically can enter in the human food chain.

Most of the microplastics uptake by *C. gigas* were egested in the depuration period (this is an obligatory practice in farms were water is classified as class B). Therefore, depuration can greatly reduce the number of microplastics of size between 100 and 500 µm. This suggests that depuration protocols should be normal procedures even when not required due to sanitary reasons such as in the case of class A waters.

## 6.3. Implications for Industry and stakeholders and limitation of this PhD study

### 6.3.1. Implications for Industry and stakeholders

The results of this PhD study give important information to increase Pacific oysters farming in Sardinian coastal lagoons. The impact of this study is already observable in the Sardinian region, indeed ShellSIM® growth prediction and Ortac units are already part of several new government projects. These projects have been developed with the scope to start new Pacific oyster farms in new sites in order to show to the stakeholders the standard procedures of this farming activity.

The advantage of using the Ortac unit in the early stages of production is important from a commercial viewpoint due to the higher survival rate but also because the use of the Ortac units in the first period of the production cycle gives the opportunity to reduce cost and manual work. In fact, the Ortac units, thanks to their swinging produced by the action of the currents need less handling for biofouling deposition clearance and no change in mesh (*pers. obs*.). Moreover it is important to underline that in the Ortac units *vs* Floating bags trial (Chapter 2), the oyster reared using the Ortac units at the end of the production cycle are slightly smaller in weight compared to the one reared in the floating bags, 50.1 ± 1.3 g and 55.8 ± 0.9 respectively, and this from a commercial viewpoint is a negligible difference, due to the fact that this difference in weight (~5g) can be gain in less than a few weeks and that the use of Ortac units during the early stages of the production cycle can have the previously discussed benefits. Furthermore, the mixed use of the two farming tools, it is also important due to the fact that the Pacific oysters grown in the floating bags have a teardrop shape, that is the oyster shape that gives an higher market value to the *C. gigas*, while in the Ortac units the oyster tend to grow with an elongated shape, that can cause a loss of marketability (Heath and Wilson, 1999).

The local stakeholders are looking for new farming protocols/tools to improve the production outputs. Indeed, the LAORE Sardinian government agency, has setup a new experimental farm in Cabras Lagoon (Oristano), to continue the work outlined in this PhD study, they requested the expert support for improving the farming protocols by using both Ortac units and floating bags. A new project sponsored with European funds for aquaculture and fisheries by FLAG (Fisheries Local Action Group) will involve the use of the Ortac units and ShellSIM® in two new sites in the south of Sardinia (Porto Pino and Nora lagoons).

Growth modelling is a key step for stakeholders to improve shellfish farming, the output of these will give important information on planning the production depending on market request and production cycle length. Moreover, growth model prediction as well as giving information within each lagoon can be used in the site selection process.

One of the main elements important for the expansion of the shellfish farming industry is the possibility to identify new suitable areas. This is important both on a small scale for example different areas within a specific site, or on a larger scale as for example between different lagoons or sites.

In this PhD study a site selection methodology for choosing Pacific oysters farming sites was developed. This is an important step within Sardinian territory because there are many lagoons that are potential *C. gigas* farming sites, but there is still not a specific spatial plan for this relatively new industry in the region. This methodology will help policy makers during costal spatial planning, but at the same time will help farmers to choose the most appropriate sites.

Due to the industry's needs of knowing the potential production of each site, the site selection process was implemented with the output of a DEB growth model to give not only information about the suitability of the site for Pacific oyster, but also their potential productivity. With this new tool, stakeholders will have prediction of the potential productivity of each site and within different areas of each site.

During stakeholder consultation, water quality control and pollution was amongst the needs clearly raised and identified as blocker to improvement and expansion of Pacific oyster farming in Sardinia. Indeed, many of the Sardinian lagoons are close to urban areas and therefore in proximity of wastewater treatment plants that discharge directly in coastal areas. This fact is obviously perceived as a risk factor for the shellfish industry, and it has already happened that waters that were previously classified as class A or B, after several years were downgraded to class C.

In recent years, associated to waste water plants and to shellfish consumption, there is the emerging problem of the microplastics presence in the environment. Many newspapers and non-scientific journals use alarming headlines about microplastics in Shellfish (BBC.com, 2019; Indipendent.co.uk, 2019), and this fact can cause concern to consumers and consequently to the shellfish aquaculture industry.

The result on the uptake and egestion dynamics of microplastics in Pacific oyster presented in this thesis, build on increasing evidence that bivalves are poor indicators of microplastic uptake (Ward *et al*., 2019) and a weak if not negligible contributor the potential trophic transfer of this type of pollution into the human food chain (Rist *et al.*, 2018). This growing body of evidence reassures producers that current depuration methods already employed by the industry are efficient in reducing microplastic associated perceived risks and suggests that depuration should be carried out even when this is not compulsory due to sanitary reason as in the case of class A waters.

### 6.3.2. Limitations of the study

The mixed use of the two farming tools, Ortac at the beginning and floating bags later, permit the improvement of the production cycle, but before these "new" tools can be fully adopted other factors must to be taken in to account. Ortac units were developed to work under oceanic currents and tides conditions, therefore these must be adapted to be used in a Mediterranean shallow lagoon contest.

In the present study, a system made of water pipes (fig. 6.1) was built up in order to keep the Ortac units on the water surface where more nutrients are present, but at the same time this structure permits keeping these tools out of the water in order to simulate ocean tides (more details in Chapter 2).

**Figure 6.1:** The structure in the picture was made with the aim to keep the attached Ortac units on the water surface, but at the same time to give the possibility to keep them out of the water simulating the ocean tides.

This structure is a limiting factor for the use of Ortac units in coastal lagoons. They take up more space than a rope with floating bags attached, and they require more maintenance. In tourist places it can also become a problem of visual impact.

The ShellSIM® growth model, was developed with the aim to have a cost-effective prediction tool to be use by farmers, regulators, teachers and scientists (Shellsim, 2011). However, in this study was seen that this requires a large data set of environmental factors to provide accurate predictions. In some cases, this fact can be a limitation on the use of this growth model due to the amount of time required to gather the data and the associated sampling and analysis cost. Moreover, it was seen that in one of the lagoons in which the model was tested, the output prediction was overestimating the real growth, probably due to the different energy content provided by the POC to the animals (see experimental

chapter 5). In lagoons where POC values are high (therefore an important source of energy for the Pacific oysters) the ShellSIM® prediction could be inaccurate in terms of production cycle length, but at the same time the growth model is still able to describe growth trends within each lagoon giving important information on how to plan production.

In this PhD work, the site selection procedure was applied only to a small number of Sardinian lagoons, due to the fact that the environmental parameters used to perform the biological score and run the DEB growth model, were collected from historical data provided by the government. This data could only be applied for the twelve lagoons, because for the other lagoons most of the important environmental data were missing. Theoretically, collection of environmental parameters could be implemented by using satellite images, but the resolution of such images is still not sufficient to accurately describe the lagoons water biological features for the scope of this study.

The result of the microplastic study of this PhD project showed that depuration is an effective method to reduce large microplastics particles in farmed Pacific oyster. However, this study was conducted on microplastics size between 100 and 500 µm, and microplastic size in the environment can vary between 5 mm and 1 µm (or less depending on different definition used in literature). This is a limitation of this study, indeed similar investigation needs to be carried out on smaller and larger microplastics size, and investigate the effect of depuration protocols for these sizes. Moreover, due to the lack of independence between treatments in this trial (Oysters were exposed to a mix of microplastics at the same time), it would have been more appropriate to add independent bead size exposure treatments to the experimental design, to investigate the uptake of each microplastics size independently from the others. Although this would not reflect the real conditions to which oysters are exposed in their environment.

## 6.4. Future Directions

The results of this PhD study leads to new questions providing the opportunity to increase the body of evidence required to boost the Pacific oyster farming industry.

In the last few years in addition to Ortac units, several new farming tools have been developed, for example OysterGro© (OysterGro) and Zapco Tumbler (Zapco Aquaculture). These systems aim at reducing manual labour, increasing growth rates and improving oysters' quality. In this study, due to time and industry constraints, it was only possible to test the Ortac units.

Moreover, a problem encountered with the use of the Ortac units was the time, space and costs associated with the deployment of these structures in a shallow lagoon environment. These problems can be avoided thanks to the fact that a new structure on which to attach Ortac units has recently been developed by the company that distributes these tools. This structure consists of ropes with floaters adapted to keep the Ortac units on the water surface and thus solving the problem of using more space than floating bags. Therefore, further investigation on different farming tools and their setup in the lagoon must to be investigated, in order to find the most performing one in the local context.

The results of this PhD study led an increasing interest, to stakeholders and policy makers, in the use of growth modelling. Indeed, the FLAG project discussed above and the already-funded second part of the "Ostrinnova" project involves the use of the ShellSIM® growth model. This will be involved in the project to study the productivity of different lagoon areas, in order to have more information on the potential sites of the lagoon to be used for the Oyster farm.

However, further studies must be performed to improve growth modelling, as identify the real digestible energy content of the Particulate Organic Carbon to modify the ShellSIM® assumption and improve its performances. Moreover, seasonality and farming tools can influence the accuracy of ShellSIM® providing scope for further tailoring of the model.

Finding new areas for aquaculture is a key factor in increasing this industry, and as the present study shows, site selection procedures are a powerful tool to find new space for shellfish farming. The possibility of accessing more biological and environmental data would give us the opportunity to assess the suitability of more areas. To continue to improve Pacific oyster farming in Sardinia, it will be important that stakeholders and policy makers will organize more frequent and methodical environmental sampling plans,

and a more accurate database that needs to be accessible to several stakeholders (for example research centres, industry, universities).

The collection of environmental parameters can be also implemented by using satellite images, but the resolution of such images is not sufficient to accurately describe the lagoons water biological features. Moreover, further work must to be done in Sardinian lagoons to validate data of environmental parameters produced by analysis of satellite or drone images, to obtain more precise data on the environmental condition of local shallow lagoons. However, in shallow waters bottom reflectance dominates the optical signal therefore the methodology to obtain biophysical data of the surface water layer must be investigated more thoroughly.

Moreover, results of the site selection methodology developed in this PhD study, pointed out that one of the main factors reducing site suitability in Sardinia is the water quality, and therefore this suggests to the policy makers and stakeholders to prepare an appropriate plan for water quality monitoring.

Water quality class A or B for bivalve cultures is a fundamental requirement to allocate a site to the shellfish industry, therefore to have more space for Pacific oyster farming and increase this industry in the region, the monitoring of the water pollution in coastal area (for example in lagoons) is an important key factor.

The last future work pointed out from the outcomes of this PhD thesis, is on the presence of microplastics in the coastal area due to the wastewater treatment plants. These are often present where other human activity as *C. gigas* farming are carried out, therefore it will be important to investigate in Pacific oyster farms which is the main source of microplastic (wastewater treatment plants, sea, farming equipment?). This will give more information to the stakeholders on food safety and on how to prevent this type of pollutions around the farms. Moreover, depuration effect on smaller microplastics that those used in the study of this PhD project, must to be investigated.

Microplastics in the marine environment are often covered by biofilms of opportunistic microbial colonisers, therefore theoretically becoming a way of dispersal for microorganisms across coastal and marine environments. Indeed, in the study of Rodrigues *et al.*, (2019) the presence of *E. coli* and *Vibrio spp*. was reported on the microplastics' surface. Therefore, with the view of improving Pacific oyster industry, further work must to be performed to investigate if the microplastics could become a way to transfer

pathogens, as for example *Vibrio aestuarianus* or *Ostreid herpesvirus 1* that are often associated at mass mortalities in Pacific oyster.

## 6.5. Concluding Remarks

Taken together, the results of this PhD thesis provide new information on how to improve Pacific oysters farming in shallow coastal Mediterranean lagoons:

- Identification of the most suitable farming systems and methods in relation to local ecological conditions and specific production phase (Grow-out or Finissage).
- Validation of a bio-energetic growth model applicable to the local farming environment.
- Identification of a methodology for site selection procedure.
- Identification and characterisation of suitable sites for the commercial production of triploid Pacific Oyster (*Crassostrea gigas*).
- New knowledge on microplastics uptake and egestion dynamics in market size Pacific oysters.

Moreover, these study outcomes are directly accessible by the industry, and stakeholders have already used some of the outcomes. All the outcomes have been transferred by meetings and workshops to the stakeholders, and new companies in the sector are now being supported in their initial efforts to farming Pacific oyster. The knowledge developed during this study has therefore the potential to support local employment and economic growth in the Sardinian region.

All the studies for this PhD were performed in Sardinian lagoons, but all the outcomes were produced to be employed in a larger Mediterranean lagoon context.

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# Appendix I – Publications

## Publication 1

**Graham, P.**, Palazzo, L., De Lucia, G. A., Telfer, T. C., Baroli, M., Carboni, S. (2019) Microplasitics uptake and egestion dynamics in dynamics in Pacific oysters, *Magallana gigas* (Thunberg, 1973), under controlled conditions. *Environmental Pollution* **252 (A)**, 742-748.


## Publication 2

**Graham, P**., Brundu, G., Scolamacchia, M., Giglioli, A., Addis, P., Artioli, Y., Telfer, T., Carboni, S. (2019) Improving pacific oyster (*Crassostrea gigas*, Thunberg, 1793) production in Mediterranean coastal lagoons: Validation of the growth model "ShellSIM" on traditional and novel farming methods. *Aquaculture* **516**, 734612.

**Publication 3**

**Graham, P.**, Falconer, L., Telfer, T., Mossone, P., Viale, I., Carboni, S. (2020) A modelling approach to classify the suitability of shallow Mediterranean lagoons for Pacific oyster, *Crassostrea gigas* (Thunberg, 1793) farming. *Ocean and Coastal Management* **191**, 105234.

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A modelling approach to classify the suitability of shallow Mediterranean lagoons for pacific oyster, *Crassostrea gigas* (Thunberg, 1793) farming

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